10

SEQUESTRATE FUNGI

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Sequestrate fungi (Fig. 10.1) are fleshy, leathery, rubbery, or cartilaginous species that sequester, or seclude, their spore-bearing tissues, preventing the spores from discharging into their surroundings (Kendrick 1992). Usually, the spore-bearing tissues are enclosed within a persistent peridium. Sequestrate fungi, with the exception of the "secotioid" species, are hypogeous, without a stipe or with only a rudimentary one (true and false truffles, respectively). The secotioid species are partly to almost entirely exposed at maturity and may have a well-developed stipe. About 150 genera and 1200 valid species of sequestrate fungi have been described in 38 families, representing 11 orders in the Ascomycetes, Basidiomycetes, and Zygomycetes. Much of the world has yet to be explored for sequestrate fungi, however, including most of Asia, Africa, and South America. New species are being discovered regularly in North America and Australia, which have been searched extensively for members of this group. Only Europe has been covered thoroughly by collectors of sequestrate fungi, and even there, new species continue to be discovered. The total number of species existing in the world is likely more than double that currently known.

In the Ascomycetes and Basidiomycetes, most sequestrate fungi appear to be ectomycorrhizal mycobionts (Molina et al. 1992). However, saprobic taxa occur in both groups, especially in New Zealand (e.g., *Paurocotylis* species and *Weraroa* species) and in tropical rain forests in Australia (e.g., *Stephensia* species). Sequestrate forms of Zygomycetes are found in the Endogonales and Glomales. Their fruiting bodies range from loose clusters of spores to dense, somewhat organized masses of spores. Most sequestrate species in the Endogonales

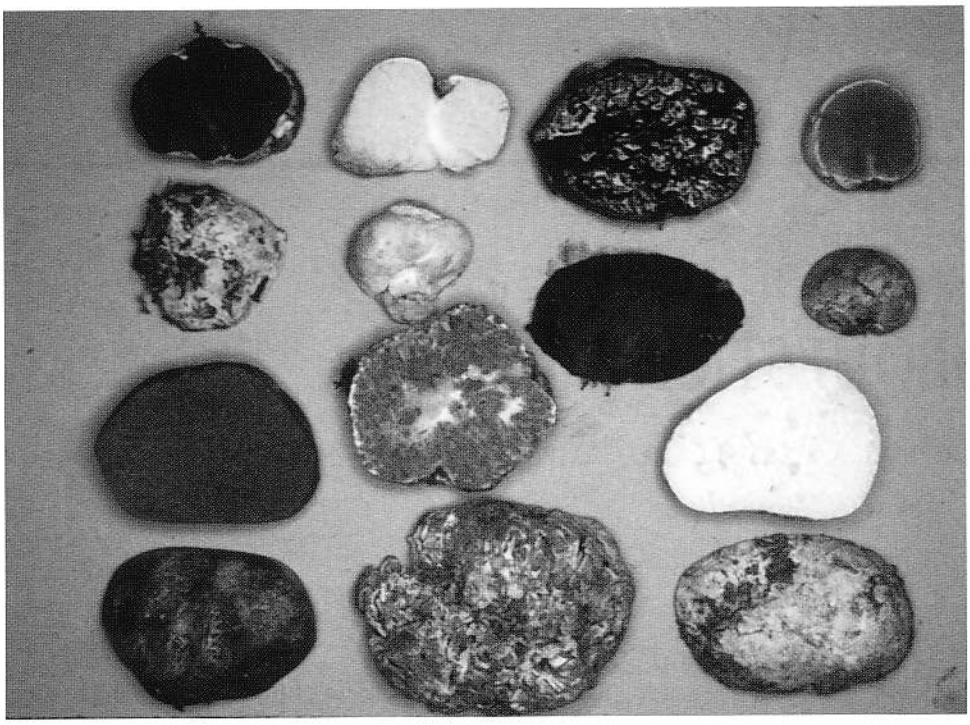

FIGURE 10.1 Sequestrate fungi.

appear to form ectomycorrhizae (Gerdemann and Trappe 1974), but *Endogone pisiformis* and probably other closely related taxa are saprobic (Berch and Castellano 1986). We do not include arbuscular mycorrhizal (AM) Zygomycetes, which fruit as individual spores in soil or roots, in our definition of "sequestrate fungi." The Glomales, in general, form arbuscular mycorrhizae with assorted host plants, but sporocarpic sequestrate forms are associated primarily with woody perennials (Gerdemann and Trappe 1974).

ECOLOGY AND EVOLUTION

Distribution and Habitats

Sequestrate fungi usually associate with trees or shrubs and hence are most commonly found in forests, woodlands, and shrublands. Ectomycorrhizal forests once were thought to occur primarily in temperate to boreal zones (Meyer 1973), but now ectomycorrhizae are known to abound in many tropical forests as well (Allen et al. 1995). Usually, wherever ectomycorrhizal hosts occur, ectomycorrhizal sequestrate fungi also occur. Similarly, sequestrate AM fungi may be found among roots of tropical to boreal AM trees and shrubs. Saprobic sequestrate species may occur similarly within this range of habitats. Some mycorrhizal sequestrate fungi occur in deserts (Gilkey 1939; Alsheikh and Trappe 1983a, 1983b; Alsheikh 1994), where they may form special types of mycorrhizae with annual or perennial hosts (Awamah et al. 1979). Some saprobic species (Zeller 1943; Singer and Smith 1958; McKnight 1985) also are found in deserts, although in general few fungal species and specimens of any type are found in this harsh habitat.

Data on species diversity of sequestrate fungi are scant, but our observations from extensive collecting of sporocarps suggest some generalities (J. M. Trappe and M. A. Castellano, unpublished data). Species diversity of all sequestrate groups can be relatively high in the tropics, but in general it seems highest in subtropical to temperate forests (Allen et al. 1995). Diversity, based on sporocarp collections, tends to decline in austral or boreal habitats with increasing proximity to the polar regions. Similarly, species diversity in mountains tends to be greater in low-elevation forests than in subalpine to treeline habitats. In alpine and arctic habitats, diversity of sequestrate fungi is low, even when suitable hosts such as krummholz conifers, willows, or birches are present (Laursen and Miller 1978; Cázares and Trappe 1990; Graf and Horak 1993; J. M. Trappe, unpublished data). Hosts suitable for sequestrate fungi are rare or lacking in Antarctica.

Origin of Life Forms

Sequestrate fungi in the Ascomycetes and Basidiomycetes share a common ancestry with or were derived from epigeous, nonsequestrate forms of cup fungi and mushrooms (Trappe 1979; Miller 1983; Thiers 1984; Bruns et al. 1989; O'Donnell et al. 1996). They have evolved along several phylogenetic lines into fungi ranging from mushroomlike to trufflelike. The Basidiomycete order Boletales exemplifies these forms particularly well (Miller 1983; Thiers 1989; Cázares and Trappe 1991). In species of the mushroom genus *Suillus,* for example, spores are produced in hymenium-lined tubes from which they are discharged forcibly. That genus is related closely to *Gastrosuillus,* which, although retaining a modified mushroom form, has hymenium-lined tubes that are contorted and plugged so that the spores are sequestered—they cannot escape to the air (Bruns et al. 1989; Thiers 1989). The stipes of *Gastrosuillus* species are much reduced, so that the spore-bearing tubes are not raised out of the ground.

Truncocolumella species represent a further progression toward reduced reproductive structures (Smith and Singer 1959). In this genus, the spore-bearing tubes found in *Suillus* and *Gastrosuillus* have been replaced by hymenium-lined chambers, and the entire basidioma is enclosed in a persistent peridium. *Truncocolumella* lacks a stipe or, at most, a vestigial ancestral stipe occasionally projects from the base of the columella. Further reduction of the sequestrate basidioma is found in *Rhizopogon* species, which resemble *Truncocolumella* in form but lack a columella (Miller 1986; Bruns et al. 1989). Finally, genera such as *Alpova* and *Melanogaster* lack the chambers and hymenia; the gleba is a gelatinous-solid mass of pockets packed with gelatinized basidia and spores (Trappe 1975).

Similar morphological progressions occur in several other families of Basidiomycetes. In the Cortinariaceae, the mushroom genus *Cortinarius* is analogous to the stipitate sequestrate genus *Thaxterogaster.* The basidiome is reduced further in the genera *Cortinomyces* and *Hymenogaster,* with some species having a columella and others losing all trace of it (Bougher and Castellano 1993). Two major progressions similarly occur in the Russulaceae: from *Russula* species to the stipitate, sequestrate *Macowanites* species, with a contorted-lamellate to loculate gleba, followed by the astipitate, loculate *Gymnomyces* species, and from *Lactarius* species to *Arcangeliella* species, followed by *Zelleromyces* species (Singer and Smith 1960). In the Tricholomataceae, the reduction from mushroom to simple sequestrate forms occurs from *Laccaria* species, through *Podohydnangium* species, to *Hydnangium* species (Bougher et al. 1993; Mueller and Ammirati 1993).

Two puffball genera, *Radiigera* and *Pyrenogaster,* are sequestrate. The sequestrate forms are hypogeous and resemble unopened specimens of their epigeous analogues such as *Geaster* species. They have lost the sutures that permit the peridium to open to produce the earth star effect of mature *Geaster* species (Toledo and Castellano 1996). The genus *Scleroderma* consists mostly of epigeous species with a peridium that opens apically to expose the powdery spore mass to the air. Some *Scleroderma* species, however, consistently remain below ground and never open (Zeller 1922, 1947; Beaton and Weste 1982). Their spore mass still may be powdery, but in some species the spores adhere to each other to produce a more or less solid mass. Even when these species happen to emerge from the soil, their peridia remain closed (J. M. Trappe, unpublished data).

Similar phylogenetic links from epigeous to sequestrate forms are seen in the Ascomycete order Pezizales. Epigeous cup fungi, such as *Humaria,* are related to the genus *Geopora,* which has sequestrate species that fruit hypogeously and have large chambers lined with hymenia that are enclosed in a peridium (Burdsall 1968; Trappe 1979). *Stephensia* is similar to *Geopora* except that its hymenia do not line open chambers but are embedded in solid tissue (Trappe 1979). *Phaeangium* represents a further simplification: the asci are packed in pockets rather than disposed in a hymenial layer (Alsheikh and Trappe 1983a). Similarly, the epigeous cup-fungus genus *Peziza* is related to the genus *Hydnoplicata,* in which the spore-bearing tissue is folded, producing sequestrate chambers lined with hymenia (Gilkey 1954). Reproductive structures are reduced further in *Hydnotryopsis* species, in which a solid gleba encloses veins of hymenia or pockets stuffed with asci. O'Donnell et al. (1996) provided molecular evidence linking other sequestrate Ascomycetes to epigeous genera and families.

Sequestrate groups in the Zygomycetes may represent evolutionary progression in the opposite direction. The fossil record of the arbuscular mycorrhizal Glomales suggests extremely early occurrence of species that form individual spores among roots in soil (Taylor et al. 1995). Reproductive structures of present-day sequestrate species range from simple, tight masses of spores to complex fruiting bodies (Gerdemann and Trappe 1974), which in this case could be regarded as an evolutionary advance. The origin of the Endogonales is obscure, but reproductive structures of sequestrate *Endogone* species also range from simple masses of spores to organized arrangements of spore-bearing tissues (Gerdemann and Trappe 1974).

These "evolutionary experiments" involve simplification of structure (except, perhaps, in the Zygomycetes) and increased protection from climatic stress accompanied by increased energy efficiency and specialized

adaptation to spore dispersal. Selection favoring protection of sporocarps from heat, drought, or freezing leads to partial or complete immersion of sporocarps in the protective, insulating soil. Consequently, the mushroom stem no longer is needed to raise spore-bearing tissues into the air, nor is the cap needed to spread those tissues for forcible spore discharge. If spores are not forcibly discharged, no orderly hymenial palisade is required to expose ascus or basidium tips to the air. Host-fungal mycorrhizal associations in sequestrate species no longer produce the nonfertile structures and thereby save resources. Although reduction from a form with an elaborate cap, stem, and hymenial tissue ultimately to a simple, truffle form with less differentiated tissue seems morphologically radical, molecular evidence indicates that such changes do not require massive numbers of mutations (Bruns et al. 1989).

Loss of forcible discharge of spores to air must be accompanied by mutations that adapt sequestrate fungi to other spore-dispersal tactics. In most cases, the dispersal agents are animals, from arthropods and gastropods to mammals and birds. The fungi have developed a means to attract animals when spores are mature and cause them to ingest the spores. The attractants are aromatic compounds, including pheromones (Claus et al. 1981). Each sequestrate species produces its own array of aromas, usually a combination of several to many compounds (Marin et al. 1984). Immature sporocarps have little or no distinctive odor. As spores begin to mature, the attractant compounds are produced, and as additional spores mature, the aroma increases in intensity and pungency (Trappe and Maser 1977).

Some species visually attract birds. In New Zealand, which lacks native mammals, birds appear to be important vectors of sequestrate fungi. *Paurocotylis pila* has a scarlet peridium. As its ascomata expand, they lift themselves to the surface of the humus, sometimes detaching completely and lying loose on the surface. Their size and color mimic fruits of nearby *Podocarpus* species and other plants that are eaten by birds; *P. pila* also matures at the same time as the *Podocarpus* fruits. *Paurocotylis* ascomata lying on the forest floor among podocarp fruits almost surely are eaten by birds. Several other New Zealand sequestrate fungi also emerge from the forest floor and are brightly colored (e.g., species of *Chamonixia, Thaxterogaster,* and *Weraroa*). *Phaeangium lefebvrei* in deserts of the Arabian Peninsula and North Africa produces different visual signals. Several to a dozen or more small ascomata cluster to form a pronounced hump on an otherwise flat desert floor. Birds spot these humps, scratch away the overlying soil, and eat the ascomata (Alsheikh and Trappe 1983a).

Animals disperse spores in response to nutritional inducements; most mammals, including carnivores, excavate and eat sequestrate fungi (Fogel and Trappe 1978; Maser et al. 1978). Sequestrate fungi contain carbohydrates, nonprotein amino acids, proteins, and elevated concentrations of some minerals (Al-Delaimy 1977); their nutritional value is evidenced by those animals that eat little but sequestrate fungi (Maser et al. 1978; Maser et al. 1985; Claridge and May 1994). All sterile tissues are digested, but the spores pass through the digestive tract unharmed. As spore-containing feces weather, the spores are washed into the soil and potentially contact receptive mycorrhizal host feeder roots (Trappe and Maser 1977).

Some sequestrate fungi such as *Gummiglobus, Mesophellia, Castoreum, Radiigera,* and *Scleroderma* species in the Basidiomycetes and *Elaphomyces* species in the Ascomycetes have a powdery gleba. The first two of these genera have a central columella or core that mammals eat; to get at it, they peel off the outer, leathery, or carbonaceous peridium. As this is done, the powdery spores are released to the air, cling to fur, or fall to the soil (Claridge and May 1994). The other genera all have thick peridia that the animals consume. They dig up the sporocarps, often taking them to a feeding spot, and then eat the peridium. They discard the powdery spore mass inside, releasing the spores to the wind (Trappe and Maser 1977).

Most desert-dwelling sequestrate fungi are adapted for passive spore dispersal by wind. Species of *Carbomyces, Terfezia,* and *Tirmania* fruit only in years when adequate rain falls at appropriate times. The fungi produce succulent ascomata composed of large, thin-walled cells ill-adapted to withstand drying. As the relatively small *Carbomyces* ascomata expand and mature, they tend to lift themselves out of the soil. When fresh, they may be eaten by animals (Zak and Whitford 1986). If not eaten, they dry and the inflated, thin-walled cells of the gleba disintegrate into a spore-bearing powder. The ascomata of *Carbomyces* species are blown about the desert floor by the wind, dispersing the spores as the peridium abrades. *Terfezia* and *Tirmania* species tend to have relatively large ascomata. As they expand, they push the overlying soil upward. The wind blows away the soil as it dries to expose the sequestrate ascomata, which quickly dry. The initially large, succulent cells of the gleba disintegrate into a spore-bearing powder, which wind-blown sand abrades away.

Development of sequestrate sporocarps in the Zygomycetes could enhance spore dispersal because, without animal mycophagy, the Endogonales and Glomales mostly are dispersed by movement of soil through the action of wind, water, landslide, or animals feet. The individual spores produced in soil by most Glomales, although large in comparison to spores of other fungi, are probably too small to attract even the smallest

mammals or birds. Large masses of spores clustered in compact sporocarps, however, are readily found and consumed by small mammals (Maser et al. 1978). Two species show adaptations that may promote dispersal by birds. *Endogone pisiformis* and *Glomus convolutus* are both bright yellow and fruit in the spring on the soil surface at the edge of melting snow banks (Gerdemann and Trappe 1974). They are spotted easily, and birds may be attracted to them or mistake them for large seeds or small fruits.

The wide distribution, considerable diversity, and local abundance of sequestrate fungi imply that this morphology confers broad evolutionary fitness. The sequestrate habit has arisen independently in parallel evolutionary lines in different places. Australia and North America provide a striking comparison. The *Suillus–Gastrosuillus–Truncocolumella–Rhizopogon* line is host-specific to members of the Pinaceae, a family that does not occur naturally in Australia. The *Descolea–Setchelliogaster–Descomyces* line appears likely to be host-specific to eucalyptus, which does not occur naturally in North America. The *Russula–Macowanites–Gymnomyces*, the *Lactarius–Arcangeliella–Zelleromyces*, and the *Cortinarius-Thaxterogaster–Protoglossum* lines are found on both continents, although no species occur on both continents.

So far as is known, no sequestrate fungi are toxic, which reflects their dependence on mycophagy for spore dispersal. Killing or discouraging one's dispersal agents hardly would confer reproductive advantage. No phylogenetic lines from toxic epigeous fungi to edible sequestrate fungi are known. The genus *Cortinarius* contains both edible and toxic species. We hypothesize that its sequestrate relatives, *Thaxterogaster* and *Protoglossum*, will prove to be related to the edible *Cortinarius* species.

It generally is believed that epigeous fruiting taxa are more numerous than sequestrate fungi, but presently no evidence suggests that one life form is more successful than the other. In all likelihood, one may be favored over the other in certain niches or habitats or in certain climates. Why the sequestrate habit occurs overwhelmingly among mycorrhizal, rather than saprobic, fungi is a matter of speculation.

Trophic Relationships and Functional Roles

The mycorrhizal or saprobic habits of the epigeous relatives of most sequestrate fungi carry over into the sequestrate fungi themselves, often to the same degree of host specificity (Molina and Trappe 1982; Molina et al. 1992). Mycorrhizal fungus–host interactions have been covered extensively elsewhere (e.g., Harley and Smith 1983; Miller and Allen 1992) and need not be repeated here. In summary, the fungus absorbs water and nutrients from the soil and transfers them to the host and, in return, receives photosynthates in the form of carbohydrates from the host. The saprobes, in contrast, produce the enzymes needed to extract energy and nutrients from dead organic matter without recourse to symbiosis with plant rootlets.

The adaptations found in organisms associated with sequestrate fungi are a tribute to interdependence and coevolution. Perhaps the ultimate example is provided by old-growth forests of Pinaceae in the Pacific Northwestern United States. The trees require ectomycorrhizal fungi for adequate mineral nutrition, and many of those fungi are sequestrate. The mycorrhizal fungi need energy from the trees to fruit, and the sequestrate fruiting bodies are a primary food of the northern flying squirrel, *Glaucomys sabrinus* (Maser et al. 1985). The squirrel, in turn, disperses the spores and is the primary food of the northern spotted owl, which nests in and hunts from the trees (Carey 1991). Once an owl captures a squirrel, it may carry it a considerable distance before gutting it; the entrails are likely to contain spores of sequestrate fungi. In summary, the trees need the fungi for their nutrition. The fungi need trees for energy, and they need squirrels for spore dispersal. The squirrels need trees for habitat, and they need fungi for their nutrition. Owls need squirrels for their nutrition, and they need trees for nesting and hunting. Owls also serve as long-distance vectors of spores.

Little is known about the trophic characteristics of the relatively few sequestrate saprobes. *Nivatogastrium nubigenum* fruits on or near brown-cubical-rotted wood in western North American mountains, as do its epigeous *Pholiota* relatives (Singer and Smith 1959). *Endoptychum depressum, Paurocotylis pila, Stephanospora* species, and *Weraroa* species fruit on forest or other humus, as do some species of their related, epigeous genera (e.g., *Agaricus, Aleuria, Lindtneria,* and *Stropharia*). *Endogone pisiformis* fruits on a variety of organic substrata, including humus, rotten wood, and dead basidiomata of various species of the Polyporaceae (Trappe and Gerdemann 1979).

Some sequestrate fungi also have special capabilities. *Hysterangium* species and *Gautieria* species, for example, form dense mycelial mats in forest soil. The mats have specific biogeochemical properties (Griffiths et al. 1991). *Rhizopogon* species typically induce prolific branching of host rootlets through production and release of auxin (Ho and Trappe 1987). It is interesting that the ancestral *Suillus* species do this as well. *Rhizopogon* species also have been shown to enhance the survival and growth of seedlings of *Pseudotsuga menziesii* in forest plantations (Castellano and Trappe 1985). *Tuber*

melanosporum produces herbicidal compounds that reduce weed competition with the host tree (Montacchini and Lomagno 1977).

Limitations to the Study of Sequestrate Fungi

Sequestrate fungi are difficult to study because most fruit hypogeously. When they fruit in compacted soil with little humus, however, they may emerge or at least raise a hump in the soil because lateral and downward expansion is difficult. Campgrounds, picnic grounds, roadsides, and old logging skid trails or landings thus are places where sequestrate fungi can be found easily when they fruit. Forest soil, in contrast, is generally not compacted and has a layer of humus and forest litter. Sequestrate fungi can expand in all directions without providing visual clues to their presence.

The holes left by animals that have excavated sequestrate fungi can indicate the location of a colony because these fungi commonly produce multiple sporocarps that often mature at different times. Raking in the vicinity of an animal dig and to about the same depth often will reveal additional specimens (Fig. 10.2). During the peak of the fruiting season, this approach can be particularly productive because production of sequestrate sporocarps often exceeds the food demands of resident animals. At the beginning and end of the fruiting season, however, when demand exceeds supply, animals remove a significant portion of the mature specimens (North and Trappe 1994).

In Europe, dogs are trained to find commercially valuable sequestrate fungi such as truffles. A collector lacking a trained dog must rely on animal digs, knowledge of the kinds of habitats that are productive, and chance to find sequestrate fungi in forests. This poses special problems for inventory and monitoring of fungal diversity or of rare species (see "Surveying Rare Species," later in this chapter).

Fruiting of sequestrate fungi varies by season and geographic area. In general, however, sequestrate species fruit at about the same time as epigeous fungi in any particular area. Fruiting seasons of sequestrate species tend to be extended because of their mostly hypogeous habit, which protects them from frost, heat, and drought.

In areas where sequestrate fungi have pronounced spring and autumn fruiting seasons, the species fruiting generally differ between the two seasons (Luoma 1988; Luoma et al. 1991). At low elevations in the Pacific Northwest United States, for example, fruiting sequestrate Ascomycetes are more abundant in spring than in autumn, and fruiting sequestrate Basidiomycetes are more abundant in autumn than spring. Those fruiting patterns mirror those of their epigeous relatives. At higher elevations, in contrast, the season is confined to a few summer months, and the seasonal separation of fruiting species is much less distinct. That is also true of areas with a single rainy season, such as much of Asia and Australasia.

FIGURE 10.2 Raking often will reveal specimens.

TAXONOMY, DIVERSITY, AND DISTRIBUTION

Synopsis of Taxa

Sequestrate fungi occur in two phyla of the Kingdom Eumycota: the Zygomycota (in the class Zygomycetes) and the Dikaryomycota (in classes Ascomycetes and Holobasidiomycetes). Presently recognized genera are listed in Table 10.1 by order and family. Assignments of genera to families differ in many cases from previous placements (e.g., Trappe 1979; Jülich 1981; Castellano et al. 1989; Trappe and Castellano 1991) because more recent data from additional collections or DNA studies have provided new insights into phylogeny. In many cases, the family to which a genus belongs is uncertain based on morphological data alone. Studies of ultrastructure and DNA are providing new evidence that is helping to resolve many of the uncertainties, but that work has only begun (O'Donnell et al. 1996). All taxonomic decisions are hypotheses that are tested repeatedly and sometimes refuted. Some genera do not fit into any described family and are listed under the heading "uncertain status." We are aware of many undescribed genera that are not included in our compilation.

Herbarium Collections

The study of sequestrate fungi began in Italy, where several pre-Linnaean botanists had a special interest in truffles. Carlo Vittadini (1831, 1842) laid the foundations for our modern generic concepts. His collections, which are housed at the Botanical Institute of the University of Torino (Torino [or Turin], Italy), comprise a seminal collection of type specimens (Mattirolo 1907). Oreste Mattirolo's types and other extensive collections also are housed there.

TABLE 10.1
Genera and Families of Sequestrate Fungi in the Kingdom Eumycota, by Class and Order

Phylum Zygomycota
Class Zycomycetes
Order ENDOGONALES: Endogonaceae: *Endogone*
Order GLOMALES: Glomaceae: *Glomus, Sclerocystis*
Order MUCORALES: Mortierellaceae: *Modicella*

Phylum Dikaryomycota
Class Ascomycetes
Order ELAPHOMYCETALES: Elaphomycetaceae: *Elaphomyces*
Order GLAZIELLALES: Glaziellaceae: *Glaziella*
Order PEZIZALES: Ascobolaceae: *Muciturbo*; Carbomycetaceae: *Carbomyces*; Helvellaceae: *Balsamia, Barssia*; Humariaceae: *Geopora, Hydnocystis, Phaeangium, Stephensia*; Discinaceae: *Hydnotrya*; Otidiaceae: *Genea, Genabea, Paurocotylis, Picoa, Sphaerosoma*; Pezizaceae: *Amylascus, Cazia, Hydnoplicata, Hydnotryopsis, Mycoclelandia, Pachyphloeus, Ruhlandiella, Sphaerozone, Tirmania*; Tuberaceae: *Choiromyces, Delastria, Dingleya, Hydnobolites, Labyrinthomyces, Loculotuber, Paradoxa, Reddellomyces, Terfezia, Tuber*
UNCERTAIN STATUS: *Fischerula: Leucangium: Petchiomyces*
Class Homobasidiomycetes
Order AGARICALES: Agaricaceae: *Endoptychum*; Amanitaceae: Torrendia; Bolbitiaceae: *Agrogaster*; Coprinaceae: *Gasteroagaricoides, Podaxis*; Cortinariaceae: *Cortinarius, Cortinomyces, Descomyces, Destuntzia, Hymenogaster, Kjeldsenia, Quadrispora, Setchelliogaster, Thaxterogaster*; Cribbiaceae: *Cribbia*; Entolomataceae: *Rhodogaster, Richoniella*; Lepiotaceae: *Neosecotium, Notholepiota*; Octavianinaceae: *Octavianina*; Russulaceae: *Arcangeliella, Cystangium, Gymnomyces, Macowanites, Zelleromyces*; Strophariaceae: *Nivatogastrium, Tympanella, Weraroa*; Tricholomataceae: *Hydnangium, Podohydnangium, Gigasperma*
Order BOLETALES: Boletaceae: *Alpova, Amogaster, Gastroboletus, Gastroleccinum, Gastrosuillus, Hallingia, Melanogaster, Rhizopogon, Royoungia, Truncocolumella*; Gomphidiaceae: *Brauniellula, Gomphogaster*; Paxillaceae: *Austrogaster, Gymnopaxillus, Paxillogaster, Singeromyces*; Strobilomycetaceae: *Austrogautieria, Chamonixia, Gautieria, Mycoamaranthus, Rhodactina, Timgrovea, Wakefieldia*
Order GASTROSPORIALES: Gastrosporiaceae: *Gastrosporium*
Order LEUCOGASTRALES: Leucogastraceae: *Leucogaster, Leucophleps*
Order LINDTNERIALES: Stephanosporaceae: *Stephanospora*
Order LYCOPERDALES: Geastraceae: *Pyrenogaster, Radiigera* Mesophelliaceae: *Andebbia, Castoreum, Gummiglobus, Malajczukia, Mesophellia, Nothocastoreum*
Order PHALLALES: Clathraceae: *Protubera*; Gelopellaceae: *Gelopellis, Phallobata, Phallogaster*; Hysterangiaceae: *Chondrogaster, Claustula, Gallacea, Hysterangium, Phlebogaster, Rhopalogaster, Trappea*
Order SCLERODERMATALES: Sclerodermataceae: *Horakiella, Scleroderma*; Sedeculaceae: *Sedecula*
Order TREMELLOGASTRALES: Tremellogastraceae: *Clathrogaster, Tremellogaster*
UNCERTAIN STATUS: *Brauniella, Hysterogaster, Mycolevis, Protogautieria, Sclerogaster, Smithiogaster*

Other important nineteenth and early twentieth century pioneers in the study of sequestrate fungi and the herbaria (Appendix III) that presently contain their collections include the brothers L. R. and C. Tulasne, Cryptogamic Museum of Paris (Paris, France); M. J. Berkeley, C. E. Broome, M. C. Cooke, and G. Massee, Royal Botanic Gardens (Kew, United Kingdom); N. Patouillard and F. Bucholtz, Farlow Herbarium, Harvard University (Cambridge, Massachusetts, United States); R. Hesse, University of Marburg (Marburg, Germany); L. Hollós, Hungarian Natural History Museum (Budapest, Hungary); H. W. Harkness and C. G. Lloyd, National Fungus Collections (Beltsville, Maryland, United States); and L. Rodway, Tasmanian Museum (Hobart, Australia). Unfortunately, the African and west Asian types and other collections of desert truffles of A. Chatin have been lost (R. Heim, personal communication).

Many mycological herbaria have a few collections of sequestrate fungi, but the most important mid- to late-twentieth century collectors and the locations of their collections are as follows (see Holmgren et al. 1990; Appendix III):

Europe—M. Soehner, Botanisches Staatssammlung, Munich (M); A. Knapp and E. Horak, Eidgenössische Technische Hochschule Zürich, Zürich (ZT); C. and L. R. Tulasne, Laboratoire de Cryptogamie, Paris (PC); M. G. Malençon and L. Riousset, Institut de Botanique, Montpellier, France (MPU); M. Lange, Botanical Museum, University of Copenhagen (C); L. Kers, Bergius Foundation, Stockholm (SBT)

North America—S. M. Zeller, New York Botanical Garden, The Bronx (NY); R. Thaxter, Farlow Herbarium, Harvard University, Cambridge (FH); H. E. Parks, University of California at Berkeley (UC); A. H. Smith and R. D. Fogel, University of Michigan (MICH); M. A. Castellano, J. W. Gerdemann, H. Gilkey, D. Luoma, and J. M. Trappe, Oregon State University (OSC); H. Saylor and H. D. Thiers, San Francisco State University (SFSU); J. S. States, Deaver Herbarium, Northern Arizona University, Flagstaff (ASC); D. R. Hosford, Central Washington University, Ellensburg (ELRG)

Australasia—J. W. Cribb, Department of Plant Industries, Indooroopilly, Queensland (BRIP); J. Cleland and J. Warcup, State Herbarium of South Australia, Adelaide (AD); G. Beaton and T. Lebel, Royal Botanic Gardens, Melbourne, Australia (MEL); N. Bougher and N. Malajczuk, C.S.I.R.O. Division of Forestry, Wembley, Perth; L. Rodway, Tasmanian Herbarium, University of Tasmania, Hobart (HO); G. H. Cunningham and R. Beever, Landcare Research, Auckland (PDD)

Live Culture Collections

Some sequestrate fungi (e.g., *Rhizopogon* species) grow reasonably well in culture and are easy to isolate. Most such fungi, however, grow poorly or not at all in culture media tried so far. This is particularly true of the Ascomycetes, Zygomycetes, and those Basidiomycetes in the Russulaceae and Cortinariaceae. The saprobic species probably can be cultured with greater success than mycorrhizal species, judging from the few attempts with which we are familiar. We are aware of only two culture collections that include several to many taxa of sequestrate fungi: that of the U.S. Forest Service, Pacific Northwest Research Station and that of C.S.I.R.O., Division of Forestry (Appendix III). Identity of any cultures not represented by voucher collections in herbaria should be regarded as questionable because misidentification of species is common due to lack of good keys for most taxa.

Patterns of Species Richness and Endemism

Much of temperate Europe, western North America, New Zealand, temperate and tropical Australia, and the deserts of North Africa and western Asia have been examined extensively for sequestrate fungi, although large areas and many habitat types in all these places are, as yet, largely unexplored. The rest of the world remains unexplored or only scantily explored.

Species richness is generally greatest at subtropical to middle latitudes in ectomycorrhizal forests. In the Northern Hemisphere, richness declines from middle latitudes northward into the boreal forest and farther north. The only equivalent forests in the Southern Hemisphere, in South America and New Zealand, are not well enough explored to draw conclusions concerning the existence of similar gradients. Sequestrate species richness appears to be greater in North America and Australia than in Europe, the same as for ectomycorrhizal hosts. In North America and Australia, the predominance of north–south-oriented mountain ranges permitted fungal and host-species migrations in response to climate changes during the Ice Ages. In Europe the predominantly east–west orientation of major mountain ranges impeded species migrations, a factor of particular importance to fungi depending on animals for spore dispersal.

Detailed data on species richness by habitat are not available, but our studies in western North America and Australia, together with studies of collections from Europe, suggest some generalities. Presence of ectomycorrhizal host plants is associated strongly with relative

richness. Because many sequestrate fungi are specific to individual host genera, a diversity of host genera in a region also contributes to richness. Moist forests appear to support greater richness than either dry or very wet forests. The *Pseudotsuga* forests of the Pacific slope of western North America support a very rich sequestrate mycota, as do the *Eucalyptus* forests of eastern Australia. Richness is reduced considerably in rain forests or strongly xeric forests in both regions.

Stand age and history probably influence sequestrate species richness. Several studies of ectomycorrhizal fungi currently in progress in the Pacific Northwest include stand age as a variable, but, so far, no general conclusions can be drawn. Many more than 100 sequestrate species are associated with mature to old-growth forests in the Pacific Northwestern United States (USDA Forest Service and USDI Bureau of Land Management 1994).

Endemism is common among the sequestrate fungi, in part because of host specificity. Pines, Douglas fir, spruce, true firs, oaks, Eucalyptus, and other ectomycorrhizal hosts harbor numerous sequestrate fungi that do not fruit in association with other host genera. In addition, the dependence of sequestrate fungi on animals for spore dispersal restricts their ability to cross major geographic barriers, such as oceans, deserts, or major mountain ranges. The combination of host specificity, narrow habitat requirements, and limited dispersal may confine successful sequestrate mutations to their region of origin for extended periods.

Sequestrate taxa known only from the type or from a few collections in a limited area are inferred to be locally endemic. Because finding ephemeral fruiting bodies is a matter of chance, at least for hypogeous species, some taxa now inferred to be locally endemic subsequently may prove otherwise. For example, when the Ascomycete *Cazia flexiascus* was described (Trappe 1989), it was known only from the type collection in southwest Oregon. It later was found 80 km to the north, and recently it has been collected in southern California, 1500 km to the south. Although still regarded as rare, this species no longer can be regarded as locally endemic.

SURVEY AND INVENTORY

Global Assessment

Sequestrate fungus communities have not been assessed adequately in any large geographic region. It is only in the last 20 years that stand-level assessments have been conducted in restricted areas of Oregon, Washington, and northern California (studies by R. Fogel, G. Hunt, D. L. Luoma, J. Smith, W. Colgan III, M. North, and J. Waters). Trappe and Castellano (personal communication) in collaboration with N. Malajczuk, N. Bougher, T. Lebel, P. Reddell, and A. Clardige recently have finished a series of 20 collecting trips over 13 years to ectomycorrhizal forests of Australia for assessment of sequestrate fungi. The volume of material collected in fewer than 400 person-days of collecting was astonishing: 7500 collections representing approximately 600 species, 90% of which are undescribed. Sampling was neither systematic nor intensive; rather, broad-based surveys were conducted across large tracts of land in just a few fruiting periods.

Minimum Resources Required

Not enough systematic baseline information has been collected to allow numbers of species to be found in "typical" temperate or tropical forests to be estimated accurately. In general, however, it appears that the diversity of sequestrate fungi increases with the diversity of ectomycorrhizal hosts and the variability of soils and topography.

A crew of six trained personnel can survey about 10 hectares in a day. Each area surveyed should be visited every 2–3 weeks during the fruiting season(s). Surveys should be conducted for a minimum of 3 years, and preferably 5 years, to increase the detection of rare and infrequently fruiting species. Three to four days of laboratory work are required for each day of fieldwork.

Sampling Across Space and Time

Most forests contain diverse microhabitats. Even in "uniform" plantations, the microtopography varies with localized wet or dry soil conditions. Distribution of woody debris is also variable and can be patchy, buried, or exposed. Some sequestrate fungi commonly are associated with or found in rotten wood (e.g., *Radiigera* species, *Hydnotrya variiformis*). The patchiness of ground cover and shrub and herb layers can dramatically affect the microclimate in restricted areas. Sites with heavy ground cover are more difficult to search for fungal specimens because laying out plots is difficult and the view is obstructed. Slope and aspect have an important effect on water relations and temperature. In the Pacific Northwest United States, steep, south-facing slopes tend to be the driest, and north-facing, gentle slopes are the wettest. All these variables must be accounted for when designing sampling procedures for each sampling objective.

Voucher Specimens, Collecting Protocols, and Permits

Some species are specially protected at the local, state, or federal level and can be collected only if authorized under scientific collecting permits. Local authorities usually can help investigators to secure permits needed. Collecting of some species (e.g., nonsequestrate polypore *Oxyporus nobilissimus*) is not permitted, and photographs, along with detailed locality and microhabitat information, should be used as a record of occurrence.

When permitted, voucher specimens must be collected to document species occurrence. The specimens should be annotated with appropriate information (see next paragraph) and then sent to a recognized herbarium for long-term storage. Except in the case of multiple collections of extremely common species from the same locality in a narrow time frame, all specimens should be kept. Large collections of common species do not provide any additional information, particularly for a location that has been collected previously. A single representative of each of the common species per collecting period is adequate to document presence over time. Most, if not all, specimens of rare or uncommon species should be harvested carefully and sent to an herbarium; such specimens may provide additional morphological information after careful study or may represent incompletely known taxa.

The date, specific location, collector, habitat, notes on the plant community, and transient attributes that may be useful for identification should be noted on the specimen label. Habitat characteristics, particularly the presence of the large woody plants, are important for determining the ecology of the fungi. In addition, identification of at least some sequestrate fungi to species requires information on fresh colors and odors, subsequent color after exposure and handling (after 10–20 minutes and again after 2–3 hours or the next day after storage in a refrigerator), color after drying, and exudation of latex from or color changes of the cut surface of a specimen. Note whether the specimens were found on the soil surface, emergent, or completely hypogeous. Specimens are best kept in waxed-paper sandwich bags under cool conditions until processed. Plastic wrap and closed "airtight" containers should never be used because they lead to anaerobic conditions that stimulate growth of resident bacteria and other microorganisms that quickly can degrade the condition of the sporocarp(s).

Each sporocarp should be cut at least in half to promote drying; large specimens (>3 cm in diameter) should be cut in several vertical slabs ±5 mm thick. Many species have hard, somewhat impermeable peridia that do not allow the specimens to dry appropriately. In other species, the sporocarp dries to the hardness of bone and disintegrates when broken open for access to the contained spores. A cross-section cut can readily be rehydrated with water or KOH (potassium hydroxide) and sectioned with a razor blade. Many species resemble one another on the surface but differ strikingly in the interior. Examining the interior of the sporocarp minimizes the chance of including more than one species in a single collection. Well-dried specimens are much easier to work with than those preserved in liquid. Specimens should be described and then dried as soon as possible, preferably within 1 day. Specimens of some species, if collected in prime condition, handled properly, and stored correctly, can be kept for up to 5 days before drying. Once begun, deterioration proceeds rapidly, and then much of a specimen's value for later study is lost.

Rapid drying at relatively low temperatures is the most successful method for preserving sequestrate fungi. A food dryer set at approximately 30°C works well. Good air circulation is critical. Specimens can deteriorate quickly when heat alone is used. When electricity is not available, specimens should be thinly sliced (±2 mm thick) and placed in a sealed, air-tight container with predried silica gel. Care should be taken to pack the specimens closely in the silica gel. Specimens should not touch each other within the container. Air space within the container should be kept to a minimum to ensure the effectiveness of this method. No more than one collection should be put in a container because, when dried, species often can be difficult to separate on the basis of macroscopic characters. One to two days will dry specimens sufficiently if the volume of silica gel is adequate. Specimens dried by silica gel should be transferred to a more conventional dryer at the first opportunity to ensure that they dry completely.

If silica gel is unavailable or impractical, specimens can be strung together with waxed dental floss and a large needle and suspended over a campfire. Alternatively, lightweight frames covered with a fine aluminum mesh screen can be used. The screens can be suspended over the campfire or exposed to a steady, but not forceful, breeze. In either case, the thin slices should be spaced to allow air to move around them, and their height above the heat should be adjusted to prevent cooking while drying.

Live Cultures

We direct the reader to Molina and Palmer (1982), Smith and colleagues (1994), and Chapter 3 for a com-

prehensive protocol for isolation and maintenance of live fungal cultures of both mushrooms and sequestrate fungi. Isolation of young sporocarps is most successful. The specimens from which isolates are obtained plus mature specimens from the same collection should serve as vouchers for confirmation of the species identity at a later time.

Abundance and Diversity Measures

Relative Importance and Abundance

Sporocarp abundance often is quantified to provide a measure of the relative importance of a species in an ecosystem. Sporocarp biomass is a direct measure of importance when considering sporocarps as a food source for animals (Fogel and Trappe 1978) or the variation in individual species' presence on mycorrhizae in different stands (Luoma et al. 1997), and it has been shown to vary within species across moisture gradients (Luoma 1988). The value of sporocarp biomass as an index to levels of ecological and/or physiological functioning of the mycelial "body" of a fungus, however, only can be inferred or assumed. The influence of sporocarp size on such variables as efficiency of nutrient uptake and translocation or competitive ability in interactions for root space are unknown. Furthermore, community structure as determined by sporocarp biomass may not reflect community structure determined by examination of ectomycorrhizae in the same stand (Gardes and Bruns 1996).

The use of numbers of sporocarps as a measure of importance is less desirable because across all species, the number of sporocarps for an individual species in a plot is poorly correlated with total sporocarp dry weight in that plot (Luoma et al. 1991). Species frequency, or presence in plots or stands, provides another mechanism for interpreting biomass values and importance and can serve as a measure of "commonness." For example, high frequency offers a different perspective on the potential "importance" of a species that has relatively less "important" biomass values for a particular season or habitat (Luoma et al. 1991). In studies of vascular plant communities, frequency traditionally evaluates the regularity of a species' distribution throughout a community (homogeneity) and has been interpreted with caution because variations in plot size, number, and vegetation structure strongly affect results (Cain and de Castro Oliveira 1959; Grieg-Smith 1983). Frequency may be used as a measure of abundance to convey importance by presence or absence at the stand level or by plot within stands. A constraint on the use of frequency in studies of fungal sporocarps is that species that fruit predominantly in spring or fall are restricted to fewer potential samples (on a habitat basis) than species fruiting in both seasons.

Methodologies used in vegetation surveys are not adequate for fungal surveys because of the need for repeated sampling of often cryptic populations. Sporocarps of sequestrate fungi vary in abundance and size. A major difficulty with using sporocarps to determine presence or importance of such fungi is the lack of data on the correlations (if any) between the size and extent of the thallus and the number or biomass of sporocarps. Some fungal species may have a relatively large thallus but produce few sporocarps; other species have a relatively small thallus but produce many sporocarps. Some species produce sporocarps irregularly or infrequently, regardless of thallus size. Some sequestrate species (e.g., *Hysterangium setchellii, H. coriaceum*, and *H. crassirhachis*) form clusters of numerous, small sporocarps; others (e.g., *Gautieria monticola*) form clusters of large sporocarps; and still other species (e.g., some *Tuber* species and many sequestrate Russulaceae) produce solitary, dispersed sporocarps. Using sporocarp biomass as the sole indicator of dominance is clearly tenuous. Variation in sporocarp production can serve, however, as an indicator of community response to environmental conditions. Luoma (1988) found that sequestrate community structure, as assessed by sporocarp biomass, changed in accordance with a vegetation/moisture gradient.

If sporocarp biomass estimates are combined with species-frequency data, comparisons between species and communities then are appropriate. Luoma and colleagues (1997) found a positive correlation between sporocarp biomass and mycorrhiza frequency for some epigeous (e.g., *Lactarius rubrilacteus*) and sequestrate (e.g., *Hysterangium coriaceum*, *Leucogaster rubescens*, and *Truncocolumella citrina*) mycorrhizal species. T. Bruns (personal communication), however, found little correlation between biomass and mycorrhiza frequency for several other fungal species.

Several investigators in the Pacific Northwestern United States have assessed the composition of sequestrate-fungus communities quantitatively (Fogel 1976; Fogel and Hunt 1979; Hunt and Trappe 1987; Luoma 1988, 1991; Luoma et al. 1991, 1996a, 1996b; Smith et al. 1996; M. North, personal communication; D. L. Luoma, unpublished data). Claridge and associates (1993) did likewise for some habitats in southeastern Australia. Fogel (1976) was the first to assess the community structure of sequestrate fungi in 50 1-m^2 quadrats, sampled every month for 3 years. Fogel (1981) reviewed techniques for quantification of sequestrate

fungi and correlated sporocarp production with temperature and moisture.

Vogt and associates (1981) reported on hypogeous sporocarp biomass production in two *Abies amabilis* stands. Each stand was sampled once a month for 6 months using 12 randomly chosen 4-m^2 quadrants to yield a total sampled area of 288 m^2. Luoma and colleagues (1991) compared sporocarp biomass values from a range of total sample areas: 5900, 2800, 1400, 800, and 500 m^2 and noted that small sample area increases the likelihood of overestimating sporocarp biomass at the stand level. They concluded that when using dispersed 4-m^2 plots, the sample is not adequate until the sample area totals between 800 and 1400 m^2. Vogt and associates et al. (1992) reviewed sporocarp production studies, including those of sequestrate species. Adequate replication and sampling intensity are keys for statistically sound quantitative comparisons (Stafford 1985).

Fungal sporocarps are relatively clustered (Fogel 1976, 1981; States 1985). Therefore, when quadrants fall in localized biomass concentrations, stand level biomass may be overestimated. Use of a relatively small number (with respect to the selected stand area) of random plots may exaggerate this overestimation because of the effects of sample clustering. An alternative to the impracticably large number of randomly distributed plots necessary for adequate sampling is the systematic placement of fewer plots, which will reduce the coincidence of plots with localized concentrations of biomass and reduce the tendency to overestimate production (Luoma et al. 1991).

Nonrandom sequestrate fungus distribution at the plot level is determined by the gregarious to caespitose sporocarp formation that many species exhibit (Fogel 1976; Arora 1987). The mycelial colony will occupy roots and soil in a discrete area of forest floor, and sporocarp formation is restricted to that area. With the growth of adequate mycelial biomass, a single colony can produce several to many sporocarps (Dahlberg and Stenlid 1994). If hierarchic scales of clustering were to be detected, then explanatory hypotheses could be developed and tested. One hypothesis to be tested is that older stands may exhibit a greater incidence of clustered sporocarp production as compared to younger stands. An older stand has had more time for colonies of different cluster-forming species to become established and may contain a greater number of host tree species. Greater host species richness will increase the number of host-specific sequestrate species present(Molina et al. 1994).

Species Richness

Richness is the total number of species counted in the unit of assessment, be it a subsample, plot, experimental unit, stand, or higher level landscape segment. A species-area curve (Cain and de Castro Oliveira 1959) commonly is used to examine adequacy of sampling for species richness. Determination of species richness is particularly problematic with fungi because their presence is not obvious. Hunt and Trappe (1987) pointed out that documenting all species of sequestrate ectomycorrhizal fungi in a forest stand requires collecting over several years. After 32 months of collecting over a total sample area of 1536 m^2, their species-area curve still had not stabilized.

Even when sporocarps are sampled over several years, mycorrhizal species richness may be greatly underestimated. During three spring and two autumn seasons, Luoma and colleagues (1996a) found 43 sequestrate species and about 100 species of ectomycorrhizal mushrooms from sporocarp production plots that covered a total area of 27,000 m^2. In marked contrast, 192 ectomycorrhizal morphological types (from 189 soil cores in the same study area) were found under a total soil surface area of only 0.45 m^2 (Eberhart et al. 1996; Luoma et al. 1997). Ectomycorrhizal morphological typing is somewhat subjective and is quite labor intensive, which prohibits its widespread use for rapid assessment of community composition. Until voucher collections of morphological types are analyzed with molecular tools to confirm species or generic identities, morphological typing remains inconclusive and cannot be compared easily between communities. Until molecular techniques become incorporated routinely into field studies, sporocarps will continue to provide the most common index of sequestrate species richness in a given area.

Inventory, Survey, and Monitoring

Definitions

We define inventory as cataloging or listing of individuals on hand, such as extant specimens in herbaria. We define survey as the determination or delineation of the extent of individual species at any one location. Monitoring is defined as the observation of extant individual populations through time. Adaptive monitoring/surveying is used following the discovery of a target species. Intensive sampling serves to delineate population extent, using intensive sampling procedures, such as strip plots or plots in concentric circles centered around a known location.

Sampling Protocols

Protocol implementation should be supervised by personnel trained in the use of the protocols and in fungal identification. Prior to sampling, personnel should famil-

iarize themselves with the general biology, ecology, habitat associations, and the specific morphological features of target species. That will improve accuracy of fungal identifications in the field and increase efficiency of field searches.

For analysis of alpha diversity, it is extremely important to restrict sample sites to relatively homogeneous habitat because of the variation associated with habitat diversity. Sampling habitat characteristics such as vegetation, stand age, stem density, elevation, aspect, and topographic position at multiple sites within an area will help to determine habitat homogeneity.

Periodicity

Sequestrate fungi can fruit any time of the year, depending on weather and substratum. Some species, for example, fruit in the middle of the dry season in buried rotten wood or near streams or standing water. For the most part, however, sequestrate fungi should be sampled during the rainy season(s). Periods of sporocarp formation in some sequestrate fungi are restricted (Fogel 1976; Hunt and Trappe 1987; Luoma et al. 1991). *Rhizopogon parksii,* for example, fruits only in late summer or autumn in western Oregon, whereas *R. vinicolor* in the same stands fruits mostly in spring and early summer. In some Ascomycetes sporocarps form in autumn but do not mature until the following winter or early spring. Sporocarps of other species found out of season may have been produced in season and then frozen (e.g., some collections of *R. parksii*) or dried *in situ* (e.g., some collections of *R. vinicolor*) rather than decaying. Temperature as well as rain can affect the fruiting season. Freezing weather truncates or delays the maturation of sporocarps, and high temperatures may accelerate drying of the substrata, thus curtailing fruiting.

Because timing of fruiting is uncertain and strongly seasonal (Luoma 1988), each site should be sampled repeatedly during 2 consecutive months of each fruiting season. Sampling within the 2-month window can be carried out at biweekly intervals because sequestrate sporocarps usually persist for about 2 weeks. When sampling across an elevational gradient, one should visit low-elevation, south-facing slopes first in spring, but last in autumn, and high elevation, north-facing slopes last in spring and first in autumn (Luoma 1988). Despite the restricted fruiting seasons of most sequestrate fungi, some sporocarps are likely to appear at any time during the year. Thus, sporocarps must be sampled throughout the year, rather than just during the peak fruiting period, to encompass diversity. Year-round sampling is particularly important in surveys for community structure.

Three or more years are needed to assess community structure or rarity of large fleshy fungi (Richardson 1970; Lange 1978; Arnolds 1981; Fogel 1981; Luoma 1991, 1996b, 1996c; Luoma et al. 1991; Vogt et al. 1992; O'Dell et al. 1999). The cryptic sequestrate fungus sporocarps, nearly all of which fruit below the litter, some within the mineral soil layer, are more difficult to detect than epigeous sporocarps. Therefore, one should not conclude that a species is absent from an area or that the area has been reasonably well surveyed until data from 2 to 4 years are available.

Surveying Community Structure

Community structure and richness can be assessed in several ways. The three preferred methods are plotless transects, line transects, or randomized plots, which can be permanent or temporary (moving). Once a clear objective is identified and the resources available for sampling have been assessed, the best method for meeting objectives with the available resources can be selected.

Line Transects

In this method sample plots are located along a line, which may or may not be straight, through the area of interest. The transects should be well dispersed in the sample area so as to intercept a wider variety of microsites than would be present in a single circular plot of the same area (Ruhling et al. 1984; Mehus 1986; Ohenoja and Metsänheimo 1982; Luoma et al. 1996b). If transects are established repeatedly in the same area, new transects should be at least 20 m from previously used transects. Plots are placed every 6 m along a 50-m transect (Luoma et al. 1996b). Usually, 25 4-m^2 sample plots are used. On slopes, transects of 8, 9, and 8 plots, respectively, are established on the upper-, mid-, and lower-slope strata. A "collection" is defined as those sporocarps of the same species from a particular 4-m^2 plot. A total area of 100 m^2 per 5- to 15-hectare stand in 25 4-m^2 circular plots gives a reasonable pooled estimate of major species diversity for that stand sample. Plots are marked with a flag or stake to avoid resampling. Another approach is to space plots 25 m apart on transects in the horizontal direction (along contours) and to space transects 75–150 m apart in the vertical direction (across contour). A statistician should be consulted about the sample design prior to sampling.

Randomized Plots

Although statistically sound, the randomized-plot method is logistically cumbersome because it is difficult to lay out a truly random array of plot samples. That logistical difficulty with the placement of plots should

FIGURE 10.3 Personnel performing a time-constrained search.

not be confused with the need, in most cases, to assign "treatments" to "experimental units" in a random fashion. To meet the requirements of a random placement of sample plots, every potential plot location in a study area must have an equal chance of being selected. To achieve that, each potential location must be identified and enumerated, often by use of a fine grid. A random numbers table then is used to select the sample points from the total pool. O'Dell and associates (1999) successfully used a stratified random method to locate permanent strip plots for mushroom sampling. They needed to locate only three plots per experimental unit, however. The location of many small plots, especially repeatedly over an extended period, is more readily accomplished by systematically dispersing them throughout the study area. Systematic placement has the additional advantage of providing better coverage of the range of variation encountered in a stand because randomly placed plots tend to cluster in some areas (Mueller-Dombois and Ellenberg 1974).

Plotless Transects (Time-Constrained Search)

A time-constrained search is one in which a survey area is searched for a set amount of time. Claridge and colleagues (2000) have outlined the procedure, which is more effective than a series of small plots of fixed size for determining the species diversity of an area or finding particular species. The personnel doing the search are free to sample any spots in the search area that they wish (Fig. 10.3), although they are instructed to include as many microhabitats (e.g., near to and far from trees, under thick and thin litter layer, under shrubs and in the open) as possible.

The time needed for a search will depend on the objectives of the survey and available resources. The number of person-minutes devoted to searching a given site is predetermined, preferably by preliminary trials of habitats representative of those to be searched. For example, a timer is set for 5 minutes for two people to search a site for the target fungi. At the end of the period, all specimens found are labeled; they represent the species and number of sporocarps of each found in 10 person-minutes. This procedure is repeated until the species curve levels off, thereby indicating how many person-minutes were needed to find all or nearly all taxa on the site. The procedure can be replicated several times for statistical analysis to produce a probability level for finding all taxa at a site. Claridge and colleagues (2000) determined that the probability of finding all sequestrate taxa in 1000-m^2 plots in the forest types they studied with 100 person-minutes of searching (four persons for 25 minutes) was very high.

To evaluate the taxonomic diversity of a site adequately by time-constrained sampling, the site must be sampled repeatedly over the fruiting season for 2–4 or more years. A truly complete assay of species diversity may require far more sampling per year for many years (Egli et al. 1997), but in the case of sequestrate fungi this is not feasible because the surface of an entire study

area likely could be disturbed by raking with that intensity and duration of sampling.

Surveying Rare Species

Surveys of rare species may focus on assessment of existing populations or detection of new ones. Two techniques, both tied to habitat assessment, are used in searching for new populations. Many sequestrate taxa have specific habitat requirements (e.g., soil type or associated host species). Rare sequestrate fungi first should be sought in habitats similar to those where extant specimens have been found. The most likely habitats are identified and located on the landscape. They then are searched during the most likely fruiting period, using plotless transects guided by ecological indicator or systematic placement of standard plots.

Plotless transects are useful for finding rare species because large numbers of target microsites can be searched by each individual collector. Indicators of fruiting sequestrate fungi include fresh digs by small animals and raised compacted humus material. Productive microhabitats generally include soils adjacent to well-decayed logs and protected areas (and areas adjacent to them) within small areas heavily disturbed by animals such as wild pigs. Small animals such as squirrels, mice, and voles commonly unearth sporocarps of sequestrate fungi one at a time as they mature, leaving a small pit 2–8 cm deep and 5–8 cm in diameter. Such digs sometimes are hard to distinguish from digs for seeds or insects or from hoof prints. Sometimes an animal will eat only the upper portion of a sporocarp, and the remaining portion can be observed at the bottom of the pit. Many sequestrate fungi fruit in clusters, so further exploration within a radius of 30–60 cm around a suspected fruiting spot often reveals additional specimens. It is best to rake into the soil to the depth of the nearby small animal dig. Needles, leaf fragments, or other debris in or a spider web across a small animal dig indicates that it is not fresh. Further exploration may reveal specimens, however, particularly if fresh digs are scattered about in the habitat. Plotless transects also can be useful in habitat with compacted soil or where the humus layer is thin. Under such circumstances even small specimens form small humps at the soil surface that look suspicious to a trained observer. Often, large specimens emerge from the small humps. Campgrounds, abandoned roads, road banks, and used or abandoned walking trails sometimes are productive.

If a particular rare species is sought, then plots subjectively placed in a microhabitat that is most likely to harbor the species have a much higher probability of including the target species than do randomly placed plots. Some species, for example, commonly fruit in association with well-decayed logs. When searching for these species, plots should be located along well-decayed wood in the appropriate habitat. As with all sampling procedures for sequestrate fungi, timing is critical, and repeated sampling over a number of years is required to assess rare fungi adequately.

Existing populations whose locations are fairly well known may be revisited to (1) assess population viability, (2) further delimit the population, or (3) detect nearby populations. A previously sampled circular plot can be resampled if enough time has elapsed to allow recovery or a concentric zone that extends 0.47 m in radius beyond the original plot can be sampled. The latter approach will provide additional information concerning the potential of the original thallus. To explore for other nearby populations, additional plots that extend away from the site of the original thallus can be installed in a starburst pattern, in which eight 0.5-m^2 plots surround the original plot.

Because the sampling procedure for sequestrate fungi is disruptive, particularly for rare species, it should be used cautiously. Disturbance of the microhabitat may have an adverse impact on the habitat and render it uninhabitable by the fungus that once was present. This is particularly evident in habitat such as coarse woody debris that is dismantled in sampling. Woody debris thus sampled does not return to its former structure rapidly, if at all. In our experience, soil substrata and their concomitant herbs and forbs return to predisturbance levels 1–2 years after sampling.

Sample Size

Total area to be sampled depends on the objective of the study. It takes less sampling to validate the occurrence of a species at a known location than to discover new locations. Likewise, less area is required when searching for new locations of rare species than when assessing community diversity of a stand or watershed. In general, a minimum total area of 800 to 1500 m^2 (0.5–2.0% of a stand area) must be sampled to assess species richness and community composition in a 5- to 15-hectare stand (Hunt and Trappe 1987; Luoma et al. 1991). That total is best achieved over 3–4 years of sampling.

Data Management

Field data sheets should be reviewed several times during the sampling process. A data sheet is checked for completeness when the plot to which it pertains is finished. At the end of each transect, data sheets are checked to

ensure that all plots were completed. When an individual stand is finished, data sheets should be collated and proofed. Questions should be resolved before moving to the next stand. In the evening, collections must be reconciled with data sheets. Unresolved questions may necessitate return to the stand or plot of concern. Castellano and colleagues (1999) provide excellent examples of data sheets for a variety of uses including documenting the field site, describing various life forms of fungi, and making field tags for collections.

Computer database design should be considered before commencement of data collection in the field to ensure that all necessary information is collected at the most appropriate and cost-effective time and recorded with the most useful notation. Consultation with a computer database specialist will ensure that the end product meets objectives.

Laboratory Procedures

In the laboratory, sporocarps should be described further and identified (see "Description of Taxa," later in this chapter). If biomass quantification is required, then after description, sporocarps are oven-dried and weighed to the nearest 0.01 g.

CHARACTERISTICS OF SEQUESTRATE FUNGI

Description of Taxa

The evolution of sequestrate fungi has tended toward morphological simplification coupled with specialization of spore-dispersal tactics. Because of their reduced morphology, sporocarps of sequestrate fungi generally are easier to describe than their epigeous relatives; spores, however, are ornamented and pigmented and tend to be more developed than in their epigeous relatives. Spore characters are, thus, important for distinguishing genera and species. Spore ornamentation also may serve as protection against chemical or physical hazards of passing through animal digestive tracts.

Careful notes on fresh material are important for complete descriptions of taxa, although some fresh characters vary so much among specimens from different habitats or collected at different seasons that they are only marginally useful in taxonomy. Developmental stages should be described to the degree possible. Sporocarps of some species, such as *Genabea cerebriformis,* are always small (never >1 cm in diameter). However, species that reach much larger dimensions, such as *Rhizopogon villosulus* (up to 10 cm in diameter), also can be less than 1 cm under some conditions. Color is also highly variable for most species. *Rhizopogon vinicolor* is white in youth and stains pink where bruised. As it matures, it develops patches of yellow and still stains pink. Later the yellow becomes mottled with reddish brown, and the pink staining becomes obscure. At full maturity, the peridium may be reddish brown to a large extent and may not stain where bruised. Standard and generalized color terms, such as those used by Kelly and Judd (1955), are accordingly more useful in describing sequestrate fungi than long lists of very specific terms from other color dictionaries. For example, Thiers and Smith (1969:532) described the pileus color of the hypogeous *Cortinarius velatus* as "near 'pale vinaceous drab' to 'pale purple drab' (lilac to pale lavender) to occasionally as dark as 'light purple drab' . . ." The color terms they used are from Ridgway (1912) and mean little unless one has a copy of Ridgway in hand. In the Kelly and Judd terminology, these terms translate into the more meaningful grayish pink, grayish purplish pink, and grayish purple, respectively. Because grayish purplish pink is intermediate between the other two colors, the pileus color of *C. velatus* can be described conveniently as "grayish pink to grayish purple." Responses of tissues to reagents such as KOH can be striking in a few genera of Basidiomycetes (particularly *Rhizopogon*), but they, too, vary so much with the developmental stage of the sporocarps that their use in species determinations is limited.

Other important characters of fresh sporocarps include ranges of vertical and lateral dimensions; shape (e.g., even and globose to ellipsoidal, flattened, irregular or furrowed, and lobed); presence of surface cavities or holes to interior; nature of surface (e.g., glabrous, scabrous, tomentose, verrucose, and, if verrucose, then size and configuration of the warts); color of peridium, including variations on single specimens (e.g., streaked, mottled) and differences between epicutis and subcutis; thickness of peridium; color changes where bruised or exposed in cross section; nature of gleba or interior (e.g., solid, chambered, enfolded, hollow, gelatinous, pulverulent); presence of columella or stipe; color of the various tissues of the interior and of spore deposits that line chambers; odor; taste of peridium and gleba (determine separately when possible); and response of peridium and gleba to a few drops of a 5% solution of KOH. Terms for the various types of ascomata have been proposed by Weber and associates (1997).

Because relatively few sequestrate fungi are macroscopically distinctive, micromorphological characters are especially important in delineating taxa. Fresh material can be mounted on microscope slides using water, 3%

KOH, or Melzer's reagent (Appendix II). The Melzer's reagent should be refrigerated and less than 1 month old for best results. Spores and tissue cells should be measured using water mounts because KOH and other reagents can cause swelling or shrinkage of cells, particularly ascospores. Some spore ornaments swell or dissolve in KOH (Trappe 1979; N. S. Weber, personal communication). Dried tissues can be mounted in KOH for rapid rehydration, but ascospores always should be measured from mounts rehydrated in water only. The color reactions of spores, asci, or other tissues to Melzer's reagent should be recorded directly (orange, red, purple, blue, black) rather than as "amyloid" or "dextrinoid." The cells or tissues that appear to react to Melzer's reagent should be compared to the analogous cells or tissues in KOH or water mounts to ensure that the color is indeed the result of Melzer's reagent and not a natural color.

Whenever possible, all micromorphological features of spores and nonfertile tissue from both young and well-matured specimens should be noted. Several "new" species have been described from specimens later demonstrated to be immature stages of an earlier-described species. Some characters, such as spore ornamentation, are absent or smaller on young spores than on mature spores. Other structures, such as cell walls, change progressively as sporocarps mature. In some cases the walls thicken; in others, they are thick and mucilaginous in youth, but thinner and firm in age. Some characters, such as the dextrinoid reaction of spores, may occur in young spores but not in mature spores. Zygomata can be judged as immature if all spores are thin walled. They are considered fully mature if all spores are thick walled and the spore attachments are occluded by wall material or a well-developed septum. Basidiomata can be judged as immature if spores are scattered over the hymenium and mature if the spores are crowded on the hymenium or in spore-bearing tissues and are of similar size, shape, wall thickness, pigmentation, and ornamentation (when present). Ascomata are judged to be immature if only a small proportion of the asci contain spores and fully mature if all asci contain spores of similar size, shape, wall thickness, pigmentation, and ornamentation (when present).

Characters to be recorded for each collection include ranges in size and shape of spores (means and standard deviations based on measurements of 50 randomly selected spores sometimes are useful); size, shape, and nature of spore ornamentation; spore-wall thickness; reaction of young and mature spore parts to Melzer's reagent (compared with spores in water or KOH); range of sizes in basidia or asci; reaction of asci to Melzer's reagent (intensity and location of color); range of sizes and shapes of other hymenial structures (e.g., paraphyses, brachybasidioles, cystidia, setae); structure, cell size and shape, cell-wall thickening, and other distinctive features of subhymenium, trama, stipe-columella, and each layer of peridium; presence, location (intracellular or extracellular), nature, and color of pigment deposits in KOH and Melzer's reagent; and additional distinctive reactions of cells or tissues to Melzer's reagent.

Habitat Assessment

Detailed information on habitat is potentially useful for understanding the ecology of fungi. The collector must decide how to allocate field time between collecting fungi and collecting habitat data for those fungi. Habitat in the broad sense includes a descriptive geographic location as well as latitude, longitude (degree, minutes, and seconds), and elevation (in meters). At a given location, the general habitat or forest type (complete overstory and major understory species) and estimated stand age, slope, aspect, and dominant ground-cover plants are useful. The specific habitat description for a given collection should include substratum (e.g., terrestrial, decayed wood), and fruiting habit (gregarious versus solitary, single versus ceaspitose).

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Inventory and Monitoring Methods

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