MONTANA STATE UNIVERSITY

Systematics of Rocky Mountain alpine Laccaria (basidiomycota, agaricales, tricholomataceae) and ecology of Beartooth Plateau alpine macromycetes
by Todd William Osmundson

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Sciences and Plant Pathology
Montana State University
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Abstract:
The alpine zone is comprised of habitats at elevations above treeline. Macromycetes (fungi that produce mushrooms) play important ecological roles as decomposers and mycorrhizal symbionts here as elsewhere. This research examined alpine macromycetes from the Rocky Mountains over 3 years, and includes: 1) a morphological taxonomic study of alpine Laccaria species, 2) a molecular phylogenetic study of alpine Laccaria using ribosomal DNA internal transcribed spacer (rDNA-ITS) sequences, and 3) a plot-based synecological study of macromycetes on the Beartooth Plateau (Montana/Wyoming, USA). The genus Laccaria is an important group of ectomycorrhizal (EM) basidiomycetes widely used in experimental and applied research on EM fungi. Five taxa are recognized in the Rocky Mountain alpine using macro- and micromorphological and culture data. All occur in Colorado, and are: Laccaria bicolor, L. laccata var. pallidifolia, L. pumila, L. montana and L. sp.(a new taxon similar to L. montana, with more elliptical, finely echinulate basidiospores). Only L. pumila and L. montana occur on the Beartooth Plateau. All are associated with species of Salix, and L. laccata also with Dryas octopetala and Betula glandulosa. Maximum-parsimony phylogenetic analysis of rDNA-ITS sequences for 16 alpine Laccaria collections provided strong support for morphological species delineations. Laccaria laccata var. pallidifolia is highly divergent relative to other taxa. Laccaria pumila and L. montana are supported as distinct species, along with a putative new taxon related to both. All taxa are supported by molecular synapomorphies except L. pumila, which exhibits a unique combination of insertion-deletions and single nucleotide polymorphisms. Alpine L. bicolor often lacks a violet basal tomentum, but differs from L. laccata by a robust, striate stipe and finely fibrillose pileus, characters supported by phylogenetic results. Interspecific ITS variation ranges from 1.6-7.3 %, and intraspecific variation from 0-1% in analyzed collections. Fifteen plots on the Beartooth Plateau, most containing a single EM host, were sampled multiple times per season. A total of 33 species (48% of estimated Beartooth Plateau total) were recorded. Small sampling plots focused on EM hosts were effective in representing most common EM fungal species; however, the EM host range of most species was not fully represented.

SYSTEMATICS OF ROCKY MOUNTAIN ALPINE *LACCARIA*

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Plant Sciences and Plant Pathology

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 2003

ii

APPROVAL

of a thesis submitted by

Todd William Osmundson

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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iv
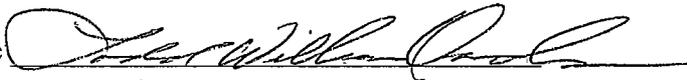
Acknowledgements

I would like to thank my thesis advisor Dr. Cathy Cripps, whose guidance and encouragement made both this project and my entering the field of mycology possible, and whose tireless enthusiasm for the Fungi serves as an inspiration for her students.

Thank you to the members of my thesis committee, Dr. Sharon Eversman, Dr. Matt Lavin and Dr. Gregory Mueller, for project and thesis guidance.

For assistance with field research, thank you to Leslie Eddington, Sarah Klingsporn, Angela Imhoff, Don Bachman, Kate Mohatt and Amy DeMers.

Assistance with molecular techniques was provided by Shirley Gerhardt, Dr. Paula Kosted, Dr. Vladimir Kanazin, Hope Talbert and Hussain Abdel-Haleen. Assistance with molecular phylogenetic methods was provided by Dr. Matt Lavin.

For loan of herbarium specimens, I wish to thank curators at the University of Tennessee Herbarium and the Field Museum, Chicago.

Assistance with scanning electron microscopy was provided by Nancy Equall in the Montana State University Imaging and Chemical Analysis Lab.

I gratefully acknowledge funding support from the National Science Foundation Biotic Surveys and Inventories Program (grant to Dr. C.L. Cripps, # 9971210), Montana State University College of Agriculture Bayard Taylor Scholarship, and Dr. Gregory Mueller and the Field Museum for travel to the Field Museum for specimen examination.

Thank you to my professional mycological mentors, Dr. Cathy Cripps, Dr. Gregory Mueller and Dr. Qiuxin Wu for countless hours of instruction and unforgettable field experiences.

TABLE OF CONTENTS

TABLE OF CONTENTS – CONTINUED

TABLE OF CONTENTS – CONTINUED

LIST OF TABLES

LIST OF TABLES - CONTINUED

LIST OF FIGURES

LIST OF FIGURES – CONTINUED

ABSTRACT

The alpine zone is comprised of habitats at elevations above treeline. Macromycetes (fungi that produce mushrooms) play important ecological roles as decomposers and mycorrhizal symbionts here as elsewhere. This research examined alpine macromycetes from the Rocky Mountains over 3 years, and includes: 1) a morphological taxonomic study of alpine *Laccaria* species, 2) a molecular phylogenetic study of alpine *Laccaria* using ribosomal DNA internal transcribed spacer (rDNA-ITS) sequences, and 3) a plot-based synecological study of macromycetes on the Beartooth Plateau (Montana/Wyoming, USA). The genus *Laccaria* is an important group of ectomycorrhizal (EM) basidiomycetes widely used in experimental and applied research on EM fungi. Five taxa are recognized in the Rocky Mountain alpine using macro- and micromorphological and culture data. All occur in Colorado, and are: *Laccaria bicolor*, *L. laccata* var. *pallidifolia*, *L. pumila*, *L. montana* and *L. sp.*(a new taxon similar to *L. montana*, with more elliptical, finely echinulate basidiospores). Only *L. pumila* and *L. montana* occur on the Beartooth Plateau. All are associated with species of *Salix*, and *L. laccata* also with *Dryas octopetala* and *Betula glandulosa*. Maximum-parsimony phylogenetic analysis of rDNA-ITS sequences for 16 alpine *Laccaria* collections provided strong support for morphological species delineations. *Laccaria laccata* var. *pallidifolia* is highly divergent relative to other taxa. *Laccaria pumila* and *L. montana* are supported as distinct species, along with a putative new taxon related to both. All taxa are supported by molecular synapomorphies except *L. pumila*, which exhibits a unique combination of insertion-deletions and single nucleotide polymorphisms. Alpine *L. bicolor* often lacks a violet basal tomentum, but differs from *L. laccata* by a robust, striate stipe and finely fibrillose pileus, characters supported by phylogenetic results. Interspecific ITS variation ranges from 1.6-7.3 %, and intraspecific variation from 0-1% in analyzed collections. Fifteen plots on the Beartooth Plateau, most containing a single EM host, were sampled multiple times per season. A total of 33 species (48% of estimated Beartooth Plateau total) were recorded. Small sampling plots focused on EM hosts were effective in representing most common EM fungal species; however, the EM host range of most species was not fully represented.

CHAPTER 1

LITERATURE REVIEW AND PROJECT OVERVIEW

Introduction

Alpine organisms live close to life's fringes, surviving under nearly constant conditions of environmental stress. While the climate, faunas, and vascular plant floras of alpine regions have been well (though by no means comprehensively) studied, fungi and other soil microorganisms, though of critical importance to ecosystem functioning, are relatively poorly known. This study is an attempt to make progress toward filling this gap in our understanding. The research described comprises two major components: a systematic study of Rocky Mountain alpine *Laccaria* species (Order Agaricales) using morphological and molecular data, and a plot-based synecological study of alpine macrofungi (Phylum Basidiomycota, Order Agaricales) on the Beartooth Plateau in southern Montana and northern Wyoming.

The term "alpine zone" refers to high mountain habitats situated at elevations above the climatic treeline, on an elevational gradient between the subalpine and nival (permanent snow cover) zones (Fig. 1). Treeline, the elevational limit for growth of the tree form in vascular plants, is situated at altitudes above which trees are physiologically unable to ripen shoots quickly enough to withstand adverse environmental conditions. Wardle (1974) comments that "timberline is therefore one of the most significant boundaries in biological nature, separating two fundamentally different ecosystems."

The alpine zone comprises approximately 4 million square kilometers, or 3% of the earth's land surface (Körner, 1999). North temperate alpine habitats are similar in terms of climate and vegetation to the Arctic, i.e., habitats situated at latitudes beyond treeline, and the two habitats are often considered collectively as comprising the "tundra," or arctic-alpine, biome that covers approximately 8 percent of the Earth's land surface (Chapin and Körner, 1995).
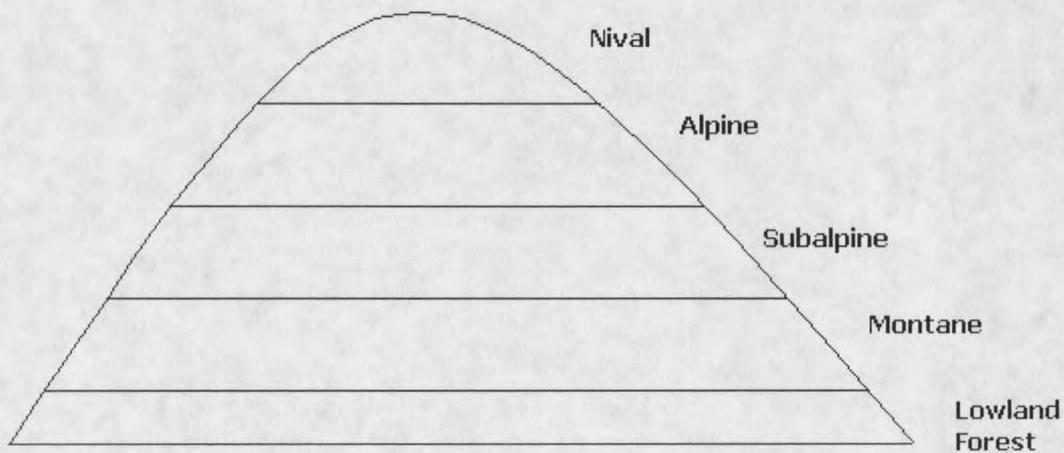
Figure 1. Schematic diagram showing elevational gradient of mountain vegetation zones.

Although most north-temperate alpine regions are geographically distant from the Arctic, similarities in climate-imposed physiological limits in addition to geologic events have shaped species distribution patterns and resulted in a high number of species having both arctic and alpine distributions. The following sections on patterns and origins of arctic-alpine biodiversity draw upon the literature pertaining to vascular plants. In

3

contrast to arctic-alpine vascular plant floras, arctic-alpine mycotas ("fungal floras" or fungal species assemblages) are poorly known in many parts of the world, precluding the ability to discern any large-scale patterns of species distribution. There are two primary reasons why a discussion of arctic-alpine vascular plant distributions may be relevant here. First, it is plausible that environmental factors have acted similarly in influencing distributions of both plants and fungi. Secondly, the presence of close associations between plants and both saprobic and mycorrhizal fungi predicts that fungal species distributions may follow those of particular plant species (or vice versa). For these reasons, the biogeographic patterns observed in arctic-alpine vascular plants provide an initial hypothesis for studies of such patterns in arctic-alpine macrofungi.

General Patterns of Arctic-Alpine Biodiversity

Although arctic and alpine habitats share a large number of plant species, the two physiographic regions differ in two main ways in regard to patterns of species diversity. First, the contribution of forest populations to overall species distributions, being a function of distance, is likely to be of greater importance in alpine than in arctic habitats (Gardes and Dahlberg, 1996). In addition, species diversity tends to be higher in alpine areas than in the arctic, both as a result of the proximity of dissimilar habitats (due to large altitudinal changes over a short distance) and the occurrence of a larger number of distinct, favorable microclimates (Billings, 1973, 1974a; Chapin & Körner, 1995; Müller & Magnuson, 1987). In general, species diversity tends to decrease with increases in altitude and latitude (Chapin & Körner, 1995).

Origins of Alpine and Arctic Floras

The present north-temperate alpine vascular plant flora is hypothesized to have a complex evolutionary history resulting from a number of factors, including adaptive radiations in older species relocated and isolated by tectonic processes, migration of arctic species during periods of glaciation, adaptations in lower elevation montane species, and ice age survival of species in glacial refugia (Körner, 1995; Löve & Löve, 1974; Murray, 1995). The floras of tropical alpine regions are vastly different from those of temperate regions, and are hypothesized to be composed largely of evolutionary lineages derived from lower elevation plants. Due to these differences in species composition and evolutionary history, tropical alpine floras will not be further discussed in this study. However, one exceptional element of these tropical floras seems worthy of mention here in relationship to the floras of north temperate alpine ecosystems: a Tertiary "remnant of an old flora of the mountain chain north of the Tethys Sea" that makes up part of the flora of both the Tibetan alpine zone and the southern Rocky Mountains (Löve & Löve, 1974).

Early Western North American Alpine Floras

Although the lack of early Mesozoic alpine plant fossils makes the early evolutionary history of alpine plants difficult to trace, evidence indicates that plants had colonized North American alpine zones at least as early as the late Cretaceous period (Billings, 1974b). Species distribution patterns and the occurrence of endemic species provide evidence that upward migration of lower elevation species and subsequent

radiation of new evolutionary lineages, as well as long-distance events or "alpine island-hopping," were important forces contributing to the present alpine flora (Billings, 1974b). Billings (1974b) recognizes a "strong endemic element" in the Rocky Mountain flora, and notes that the flora of the Beartooth Plateau of southern Montana and northern Wyoming consists of approximately 50% arctic-alpine and 50% western North American endemic species. The percentage of arctic-alpine species exhibits a southward decrease, with only about 25% of southern Rocky Mountain species having arctic-alpine distributions; a majority of species are related either to central Asian alpine species, southwest desert species, or represent endemic taxa.

Development of the Early Arctic Flora

Fossil evidence indicates that no representatives of current arctic-alpine plant genera were present in the northern plains during the early Tertiary period (Löve & Löve, 1974). Rather, it appears that by the early Eocene, the vegetation of the northern latitudes was dominated by elements of the Tertiary nemoral flora as the result of northward continental drift. Evolution of elements of the present arctic-alpine flora from these Tertiary species appears to have occurred in latitudes north of the conifer zone by the Late Miocene, and an early arctic flora of approximately 1,500 species had achieved a circumpolar distribution prior to the beginning of the Pliocene glaciation events (Löve & Löve, 1974).

Effects of Pliocene/Pleistocene Glaciation

The glacial events of the Pliocene and Pleistocene periods profoundly influenced the distribution of arctic-alpine floras through the combined effects of climate change, physical disruption of species ranges, migration barriers, and occurrence of glacial and interglacial refugia. During periods of glaciation, ice cover resulted in the physical disruption of formerly circumpolar species distributions, and climatic cooling resulted in the southward displacement of species ranges. It is these southward displacements, and subsequent upward altitudinal migration of species, that is hypothesized to result in the observed patterns of shared species between arctic and alpine habitats (Löve & Löve, 1974). Of those species that did not extend southward, some, it can be presumed, suffered extinction. Some species, however, survived in northern regions in glacial refugia such as coastlines, islands, or nunataks (unglaciated mountain peaks surrounded by glacial ice) that escaped glaciation. These species, in addition to lower-latitude alpine species that experienced northward range extensions during interglacial periods, then colonized or recolonized arctic habitats during late Pleistocene interglacial periods (Abbott et al., 2000; Billings, 1974b; Ives, 1974b; Müller & Magnuson, 1987). The observation that most alpine populations of arctic-alpine species differ at the ecotype level from their arctic counterparts underscores the effect of barriers to gene flow during glacial periods, and suggests that populations surviving in glacial refugia may have been more important than northward-expanding populations in serving as interglacial source populations (Billings, 1974b; Löve & Löve, 1974). Billings (1974b) stresses the

importance of interglacial as well as glacial refugia in affecting the evolution of alpine species. In the former, alpine species were able to survive during upward elevational forest migrations during periods of deglaciation when climates became warmer. In addition to displacing ranges southward and isolating populations in refugia, glaciation events led to accelerated paces of evolution by fragmenting once-circumpolar species into isolated, independently evolving lineages (Löve & Löve, 1974).

An alternate hypothesis states that a widely distributed Northern Hemisphere arctic-alpine flora (in contrast to the solely Arctic flora mentioned above) was present prior to Pleistocene glaciation events, and that Pleistocene glaciation resulted in dissecting this arctic-alpine flora rather than forcing southward migrations of species. The presence of plants having Rocky Mountain – central Asian disjunct distributions seems to provide evidence supporting this hypothesis (Blair, 1996).

In regard to contemporary forces, work by Riebesell (1982) suggests that plant migration and extinction are ongoing processes in the alpine, comparable to island communities, and that alpine plant populations are dynamic rather than representing strictly relict populations. In summary, alpine vascular plant floras consist of elements having a complex mixture of historical origins: endemic species evolved from adapted populations of lower elevation species coexisting with local populations of more widely distributed species with distribution patterns resulting from a number of geological phenomena.

Arctic-Alpine Mycotas

The biogeographic and evolutionary patterns of alpine macrofungi are much more poorly understood than are those of vascular plants; for instance, the level of endemism in western North American alpine species cannot yet be determined due to the lack of any previous large-scale studies of the mycota of this region, and a lack of baseline biogeographic data makes overall evolutionary patterns difficult to discern. Watling (1987) posed the question of whether the distributions of macrofungi are directly related to the migration of plant species, mirror patterns of plant distributions on the basis of similar physiological tolerance zones rather than on directly following plant migrations, or are, in fact, not similar to patterns of arctic-alpine plant distributions. An addition to these questions is whether saprobic fungal lineages follow the same evolutionary patterns as symbiotic or parasitic lineages. The biogeographic and evolutionary patterns observed for vascular plants can serve as a template for investigations of the evolution of alpine fungal taxa; however, more baseline biodiversity data are necessary before patterns for fungi can be reliably interpreted.

As mentioned earlier, the presence of mycorrhizal associations may predict that the distribution patterns of mycorrhizal macrofungi follow that of their plant hosts (or vice versa). Mycorrhizae are mutualistic associations between certain species of fungi and the roots of certain species of vascular plants. Ectomycorrhizae are a type of mycorrhizae in which the fungal hyphae form a sheath around the fine root tips of the plant. Nutrient exchange takes place in a netlike-structure (Hartig net) that surrounds the

plant epidermal cells. Ectomycorrhizae are generally formed by macrofungi in the Basidiomycota, and occasionally Ascomycota. Other types of mycorrhizal fungi are not considered in the present study.

Most mycorrhizal fungi are obligant symbionts, able to complete their life cycles only when associated with a suitable host plant. Ectomycorrhizal (EM) fungi exhibit various degrees of host specificity, with most having a wide host range while others (e.g. *Suillus* spp.) are restricted to a single host genus or species within a genus (Smith & Read, 1997). Watling (1992) notes that most EM macrofungi associated with willows (*Salix* spp.) in Great Britain are species having broad host ranges; however, he also notes that willow-dominated regions in Great Britain share many EM fungal species with willow-dominated regions in continental Europe. This finding suggests that the distributions of EM fungi are influenced by, or at least mirror, those of EM host plants.

Previous arctic-alpine fungal inventories indicate that between 28% and 60% of these mycotas comprise EM fungi (Cripps et al., 2001; Gulden, 1996); these fungi are associated with a limited number of host plant species (Table 1). While historical factors may explain broader patterns of species distributions, localized distributions of EM host plants, especially *Salix* spp.(the most common EM host plants in the North American alpine zone), may be the most important factor affecting Rocky Mountain alpine EM fungal distributions (C. Cripps, personal communication).

Because saprobic fungi appear to exhibit a degree of substrate specificity, it is likely that their distributions are influenced by the distribution of host plants as well. Preliminary evidence suggests that some saprobic macrofungi are host specific. A study

Table 1. Ectomycorrhizal host plants documented in arctic alpine habitats. Adapted from Gardes & Dahlberg (1996) with additions by T. Osmundson.

Species	References
Arctostaphylos alpina	Michelsen et al. 1996
Arctostaphylos rubra	Miller et al. 1982
Betula glandulosa	Eddington & Cripps (manuscript in preparation), Lange 1957
Betula nana	Michelsen et al. 1996, Miller et al. 1982, Treu et al 1996, Väre et al. 1997
Cassiope tetragona	Miller 1982, Miller & Laursen 1974, Kohn & Stasovski 1990, Stutz 1972
Crepis aurea	Read & Haselwandter 1981
Daphne striata	Read & Haselwandter 1981
Dryas integrifolia	Bledsoe et al. 1990, Kohn & Stasovski 1990, Miller & Laursen 1974, Read & Haselwandter 1981, Stutz 1972, Väre et al. 1992
Dryas octopetala	Bledsoe et al. 1990, Debaud et al. 1981, Haselwandter & Read 1980, Lesica & Antibus 1986, Miller 1982, Read & Haselwandter 1981, Treu et al. 1996, Väre et al. 1992
Festuca rubra	Read & Haselwandter 1981
Helianthemum oelandicum	Read & Haselwandter 1981
Homogyne alpina	Read & Haselwandter 1981
Kobresia bellardii (=K. myosuroides)	Haselwandter & Read 1980, Kohn & Stasovski 1990, Massicotte et al. 1998, Read & Haselwandter 1981
Pedicularis capitata	Kohn & Stasovski 1990
Pedicularis dasyantha	Väre et al. 1992
Pedicularis hirsuta	Stutz 1972
Polygonum viviparum	Haselwandter & Read 1980, Lesica & Antibus 1986, Massicotte et al. 1998, Michelsen et al. 1996, Read & Haselwandter 1981, Treu et al 1996
Potentilla hyparctica	Bledsoe et al. 1990, Haselwandter & Read 1980
Potentilla reptans	Read & Haselwandter 1981
Potentilla stricta	Read & Haselwandter 1981
Pyrola grandiflora	Kohn & Stasovski 1990
Salix spp.	Bledsoe et al. 1990, Dhillion 1994, Haselwandter & Read 1980, Kohn & Stasovski 1990, Laursen & Chmielewski 1982, Linkins & Antibus 1982, Michelsen et al. 1996, Miller 1982, Read & Haselwandter 1981, Stutz 1972, Treu et al. 1996, Väre et al. 1992, 1997
Saxifraga oppositifolia	Kohn & Stasovski 1990, Stutz 1972
Saxifraga paniculata	Read & Haselwandter 1981
Silene acaulis	Read & Haselwandter 1981
Vaccinium uligionosum	Stutz 1972

by Horak and Miller (1992) indicates that species of *Galerina,* a genus of moss-decomposing fungi, are restricted to certain mosses. In addition, the saprobic species *Marasmius epidryas* is reported only on dead leaves of *Dryas* spp.

In addition to historical and biotic factors, abiotic factors such as soil type and microclimate may influence macrofungal distributions. Lesica and Antibus (1986) found that levels of root colonization by arbuscular mycorrhizal fungi were significantly higher on calcareous than on acidic soils, speculating that this phenomenon is due to the fact that phosphorous availability can be limiting at pH levels higher than 7 and that mycorrhizal associations may therefore be particularly advantageous for plant growth on calcareous soils. It has not been established whether a similar pattern exists for EM fungi.

Alpine Climate

Perhaps the most common characterization of the temperate alpine zone is as a cold-dominated region: the effects of low average temperatures are in fact widespread, including short growing seasons, high incidence of water stress, and low soil nutrient availability due to reduced rates of weathering and mineralization processes, low biomass production, low decomposition rates and high levels of aeolian erosion (Gardes & Dahlberg, 1996; Körner, 1999; Lesica & Antibus, 1986). However, other factors such as high maximum solar radiation and reduced atmospheric pressure may be more common among alpine regions on a global scale; therefore, it can be said that cold temperatures alone do not account for patterns of adaptation exhibited by alpine plants (Körner, 1999).

In the Rocky Mountain temperate alpine zone, distribution of plant (and presumably fungal) species may be further influenced by low water availability due to high winds that remove snow cover in winter and increase evapotranspiration in plants during the growing season (Lesica & Antibus, 1986), and by low annual precipitation due to the influences of a continental climate type.

Perhaps the most critical determinant of species diversity levels and local distribution patterns is the occurrence of numerous, diverse, topologically influenced microclimates, or "topoclimates" (Barry and Van Wie, 1974; Billings, 1974a; Körner, 1999). Local differences in solar radiation, slope, and exposure (in turn influenced by wind speed, air temperature and soil type) influence soil temperatures, snow drift and cover patterns and melt rates, depth of soil thaw, effects of wind and local differences in plant canopy structure, and may in fact be more important than the more generalized "alpine climatic conditions" in determining species distributions (Billings, 1974a; Billings & Bliss, 1959; Körner, 1999). Billings (1974a) draws a sharp contrast between arctic-alpine and forested habitats in terms of the relationship between vegetation and the physical environment, stating that, "unlike the situation within a forest, the modification of microclimate by vegetation is minimal and the physical environment dominates the vegetation." Distinct microclimates can result from the location of a number of physical features, including rocks, solifluction terraces, soil polygons, or, for soil organisms or small-statured plants, from shelter offered by shrubs or other plants (Billings, 1974a).

Adaptations in Arctic-Alpine Plants and Fungi

The origin of alpine life forms required adaptations to the unique climatic challenges associated with altitudinal extremes. In plants, these adaptations include avoidance of low temperature extremes through genetically determined patterns of growth form (e.g., cushion, prostrate shrub and rosette morphologies), phenological and life history patterns (e.g., dormancy, responses to temperature and photoperiod, predominance of perennial over annual species) and microhabitat selection (Körner, 1999). While compiling data on arctic-alpine fungal adaptations is outside the scope of the present research, a brief discussion of hypothesized adaptations is presented here because a better understanding of these mechanisms will ultimately enhance the understanding of evolutionary patterns in arctic-alpine fungi.

In terms of temperature adaptation, fungi must be able to endure freezing temperatures even during the growing season and resume metabolism without having to undergo an extended dormancy phase (Savile, 1982). Putative fungal adaptations to arctic-alpine and cold-dominated environments include physiological adaptations allowing growth at low temperatures, biochemical adaptations such as the production of cryoprotectant compounds, spore germination requiring incubation at sub-freezing temperatures, the ability of basidiocarps to resume spore production following freezing, production of dormant spores, thick and/or pigmented cell and spore walls, spores having a gelatinous outer coating, and self-compatible mating systems (Aragno, 1981; Gardes & Dahlberg, 1986; Ingold, 1982; Müller & Magnuson, 1987; Robinson, 2001). Research on

cold tolerance in fungi indicates that cold-adapted ecotypes exist within species (Cline et al., 1987; Tibbett et al., 1998), a phenomenon well known in vascular plant species (e.g., Billings & Mooney, 1968; McGraw, 1985); whether this ecotypic variation in fungi is the result of phenotypic plasticity or genetic drift is a question open to further research.

The aforementioned occurrence of self-compatible mating systems may be of particular importance in certain ectomycorrhizal basidiomycetes. The occurrence of fungi that produce basidia having a two-sterigmate condition (two spores borne on each basidium), instead of the typical four-sterigmate condition (resulting from meiosis and subsequent migration of one nucleus into each spore), is hypothesized to occur more frequently in arctic-alpine than in temperate species (Gardes and Dahlberg, 1996). This state occurs in genera such as *Laccaria* and *Inocybe* that are commonly found in disturbed and primary successional habitats as well as in arctic-alpine regions. In the two-sterigmate condition, basidiospores normally contain twice the normal number of nuclei (Mueller et al., 1993) and may therefore be secondarily homothallic, i.e., contain both mating type alleles and therefore be self-fertile. Secondary homothallism may allow rapid colonization of disturbed or extreme habitats, since mycelia from two spores of opposite mating types do not have to find each other for successful mating to occur.

The association of fungi in symbioses may represent an additional means of adaptation to conditions (in arctic-alpine or non-arctic-alpine habitats) of water stress or high ultraviolet radiation. The occurrence of Ascomycete and (very few) Basidiomycete fungi with algae or cyanobacteria in lichen symbioses is plentiful in arctic-alpine habitats, suggesting a potentially adaptive situation (Billings & Mooney, 1968; Larcher & Bauer,

1981; Redhead & Kuyper, 1987). Basidiolichens are comparatively abundant in arctic-alpine habitats, and represent a recently evolved symbiosis compared to ascolichens (Lutzoni & Vilgalys, 1995).

Studies of roots of arctic-alpine plants indicate that the incidence of arbuscular mycorrhizal colonization decreases with altitude (Körner, 1999). However, formation of ectomycorrhizae with woody dwarf and shrub plants (e.g., *Salix* spp., *Betula* spp., *Dryas* spp.) is nearly ubiquitous in arctic-alpine habitats (Eddington and Cripps, manuscript in preparation; Gardes & Dahlberg, 1996). This finding concurs with the observation by Moser (1966) that conifer species at treeline are almost universally ectomycorrhizal. These observations suggest that the formation of ectomycorrhizae may be critical for survival of woody plant species at high altitudes and latitudes. Gardes and Dahlberg (1996) suggest that greater knowledge of the population structures of arctic and alpine ectomycorrhizal fungi would be valuable in understanding these and other possibly advantageous adaptations.

What is an Alpine Species?

In discussing distribution patterns in alpine plant species, Körner (1999) notes that species observed in the alpine zone can represent: 1) Species with ranges centered at low elevations but extending above treeline, 2) Species with ranges centered in the montane zone and extending both to lower elevations and into the alpine zone, 3) Species with ranges predominantly in the alpine zone but extending into lower altitudes, and

4) Species restricted to the alpine zone. Previous studies indicate that these patterns apply to distributions of macrofungi as well (Gardes & Dahlberg, 1996; Moser, 1982, 2002; Singer, 1954). Of these categories, Körner considers only the last two to represent "true" arctic-alpine species. However, from a standpoint of identifying cold-adapted ecotypes, inferring evolutionary and biogeographic patterns, and identifying the physiological and host ranges of species, the study of species potentially belonging to any of these categories is important. In addition to these reasons, study of species occurring in the first two categories is of importance because these species may represent a significant component of the alpine mycota. To cite an example from the Arctic, a study of the saprobic fungal genus *Galerina* on the arctic island of Svalbard by Gulden (1987) reported the occurrence of 12 species: three having predominantly boreal distributions extending into the Arctic, four with wide (temperate to arctic) distributions, and five with predominantly arctic-alpine distributions. Of these latter five species, only two appear to be restricted to arctic-alpine habitats.

Distinguishing true arctic-alpine species may be complicated by the occurrence of environmentally - influenced morphological modifications (Bendiksen et al., 1993; Gardes and Dahlberg, 1996) that result from phenotypic plasticity rather than speciation events. Recognizing true arctic-alpine species from ecotypes of more widely distributed species can be approached with more confidence using molecular identification and/or mating studies. The present project attempts to take this approach toward better elucidating species identities in Rocky Mountain alpine species of *Laccaria* in hopes of

leading to a better understanding of the evolution of arctic-alpine mycorrhizal fungi in general.

Arctic-Alpine Macromycete Studies

The study of arctic and alpine macromycetes (fungi, particularly Basidiomycetes but including Ascomycetes, forming conspicuous fruiting structures) is a relatively immature field of study compared to arctic-alpine studies involving vascular plants. While several small-scale examinations of arctic and alpine collections were conducted in the late nineteenth century, the first major works on arctic (Greenland: Lange, 1948-1957) and alpine (Swiss Alps: Favre, 1955) macromycetes were not carried out until the mid-twentieth century (Gulden, 1996, Horak et al., 2002). More recently, arctic-alpine mycotas have been documented for Europe, Iceland, Svalbard, and the Canadian Arctic but relatively little work has been done in North America or in the tropical alpine zones. Of macrofungi in general, Billings (1974b) states that "little is known of their ecology in alpine ecosystems." More recent studies have begun to elucidate the community structures and host relationships of alpine ectomycorrhizal basidiomycetes, but clearly more work is needed in this regard (see Gardes & Dahlberg, 1996 for review and discussion).

There are a number of reasons to explain this discrepancy between the states of knowledge pertaining to arctic-alpine fungi and vascular plants. Studies of macromycete systematics and biodiversity has historically lagged far behind that of vascular plants in general. In addition, relatively few mycological studies have been conducted in arctic-

alpine areas, most likely due to the relative inaccessibility of these habitats and their geographic distance from most centers of academic research.

A number of challenges are encountered in studying macromycetes in arctic-alpine habitats. First, the fruiting of macromycetes occurs during a short window of time and can be unpredictable due to the occurrence of summer freezing and potentially dry conditions. In a 3-year study of macrofungi in Rocky Mountain subalpine forests, Keck (2001) noted that 65% of the species recorded fruited only in a single year with above-average precipitation. Second, the basidiocarps of arctic-alpine macromycetes tend to be small and often hidden under shrubs or other vegetation, making collecting difficult. Third, the presence of diverse topoclimates results in patchy patterns of plant species distribution, resulting in small areas of species establishment and making placement of large research plots for quantitative studies difficult. However, the existence of diverse topoclimates probably serves to increase overall species diversity (Körner, 1999), and discrete vegetation units can be beneficial for inferring mycorrhizal host-symbiont associations and choosing appropriate comparative sites using small sampling plots. Two general types of approaches have been used in previous arctic-alpine macromycete studies to account for habitat patchiness: wide-scale sampling over diverse topoclimates (e.g., Lange, 1957), and focused sampling in specific habitat types of high fungal diversity, e.g. snowbed (Graf, 1994; Senn-Irlet, 1988) or mire (Senn-Irlet, 1993) communities.

In addition to fostering a better understanding of the ecology of cold-adapted organisms, further studies of arctic-alpine mycorrhizal fungi may have implications for

the overall understanding of mycorrhizal symbioses. As stated by Gardes and Dahlberg (1996), "cold-dominated environments provide extreme conditions for the establishment and functioning of mycorrhizal associations. Therefore, such systems are simple models to address the ecology and evolution of mycorrhizal symbioses."

The present review covers the body of arctic-alpine literature on two general types of studies: biotic inventories and mycosociological studies. As previously mentioned, a number of arctic-alpine regions have been the subject of macrofungal biotic inventories. Table 2 provides a summary of species numbers (fleshy Basidiomycetes only) reported in these biotic inventories; studies restricted to a single fungal genus are numerous and are not included here. As evidenced by these figures, many of the fungal inventories involving cold-adapted species has been conducted in Arctic ecosystems. Data suggest that macrofungal species diversity is greater in arctic than in alpine regions, in contrast with patterns observed in vascular plants. Whether this difference represents a true pattern resulting from historic or climatic factors or is simply an artifact of sampling effort will only become clear with further intensive surveys in alpine ecosystems.

While biotic inventories are primarily concerned with documenting the biodiversity of fungi within a study region, mycosociological studies are more concerned with elucidating aspects of community structure and ecological interactions between fungal species, between fungi and other organisms, and between fungi and environmental factors. Pertaining to the arctic-alpine biome, perhaps the most well-known study in this regard is that of Lange (1957), who documented distributions of Greenland macrofungi in relationship to plant community types.

Table 2. Summary of arctic-alpine macrofungal biotic inventories. Species numbers include fleshy Basidiomycetes (Agaricales, Boletales, Russulales, Gasteromycetes) only. Compilations include checklists or reviews not referring to single studies.

Study Location	Arctic/Alpine	Study Duration	Number of Species	Reference
Alaska, USA	Arctic	1 yr.	53	Kobayasi et al. 1967
Alaska, USA	subarctic tundra/taiga	10 yrs.	28	Miller 1982b
Alaska, USA	Arctic	10 yrs	22	Miller et al 1982, Gillman & Miller 1977
Baffin Island	Arctic	1 yr	18	Parmelee 1969
Greenland	Arctic	1 yr.	28	Watling 1977
Greenland	Arctic	Compilation	25	Watling 1983
Greenland	Arctic	1 yr.	44	Kobayasi et al. 1971
Greenland	Arctic	Compilation	560	Borgen et al. 2000
Greenland	Arctic	9 yrs.	218	Lange 1948-57
Godhavn area, W Greenland	Arctic	5 yrs	150	Lamoure et al. 1982
Iceland	Arctic	1 yr.	140	Christiansen 1941
Iceland	Arctic	11 yrs.	60*	Hallgrímsson 1981
Iceland	Arctic	Compilation	466	Hallgrímsson 1998
Iceland	Arctic	Compilation	13	Watling 1983
Northern Norway	Arctic	Compilation	212	Lange & Skifte 1967
Norway	Arctic	Compilation	15	Watling 1983
Svalbard	Arctic	Compilation	155	Ohenoja 1971, Gulden & Torkelsen 1996
Svalbard	Arctic	1 yr.	28	Kobayasi et al. 1968
Svalbard	Arctic	Compilation	19	Watling 1983
Fennoscandia	Arctic, Alpine	compilation	406	Bendiksen & Ohenoja, unpublished
Alberta, Canadian Rockies	Alpine	5 yrs.	13 **	Kernaghan & Currah 1998
Fiera di Primiero, Italian Alps	Alpine	Single foray	22	Bon 1987
Rhaetian Alps	Alpine	Single foray	22 **	Senn-Irlet 1992
Swiss Alps	Alpine	3 yrs	69	Graf 1994
Swiss National Park, Swiss Alps	Alpine	13 yrs.	213	Favre 1955
Southern and Central Rocky Mountains, USA	Alpine	4 yrs	150+	Cripps et al 2002

* Family Tricholomataceae only.
** Study included subalpine as well as alpine areas; only alpine species are included here.

The ecological aspects of these studies are reviewed in Chapter 4 (Beartooth plot studies); only the inventory data related to these studies are summarized here (Table 3). In contrast to the body of fungal inventories, the majority of mycosociological studies have been conducted in alpine areas, especially the European Alps.

Table 3. Summary of species inventory data generated during arctic-alpine mycosociological and ecological studies. Species numbers include fleshy Basidiomycetes (Agaricales, Boletales, Russulales, Gasteromycetes) only.

Study Location	Arctic/Alpine	Study Duration	Number of Species	Reference
Alps	Alpine	2 yrs.	39	Eynard 1977
Swiss Alps	Alpine	3 yrs	69	Graf 1994
Swiss Alps	Alpine	3-5 yrs.	88	Senn-Irlet 1988
Swiss Alps	Alpine	5 yrs	25	Senn-Irlet 1987
Western Italian Alps	Alpine	5 yrs	99	Lo Bue et al 1994
Eagle Summit, Alaska, USA	Arctic	Not listed	16 (EM only)	Miller 1982
Godhavn area, W. Greenland	Arctic	3 yrs	65	Petersen 1977
Greenland	Arctic	9 yrs.	218	Lange 1948-57
NW Finnish Lapland	Arctic	8 yrs.	56	Metsanheimo 1987

Further research in two areas is suggested by the studies cited here: additional inventories in previously under-investigated regions, and further research into the ecology of arctic-alpine fungi. Many of the alpine fungal inventories have been focused on the Alps; investigations of mountain ranges in regions such as North America and temperate Asia are necessary for better understanding biogeographic patterns. Lack of knowledge of the latter region could represent language biases in the literature rather than true gaps in research. Among agaric fungi, ectomycorrhizal fungi are dominant in arctic-alpine habitats in terms of species richness, comprising between 28 and 60 percent of macromycete species observed (Cripps et al., 2001; Gulden, 1996). Further investigations on mycorrhizal community structure, host associations, and factors affecting species composition and diversity will help elucidate this important aspect of arctic-alpine ecology.

The Rocky Mountain Alpine Mycota Project

As mentioned above, the arctic-alpine mycota has been the subject of biotic inventories in several areas, but that of the North American alpine is virtually unknown. The present study was conducted as a part of the Rocky Mountain Alpine Mycota project, a National Science Foundation Biotic Surveys and Inventories Program–sponsored project dedicated to conducting the first large-scale survey of macrofungal biodiversity in the North American alpine zone (Cripps & Horak, 1999; Cripps et al., 2002).

The importance of better understanding species biodiversity in the Rocky Mountain and other alpine regions is underscored by the function of these areas as repositories for winter precipitation (of particular importance to the arid regions of western North America), as potential indicator biomes responding to climate change events, and as understudied physiographic regions.

Because of their high latitudes/altitudes and areas of perennial snow cover, arctic-alpine areas are likely to be particularly sensitive to the effects of large-scale climate change events (Grabherr et al., 1995; Smaglik, 2000). Such events could have pronounced effects on arctic-alpine habitats by changing snowmelt timing and/or patterns, affecting organismal physiology through higher levels of ultraviolet radiation, allowing upward elevational shifts in treeline, causing melting of permafrost layers (either directly through higher mean air temperatures or indirectly through modifications to plant and soil community composition), changing the abundance of shrubby vegetation and altering levels of net CO_2 emissions. Warmer mean annual temperatures could affect