AN ABSTRACT OF THE DISSERTATION OF

Susie M. Dunham for the degree of Doctor of Philosophy in Forest Science presented on February 12, 2003.
Title: Population Genetics, Systematics, and Habitat Associations of Chanterelles in the Pacific Northwest.

Abstract Approved

Signature redacted for privacy.

Dr. Thomas E. O'Dell

Signature redacted for privacy.

Dr. Randolph J. Molina

Balancing resource extraction with protection of Pacific Northwest forest ecosystems requires understanding the population biology of ectomycorrhizal fungi. Design of landscape-scale management and research strategies requires understanding genet size, habitat requirements, and dispersal capabilities. The purpose of this dissertation was to gain knowledge about the distribution of genetic variation, systematics, and habitat associations of chanterelles (*Cantharellus* sp.). Population genetic markers were required to complete this research. I developed and characterized five co-dominant microsatellite markers and used them to study Pacific golden chanterelle (*C. formosus)* genet size and its relation to forest disturbance. Genetic data indicated that *C. formosus* collections included a cryptic chanterelle species. Small (<4 m diameter) genets were characterized for both genetic types and there was no evidence that genet size differed across disturbance treatments. Three genetic and one morphological data

set were colleted to determine if the genetic variability observed during the genet study was indicative of species boundaries. These data were used in combination to characterize a new species of yellow chanterelle, *C. cascadensis* nom. prov. Microsatellite data provide evidence that *C. subalbidus*, *C. cascadensis* nom. prov., and *C. formosus* do not interbreed when they co-occur spatially. Morphological data indicate that pileus color and stipe shape can be used to separate fresh collections of *C. formosus* and *C. cascadensis* nom. prov.. I also determined the habitat associations of three chanterelle species with respect to stand age. At the watershed scale stand age is a good predictor of the distribution of *C. subalbidus* and *C. formosus*, but is only marginally important for *C. cascadensis* nom. prov. To characterize chanterelle dispersal capabilities I used spatial autocorrelation analysis to examine the within-population genetic structure of *C. formosus*. Positive spatial autocorrelation was detected estimating a 200 m radius for genetically homogeneous patches for *C. formosus* indicating that limited spore dispersal possibly coupled with inbreeding works to maintain fine scale genetic structure in this species.

Population Genetics, Systematics, and Habitat Associations of Chanterelles in the Pacific Northwest.

By

Susie M. Dunham

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Doctor of Philosophy

Presented February 12, 2003

Commencement June 2003

ACKNOWLEDGEMENTS

The author thanks Jason Dunham, Thom O'Dell, Randy Molina, Dave Pilz, George Leinkaemper, Lisa Ganio, Manuela Huso, Julia Jones, Tom Horton and Annette Kretzer for many useful discussions regarding sampling design and data analysis, Dylan Nelson for countless DNA extractions, Mike Pfrender, Tom Mullins and Adam Jones for help developing the microsatellite loci, Nancy Adair and Caprice Rosato for running many genescan and sequencing gels on short notice. Joey Spatafora, Mike Blouin, Kirsten Monsen, and Mollie Manier provided laboratory access and assistance. Efren Cazares helped with morphological descriptions and Jim Trappe provided the Latin diagnosis for *C. cascadensis* sp. nov and advice on characterizing mushroom colors. Mike Taft, Teresa Lebel and Rita Claremont were indispensable during field sampling and laboratory processing of samples. Final thanks go to Kermit Cromack Jr. and Aaron Liston for their help and support with fulfilling departmental requirements and committee work. This research was funded by cooperative agreement PNW 97-5090-2-CA between the USDA Forest Service and Oregon State University.

CONTRIBUTION OF AUTHORS

Annette Kretzer provided training on the DNA cloning procedures required to develop microsatellites and practical advice on may other aspects of molecular lab work. Mike Pfrender contributed significant time helping the author isolate and characterize microsatellites loci. Kelly Collins assisted with data collection during the second season of the habitat association study.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (Continued)

Population Genetics, Systematics, and Habitat Associations of Chanterelles in the Pacific Northwest.

INTRODUCTION

Over the last decade concern for the long-term population viability of forest fungi has increased in the Pacific Northwest for two primary reasons. First, approximately 200 species of rare, old growth dependent fungi currently are listed for protection under the Northwest Forest Plan (NFP). Second, the sustainability of commercial harvest of edible forest mushrooms is in question because mushroom harvesting has increased as tree harvest activities have been curtailed. The purpose of this dissertation was to gain basic knowledge about the distribution of genetic variation in populations of chanterelles (*Cantharellus* sp.) and explore how this information can be used to improve management activities focused on forest fungi. My research addresses the genetic structure of populations and habitat associations of closely related species at different scales (species, populations, and watersheds) so that the results are relevant to ecosystem management and conservation planning. This introduction outlines the major conservation issues in the Pacific Northwest, my specific research questions, and potential applications of this research to the conservation and management of forest fungi.

The drafting and implementation of the NFP represents a significant paradigm shift in forest management from an emphasis on extractive activities like timber harvest to managing for ecosystem function and stability. The primary focus of the NFP (USDA & USDI 1994a) is to provide for the persistence of old growth forest dependent species while also allowing for sustainable resource extraction. Although

the NFP is not integrated with the Endangered Species Act, it does represent the first Federal effort to formally protect fungal species and highlights the need for research addressing poorly understood population biology of forest fungi.

Fungi play a fundamental role in ecosystem function because they are integral to nutrient cycling pathways, food webs, forest disease cycles, and mutualisms. Many fungi form mycorrhizal associations with a diverse array of plant species. The mutualistic association formed between ectomycorrhizal (EM) fungi and most forest trees is one of the most common forms of symbioses in western forest ecosystems (Molina *et al.* 2001). Benefits of the EM association to plant hosts are well documented and include increased efficiency in nutrient uptake, resistance to drought stress, and protection from root pathogens (Smith & Read 1997). EM hosts in the western United States include all species in the commercially important Pinaceae family (*Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, and *Tsuga*), as well as the Fagaceae (*Castinopsis*, *Lithocarpus*, *Quercus*), Salicaceae (*Populus*, *Salix*), and Betulaceae (*Alnus*, *Betula*, *Corylus*). These species dominate western forests and are associated with thousands of EM fungal symbionts.

Over the last two decades significant declines in the productivity and diversity of forest fungi have been documented in Europe (Arnolds 2001) and Japan (Hosford *et al.* 1997). Over the same time frame the harvest of wild, edible mushrooms has dramatically increased in the Pacific Northwest along with export of these forest products to Europe, Japan, and elsewhere. In 1992 alone, nearly 2 million kg of edible mushrooms, worth ~ $40 million US dollars, were harvested in the Pacific Northwest

(Schlosser & Blatner 1995). Our knowledge of the reproductive biology and habitat requirements of these species is not sufficient to determine if such harvest levels are sustainable. Although commercially harvested species are not rare, their conservation is also important to consider in the context of forest management.

Selection of Chanterelles as Study Organisms

Addressing management-oriented questions about fungal population dynamics requires the design of non-arbitrary, objective research to determine the scales at which important evolutionary processes operate. Observations from mismatched spatial scales (scale of measurements differs from scale at which the process of interest is acting) can result in erroneous identification of pattern-processes relations because the types or relative influences of processes may change as new spatial domains are entered (Wiens 1989). For example, at fine scales, biotic factors such as inter- and intra-specific competition, forest age, species composition, and disturbance history may be strongly correlated with patterns of genetic variability associated with population viability. At broad geographic scales, abiotic factors such as climate, rainfall patterns, exposure, and elevation may strongly influence patterns of genetic variability associated with speciation (evolutionarily significant units). Fine-scale processes may exert control over the total amount of genetic variability on which large-scale processes can act, and thus may be more relevant to management issues. Identifying fungal population boundaries and dispersal barriers requires the development of sampling strategies that estimate gene flow at forest stand, watershed,

and regional spatial scales. The ultimate goal of such a multi-scale sampling approach is to ensure that the scale of measurement and the response of fungal populations to some variable of interest (for example disturbance) fall within the same spatial domain.

Variability across EM fungal species in genet size and dispersion, mating system, and dispersal capability makes it impossible to apply a single sampling protocol to all species. Building a strong knowledge base requires that initial genetic research be done on species that will yield large sample sizes and exhibit reliable fruit-body production from year to year. Initial research on such common species will provide the statistical power sufficient to correctly identify evolutionarily important pattern-process relations (Dizon *et al.* 1995). The Pacific golden chanterelle (*Cantharellus formosus* Redhead *et al.* 1997) is a commercially harvested basidiomycete that forms EM associations with a wide range of economically important host trees (Molina *et al.* 1993; Redhead *et al.* 1997). This species fruits abundantly in several different habitats over a broad geographic range and is a good candidate for base-line genetic research because it possesses a distinct set of life history characteristics with respect to potential spore dispersal. In addition, chanterelles also are one of the most important edible mushrooms harvested from PNW forests (Pilz & Molina 2002). Because they are both economically and ecologically important in the Pacific Northwest region species in the genus *Cantharellus* are of particular interest to forestland managers.

Dissertation Scope and Relation to Management

Chanterelle Genet Size - The design of landscape-scale sampling strategies requires understanding how individuals (genets) of EM fungi are distributed. This presents a difficult obstacle with fungi because mycelia that give rise to fruit-bodies are hidden in soil or wood and cannot easily be observed. Because studies characterizing population-level variation in EM fungi typically rely on fruit-body sampling, understanding the mean and variance in genet size for different EM species is critical to avoid re-sampling genets and associated problems with lack of sample independence. Past research on EM fungi has identified basidiomycete species capable of producing genets 20-40 m in diameter (Dahlberg & Stenlid 1990, 1994; Dahlberg 1997; Bonello *et al.* 1998). Similar studies also have identified species that form much smaller genets likely because individuals persist for shorter time periods (Gherbi *et al.* 1999; Zhou *et al.* 1999; Redecker *et al.* 2001). Collectively, these studies have demonstrated significant differences in age structure, clonality, and genet recruitment patterns among the many EM species examined (Dahlberg 2001). The range in genet size unique to each EM fungal species and environment will dictate the scale at which population boundaries should be encountered, and set the minimum study area size.

Chapter 1 of this dissertation describes research aimed at determining the genet size of *C. formosus* that was initiated during the fall of 1997. Fine-scale research plots were established in three forest types including old growth, naturally regenerated 40-60 year old second growth, and second-growth recently disturbed by forest harvest. Characterization of genet sizes under these stand conditions is intended to determine

whether propagation strategies (sexual or vegetative) of chanterelles are altered by forest age or harvesting disturbance. Delineating genets of EM fungi requires multiple, independent, hypervariable markers that measure variation within the nuclear genome. Comparative studies of population-level markers have shown that the use of short, tandemly repeated DNA sequences (microsatellite repeats) is an effective method for detecting variability where other genetic markers fail (Hughes & Queller 1993). These valuable molecular markers exist for only a few EM basidiomycete species. Chapter 1 also describes the development of five co-dominant microsatellite markers for *C. formosus* and characterizes the variability of these loci using a sample of chanterelles collected from the 6,400 ha HJ Andrews Experimental Forest. Application of these markers in the study of *C. formosus* genet size tested their utility for subsequent population genetic research.

Species Concepts in Cantharellus - Solid taxonomic knowledge is an essential component of population genetic research. The taxonomy of EM fungi is still a work in progress with only a small fraction of biological species (sensu Mayr 1963) described in the scientific literature (Hawksworth 1991). Even in groups that are commercially important, new species have recently described and species boundaries are not well defined (e.g. Redhead *et al.* 1997; Hughes *et al.* 1999; Sime & Petersen 1999). For example, until recently, morphologically similar yellow chanterelles throughout North America were lumped under a single Latin binomen and considered synonymous with the European yellow chanterelle (*Cantharellus cibarius* Fr.). In

contrast, throughout the 1900's, mycologists have noted that yellow chanterelles found in North America not only are morphologically distinct from European species (Murrill 1912; Smith & Morse 1947; Corner 1966; Petersen 1979) but also exhibit intra-specific variation across North America (Feibelman *et al.* 1997) and at regional scales (Smith & Morse 1947; Redhead *et al.*1997). Genetic studies of North America chanterelles with *C. cibarius*-like morphology have demonstrated significant length variability in the nuclear ribosomal internal transcribed spacer (ITS), which suggests that this common morphology masks a species complex (Feibelman *et al.* 1994). In the study of genet size (Chapter 1), putative *C. formosus* collections exhibited two genotypes that varied in the total length of the ITS region, ITS-RFLP patterns and microsatellite variability. Multiple ITS genotypes in what was considered to be a single taxon indicated that a re-examination of species concepts in PNW chanterelles was required prior to proceeding with further population genetic studies. Species in the genus *Cantharellus* exhibit a limited number of conserved morphological characters (Smith & Morse 1947; Thiers 1985), thus application of molecular techniques to a much larger sample of fruit bodies was required to define genetic lineages subsequently examined for previously undetected phenotypic differences.

The research presented in Chapter 2 explores the evolutionary relationships among PNW chanterelle species and uses this information to determine if observed ITS and microsatellite variability found during genet size research is indicative of biological species boundaries. Specific objectives were to (1) analyze nrDNA sequences to determine the phylogenetic relationships among PNW chanterelle

collections with alternate ITS genotypes and European *C. cibarius*, (2) analyze microsatellite allele frequencies from sympatric chanterelle collections that represent multiple ITS genotypes to test for gene flow among ITS genotypes, and (3) identify morphological characters useful in differentiating cryptic species within the *C. formosus* morphology. This research culminated in the description of a new species *C. cascadensis* nom. prov. based on these genetic and morphological characters.

Habitat Associations of Chanterelle Species - Knowledge of species habitat requirements and how populations respond to disturbance events within a habitat allows for more rigorous comparison of the ecological effects of alternative forest management scenarios. Because detection of fungal individuals usually depends on the production of reproductive structures, we only poorly understand the specific habitat requirements of a handful of species (Dreisbach *et al.* 2002). This lack of information makes it difficult to include fungi in land management schemes that provide for persistence of habitat and therefore populations and species. Survey and manage guidelines within the NFP record of decision outline four alternative strategies for protecting viable populations of listed species. Although the objectives of these four strategies differ, each requires information on the distribution and abundance of listed species with high priority placed on site-specific habitat information. Such information is useful in habitat-based models that can be used to guide expensive survey efforts conducted at watershed and regional landscape scales to further our knowledge of fungal species distributions.

Defining the habitat associations of EM fungal species requires knowledge of interactions and interdependencies among host trees and their symbionts. Because of the intimate link between EM fungi and their host associates, plant community composition is an important factor for predicting EM species occurrence at regional scales. At the watershed scale, forest stand age, structure, and disturbance patterns are known to influence the composition of EM communities (Molina *et al.* 1992; Vogt *et al.* 1992; Waters *et al.* 1997; Baar *et al.* 1999; Colgan *et al.* 1999). The impacts of forest management activities on EM fungal communities have been characterized by few studies, many of which are descriptive (Miller & Lodge 1997). The severity of disturbance associated with forest management can also affect the persistence of EM fungi (Pilz & Perry 1984; Amaranthus & Perry 1987). In Sweden, where 95% of forests are used for commercial purposes (Berg *et al.* 1994), the high proportion of threatened macrofungi in spruce forests demonstrates the negative impact of forestry management practices (Rydin *et al.* 1997). To effectively manage *Cantharellus* populations it is necessary to understand the impacts of both anthropogenic and natural disturbances on habitat availability. Research presented in Chapter 3 characterizes the habitat associations of *C. formorsus, C. subalbidus,* and *C. cascadensis* nom. prov. in Douglas-fir dominated forests at the watershed scale with respect to stand age and harvest disturbance.

Spatial Autocorrelation Analysis of Multilocus Genotypes - Broad-scale patterns in genetic variability are widely used to prioritize intra-specific units for conservation

efforts (Crandall *et al.* 2000). Studies of population-level variation in EM fungi have primarily focused on characterizing genet size for various basidiomycete species. These studies have yielded insights into the relative roles of basidiospore dispersal and vegetative growth in the life histories of EM fungi. Several have shown that genet establishment from basidiospores occurs frequently (Guidot *et al.* 2002), indicating that spore dispersal may be an important for the maintenance of genetic diversity in EM fungal populations. However, little is known about the long-distance dispersal capabilities and population establishment patterns of EM fungi across watershed and landscape spatial scales. Information of this nature is required for effective management of EM fungal populations.

Patterns of genetic isolation and indirect estimates of gene flow among populations frequently are estimated using the variance in allele frequencies within and among sampling units (Wright 1951). Precision of these statistics requires that sampling units do not encompass multiple random breeding units (neighborhoods). Wright (1969) showed analytically that as more neighborhoods are pooled within sampling units, the genetic variance among sampling units relative to the total variance declines, and the power to detect spatial genetic structure is lost. Progressing to from genet studies to landscape-scale studies requires that we understand the scale at which random mating occurs in EM fungal populations. For organisms where population boundaries are difficult to delineate because individuals are continuously distributed or difficult to detect a useful alternative for defining independent sampling units is to characterize genetic variability using spatial autocorrelation analysis

(Epperson 1993; Smouse & Peakall 1999). These methods are powerful tools for detecting genetic differentiation over a range of spatial scales and allow simultaneous definition of appropriate sampling networks and characterization of dispersal capabilities.

Chapter 4 describes the use of microsatellite markers (Chapter 1) and spatial autocorrelation analysis in characterizing the fine-scale genetic structure and spore dispersal distances in *C. formosus*. This research required the development of methods for assigning fruit-body collections to genets, allowing assessment of the contribution of both vegetative spread and isolation-by-distance to fine-scale genetic structure in a 50 ha second growth stand dominated by Douglas-fir. Results of this research will aid in the design of sampling methods that will best characterize genetic patterns and processes at landscape scales and facilitate identification of geographic areas important to the conservation of genetic diversity.

References

Amaranthus MP, Perry DA (1987) Effect of soil transfer on ectomycorrhiza formation and survival and growth of conifer seedlings in disturbed forest sites. *Canadian Journal of Forest Research*, **17**, 944-950.

Arnolds E (2001) The future of fungi in Europe: threats, conservation and management. In Moore D, Nauta MM, Evans SE, Rotheroe M. *Fungal Conservation: Issues and Solutions*. Cambridge University Press.

Baar J, Horton TR, Kretzer AM, Bruns TD (1999) Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. *New Phytologist,* **143**, 409-418.

Berg Å, Ehnström B, Gustafsson L, Hallingbäck T, Jonsell M, Weslien J (1994) Threatened plant, animal, and fungus species in Swedish forests: Distributions

and habitat associations. *Conservation Biology*, **8**, 718-731.

Bonello P, Bruns TD, Gardes M (1998) Genetic structure of a natural population of the ectomycorrhizal fungus *Suillus pungens*. *New Phytologist,* **138**, 533-542.

Colgan W, Carey AB, Trappe JM, Molina R, Thysell D (1999) Diversity and productivity of hypogeous fungal sporocarps in a variably thinned Douglar-fir forest. *Canadian Journal of Forest Research*, **29**, 1259-1268.

Corner EJH (1966) *A monograph of Cantharelloid fungi*. Oxford University Press, London 255 pgs.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000). Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution*, **15**, 290-295.

Dahlberg A (1997) Population ecology of *Suillus variegatus* in old Swedish Scots pine forests. *Mycological Research,* **101**, 47-54.

Dahlberg A (2001) Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. *New Phytologist,* **150**, 555-562.

Dahlberg A, Stenlid J (1990) Population structure and dynamics in *Suillus bovinus* as indicated by spatial distribution of fungal clones. *New Phytologist,* **115**, 487-493.

Dahlberg A, Stenlid J (1994) Population ecology of *Suillus variegatus* in old Swedish Scots pine forests. *Mycological Research,* **101**, 47-54.

Dizon AE, Taylor BL, O'Corry-Crowe GM (1995) Why statistical power is necessary to link analyses of molecular variation to decisions about population structure. In *Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation* (ed. Nielsen JL, Powers DA). American Fisheries Society: Bethesda, Maryland.

Dreisbach TA, Smith JE, Molina R (2002) Challenges of modeling fungal habitat: when and where do you find chanterelles? In *Predicting Species Occurrence: Issues of Scale and Accuracy* (eds. Scott JM, Heglund PJ, Morrision M, Raphael M, Haufler J, Wall B). Island Press, Covello, CA.

Epperson BK (1993) Recent advances in correlation studies of spatial patterns of genetic variation. *Evolutionary Biology,* **2,**: 95-155.

Feibelman T, Bayman P, Cibula WG (1994) Length variation in the internal

transcribed spacer of ribosomal DNA in chanterelles. *Mycological Research,* **98**, 614-618.

Feibelman TP, Doudrick RL, Cibula WG, Bennett JW (1997) Phylogenetic relationships within the Cantharellaceae inferred from sequence analysis of the nuclear large subunit rDNA. *Mycological Research,* **101**, 1423-1430.

Gherbi H, Delaruelle C, Selosse M-A, Martin F (1999) High genetic diversity in a population of the ectomycorrhizal basidiomycete *Laccaria amethystina* in a 150 year-old beech forest. *Molecular Ecology,* **8**, 2003-2013.

Hawksworth DL (1991) The fungal dimension of biodiversity: its magnitude and significance. *Mycological Research,* **95**, 441-456.

Hosford D, Pilz D, Molina R, Amaranthus M (1997) *Ecology and Management of the Commercially Harvested American Matsutake Mushroom.* General Technical Report. PNWGTR-412. US Department of Agriculture, Forest Service, Pacific Northwest Research Station: Portland, Oregon.

Hughes KW, Mcghee LL, Methven AS, Johnson JE, Petersen RH (1999) Patterns of geographic speciation in the genus *Flammulina* based on sequences of the ribosomal ITS1-5.8S-ITS2 area. *Mycologia,* **91**, 978-986.

Hughes CR, Queller DC (1993) Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Molecular Ecology,* **2**, 131-137.

Mayr E (1963) *Populations, Species, and Evolution.* Harvard University Press, Cambridge, Massachusetts.

Miller RM, Lodge DJ (1997) Fungal Responses to Disturbance: Agriculture and Forestry. In *The Mycota IV: Environmental and Microbial Relationships* (Eds. Wicklow DT, Söderström B) Springer-Verlag, Berlin.

Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In. Allen M (ed.) *Mycorrhizal functioning, and integrative plant-fungal process.* New York, Chapman and Hall. Pp. 357-423.

Molina R, O'Dell T, Luoma D, Amaranthus M, Castellano M, Russell K (1993) *Biology, Ecology, and Social Aspects of Wild Edible Mushrooms in the Forests of the Pacific Northwest: A Preface to Managing Commercial Harvest.* General Technical Report PNWGTR-309. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station: Portland, Oregon.

Molina R, Pilz D, Smith J, Dunham S, Dreisbach T, O'Dell T, Castellano M (2001) Conservation and management of forest fungi in the Pacific Northwestern United States: an integrated ecosystem approach. In Moore D, Nauta MM, Evans SE, Rotheroe M. *Fungal Conservation: Issues and Solutions.* Cambridge University Press.

Murrill WA (1912) The Agaricaceae of the Pacific Coast – I. *Mycologia,* **4**, 205-217.

Petersen RH (1979) Notes on cantharelloid fungi. IX. Illustrations of newly or poorly understood taxa. *Nova Hedwigia,* **31**, 1-23.

Pilz D, Molina R (2002) Commercial harvests of edible mushrooms from the forests of the Pacific Northwest United States: issues, management, and monitoring for sustainability. *Forest Ecology and Management,* **155**, 3-16.

Pilz D, Perry DA (1984) Impact of clearcutting and slash burning on ectomycorrhizal associations of Douglas-fir seedlings. *Canadian Journal of Forest Research,* **14**, 94-100.

Redecker D, Szaro TM, Bowman RJ, Bruns TD (2001) Small genets of *Lactarius xanthogalactus, Russula cremoricolor* and *Amanita francheti* in late-stage ectomycorrhizal successions. *Molecular Ecology,* **10**, 1025-1034.

Redhead SA, Norvell LL, Danell E (1997) *Cantharellus formosus* and the Pacific Golden Chanterelle harvest in Western North America. *Mycotaxon,* **65**, 285-322.

Rydin H, Martin D, Hallingbäck T (1997) Biological characteristics, habitat associations, and distribution of macrofungi in Sweden. *Conservation Biology,* **11**, 628-640.

Schlosser WE, Blatner KA (1995) The wild edible mushroom industry of Washington, Oregon and Idaho. *Journal of Forestry,* **93**, 31-36.

Sime AD, Petersen RH (1999) Intercontinental interrelationships among disjunct populations of *Melanotus* (Strophariaceae, Agaricales). *Mycotaxon,* **71**, 481-492.

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd ed. Academic Press.

Smith AH, Morse EE (1947) The genus *Cantharellus* in the western United States. *Mycologia,* **39**, 497-534.

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele

and multilocus genetic structure. *Heredity,* **82**, 561-573.

Thiers HD (1985) *The Agaricales (Gill Fungi) of California. 2. Cantharellaceae.* 34 pgs. Mad River Press, Eureka.

U.S. Department of Agriculture, Forest Service; U.S. Department of the Interior, Bureau of Land Management. 1994. Record of decision for amendments to Forest Service and Bureau of Land Management planning documents within the range of the northern spotted owl. (place of publication unknown).

Vogt KA, Bloomfield J, Ammirati JF, Ammirati SR (1992) Sporocarp production by Basidiomycetes, with emphasis on forest ecosystems. In Carroll GC, Wicklow DT (eds.) *The Fungal Community: Its Organization and Role in the Ecosystem*, 2nd Edition. Marcel Dekker, New York, NY, pp. 563-581.

Waters JR, McKelvey KS, Luoma DL, Zabel CJ (1997) Truffle production in old-growth and mature fir stands in northeastern California. *Forest Ecology and Management*, **96**, 155-166.

Wiens JA (1989) Spatial scaling in ecology. *Functional Ecology,* **3**, 385-397.

Wright S (1951). The genetical structure of populations. *Annual Eugenetics,* **15**, 323-354.

Wright S (1969) *Evolution and the Genetics of Populations*. Vol. 2. The Theory of Gene Frequencies. University of Chicago Press, Chicago.

Zhou Z, Miwa M, Hogetsu T (1999) Analysis of genetic structure of a *Suillus grevillei* population in a *Larix kaempferi* stand by polymorphism of inter-simple sequence repeat (ISSR). *New Phytologist,* **144**, 55-63.

CHARACTERIZATION OF PACIFIC GOLDEN CHANTERELLE (*CANTHARELLUS FORMOSUS*) GENET SIZE USING CO-DOMINANT MICROSATELLITE MARKERS

Susie. M. Dunham

Department of Forest Science, Oregon State University, Corvallis, OR 97331.

Annette. Kretzer

Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

Mike E. Pfrender

Department of Biology, 5305 Old Main Hill Rd., Utah State University, Logan, UT, 84332.

Molecular Ecology

Blackwell Scientific

In Press

Abstract

We characterized five co-dominant microsatellite markers and used them to study Pacific golden chanterelle (*Cantharellus formosus)* genet size and its relation to forest age and disturbance. Fruit-bodies were mapped in and collected from nine replicate study plots in old-growth, recently thinned, and unthinned 40-60 year old second-growth stands dominated by Douglas-fir (*Pseudotsuga menziesii*). Information from microsatellite loci, combined with RFLP analysis of the nrDNA internal transcribed spacer indicates that putative '*C. formosus*' fruit-body collections may include a cryptic chanterelle species. Small genets were characterized for both genetic types with mean maximum widths of 3.2 m ± 3.6 m for *C. formosus* and 1.5 m ± 1.7 m for the alternate genetic group. Variance in genet size was high and some multilocus genotypes were observed on multiple plots separated by 0.3 kilometers or more, indicating that genets were not fully resolved by the loci described here. There was no evidence that genet size differed across the three disturbance treatments.

Introduction

Concern for the population viability of ectomycorrhizal (EM) forest fungi has drawn attention to the need for considering fungi in Pacific Northwest forest management plans (Molina *et al.* 2001). Achieving this goal requires understanding how evolutionary processes act within fungal populations at different spatial and temporal scales. At a more basic level, public land managers require knowledge of spatial patterns in intra-specific genetic diversity. These patterns can reveal important clues about the evolutionary significance of past and present ecological and demographic events (Avise 1994), particularly for species not easily observed during most of their life cycle.

Proper analysis and interpretation of genetic spatial patterns requires study areas large enough to encompass populations governed by independent evolutionary processes and samples dispersed such that the variance of process effects can be quantified (Wiens 1989). Several characteristics of fungi impede our ability to select study areas of appropriate size and geographic orientation for population genetic studies. The design of landscape-scale sampling strategies requires understanding how individuals are distributed. This presents a difficult obstacle with fungi because mycelia that give rise to fruit-bodies are hidden in soil or wood and cannot easily be observed. For fungi, individuals possessing unique genotypes are usually referred to as genets. EM basidiomycete fungi establish new genets by fusion of two monokaryotic mycelia produced by germination of sexual basidiospores. Newly formed dikaryotic

mycelia undergo vegetative growth and produce fruit-bodies that continue the reproductive cycle (Todd & Rayner 1980).

Studies characterizing population-level variation in EM fungi typically rely on fruit-body sampling. Understanding the mean and variance in genet size for different EM species is critical to avoid re-sampling genets and associated problems with lack of sample independence. Past research on EM fungi has identified basidiomycete species capable of producing persistent genets (Dahlberg & Stenlid 1990; De La Bastide *et al.* 1994; Selosse *et al.* 1998, 1999; Sawyer *et al.* 1999; Gryta *et al.* 2000) that can grow to 20-40 m in diameter (Dahlberg & Stenlid 1994; Dahlberg 1997; Bonello *et al.* 1998). Similar studies also have identified species that form much smaller genets likely because individuals persist for shorter time periods (Baar *et al.* 1994; Gryta *et al.* 1997; Gherbi *et al.* 1999; Zhou *et al.* 1999; Redecker *et al.* 2001). To better understand the relative roles of basidiospore dispersal and vegetative growth in the life histories of EM fungi, many studies have characterized genet size of EM species across different habitats. Results from these studies demonstrate that environmental factors like forest age and disturbance are associated with variation in genet lifespan and size and that larger genets typically occur in older or less disturbed forests (Dahlberg & Stenlid 1990, 1995; Dahlberg 1997; Guidot *et al.* 2002). Exceptions to this pattern have also been identified indicating that genetics may play a larger role in longevity and genet size for other EM species (Fiore-Donno & Martin 2001; Redecker *et al.* 2001). Knowledge of genet size unique to each EM species and

environment will facilitate our understanding of the scale at which population boundaries should be encountered, and help determine minimum study area size.

A practical approach to expanding our understanding of EM fungal population genetics to landscape spatial scales involves research on species that fruit reliably and are broadly distributed. Pacific golden chanterelles (*Cantharellus formosus*, Redhead *et al.* 1997) are commercially harvested basidiomycetes that form EM associations with a wide range of economically important host trees (Molina *et al.* 1993; Redhead *et al.* 1997). This species fruits abundantly in several different habitats over a broad geographic range and is a good candidate for landscape-level population genetic studies. Population genetic research also requires genetic markers that exhibit levels of variability appropriate for use in studies ranging from parentage determination to characterization of landscape-level genetic patterns. This paper describes the development of five co-dominant microsatellite markers for *C. formosus* and characterizes the variability of these loci using a sample of chanterelles from an area of 6,400 ha. To test the utility of these microsatellite markers, we applied them in a study aimed at determining the size of chanterelle genets in Douglas-fir (*Pseudotsuga menziesii*) / western hemlock (*Tsuga heterophylla*) habitat in central Oregon. Three forest types were sampled including old-growth, naturally regenerated 40-60 year old second-growth, and second-growth recently disturbed by forest harvest. Characterization of genet sizes under these stand conditions is intended to determine whether propagation strategies (sexual or vegetative) of chanterelles are altered by forest age or harvesting disturbance.

Materials and Methods

Study Area and Sampling - Fruit-bodies were collected from 18 forest stands distributed over 6,400-ha in and around the HJ Andrews experimental forest (HJA; 44.2° latitude by 122.2° longitude). Once in October and once in November of 1998 fixed length searches of each stand were conducted and data from these two months were pooled for analysis. To avoid re-sampling genets all collection sites were marked and a minimum of five meters was kept between any two fruit-body collections. This distance is based on a preliminary study of genet size using Inter Simple Sequence Repeat (ISSR) markers and the fine scale samples described below (data not shown).

Samples used to characterize genet size were taken from nine plots located in 40-60 year old second-growth (SG) and 350+ old-growth (OG) stands along the southeast boundary of the HJA. Plots located in SG stands are part the Young Stand Thinning and Diversity Study (YSTDS), a joint effort between the Cascade Center for Ecosystem Management, Oregon State University, USDA Forest Service Pacific Northwest Research Station, and the Willamette National Forest McKenzie, Blue River and Middle Fork Ranger Districts. The goal of the YSTDS is to investigate the effects of SG thinning techniques on several ecosystem variables including chanterelle production. To characterize genets we selected seven chanterelle productivity plots from two YSTDS sites (18 km apart) closest to the southeast boundary of the HJA. The two YSTDS treatments sampled in this study included unthinned control stands (250 trees per acre, tpa) and lightly thinned stands (~100-120 residual tpa). In addition to the YSTDS plots we installed plots in OG stands adjacent to each replicate site.

This sampling encompasses three disturbance treatments including undisturbed OG, SG control stands disturbed once by clear-cut harvest 40-60 years ago, and thinned SG stands similarly disturbed by clear-cut harvest 40-60 years ago, and recently by a light thin harvest.

In total, we sampled two plots in OG stands, three plots in two YSTDS control stands, and four plots in two YSTDS light thin stands with a minimum distance of 0.3 km between any two plots. Plot locations were selected non-randomly by searching stands for areas where chanterelles fruit-bodies occurred in high abundance. When such an area was located a 16 m diameter plot encompassing the greatest number of fruit-bodies was installed. Fine-scale samples used to characterize genets were collected two weeks after the first fall rain of 1997. Within each plot the spatial coordinates of all chanterelle fruit-bodies were mapped to the nearest decimeter and azimuth (0-360) direction from plot center. Each mapped fruit-body was collected and samples were taken for subsequent genetic analyses.

DNA Extraction and Amplification of the Internal Transcribed Spacer (ITS) region - To aid in the detection of possible multiple taxa within our chanterelle sample we analyzed all fruit-bodies using restriction fragment length polymorphism analysis of the internal transcribed spacer region of the nuclear ribosomal repeat (ITS-RFLP), a technique commonly used to characterize community level species diversity (Gardes & Bruns 1996; Horton & Bruns 2001). After grinding approximately 5 mm^3 of fresh tissue in lysis buffer (100 mM Tris, 10mM EDTA, 2% sodium dodecyl sulfate, pH

8.0, 1mg/ml proteinase-K), each sample was incubated for 1.5 hours at 55° C and DNA was extracted using phenol/chloroform emulsification and precipitation (Sambrook *et al.* 1989). Unquantified DNA samples were diluted 10 to 100-fold, and the polymerase chain reaction (PCR) was attempted on successive ten-fold dilutions until amplification of ITS region was achieved. The region spanning the 3' end of the 18S rDNA, ITS-1 spacer, 5.8S, ITS-2 spacer, and 5' end of the 28S rDNA was amplified with the fungal specific primer ITS1-F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). PCR mix components were 1X Assay Buffer A (Fisher Scientific), 200 μM dNTPs each, 0.2 μM of each primer, 0.75 units *Taq* DNA polymerase (Fisher Scientific). Reactions were subject to initial denaturation at 95° C for 3 min followed by 35 PCR cycles (95° C, 45 s, 52° C, 55 s, 72° C, 90 s) in a PTC-100 Programmable Thermal Controller (MJ Research Inc.). Following the 35th cycle, samples were subjected to a 10 min 72° C extension.

RFLP Analysis of the ITS region - Unpurified PCR products were digested separately with four restriction enzymes, *Alu*I, *Hinf*I, *Dpn*II, and *Hae*III, according to the manufacturer's recommendations (GIBCO BRL, Grand Island, NY). These enzymes were chosen because they cut at 4 base-pair (bp) recognition sites and have demonstrated utility in the molecular typing of ectomycorrhizal root tips (Gardes & Bruns 1996). Restriction fragments were electrophoretically separated on 3% agarose gels (2% Nu-Sieve agarose, 1% SeaKem LE agarose; FMC BioProducts), and scored against a 100 base-pair molecular weight standard using the Alpha Imager 2000

documentation and analysis system (v. 3.2, Alpha Innotech Corp.). Each collection was assigned an ITS type that summarised information from the four restriction enzyme profiles. Collections with the same ITS type designation share identical restriction fragment patterns across all four enzymes.

Microsatellite Enrichment Protocols and Primer Development - The enrichment largely followed the protocol of Fleisher & Loew (1995) with minor modifications. Genomic DNA was extracted from a dried *C. formosus* fruit-body and remaining voucher material was placed in the Oregon State University herbarium under accession number OSC 76037. Approximately ten µg of DNA were digested with TaqI and size fractionated by agarose gel electrophoresis. Fragments in the 500-1000 bp size range were recovered from the gel and ligated to SAULA (5'-GCG GTA CCC GGG AAG CTT GG-3') / TaqB (5'-CGC CAA GCT TCC CGG GTA CCG C-3') oligonucleotide linkers. The SAULA linker was used as a primer to amplify ds ligation products. Ligation PCR products were hybridized to three biotinylated probes, $(GGAT)_7$ and $(GACA)_7$ (mixed in a single reaction) and $(CAC)_{10}$ (used separately) and the products were immobilized onto Dynabeads M-280 Streptavidin (DYNAL, France). Immobilized hybridization reactions were washed repeatedly at increasing temperatures with final washes at 95° C to remove the single stranded (ss) fraction enriched for microsatellite repeats. Retained ss fragments were amplified using the SAULA primer and the ds products were digested with TaqI to partially remove linkers and prepare fragments for ligation into the pUC19 *AccI* restriction site.

Plasmids were transformed into *Escherichia coli* DH5α and transformants were detected on $LB_{Amp+X\text{-}Gal}$ plates. Transformants containing microsatellite repeats were detected by hybridization to ^{32}P-end labeled oligonucleotide probes. Nucleotide sequences for positives were generated from the M13 forward primer using the BigDye Terminator sequencing kit and an ABI 377 automated sequencer (PE Applied Biosystems). Sequences flanking microsatellites five repeats or longer were used to design locus-specific PCR primers with annealing temperatures at 55° C.

Microsatellite Screening - Allelic diversity of co-dominant microsatellite loci was characterized using a sample that included both collections from the HJA and selected samples representing unique multilocus genotypes on circular plots. PCR reaction conditions were as described for ITS amplification. The PCR profile consisted of initial denaturation at 95° C for 3 min followed by 35 cycles (95° C, 45s; 55° C, 60 s; 72° C, 60 s). Following the 35th cycle, samples were subjected to a final 72° C extension of 60 min to maximize the proportion of complete PCR products. PCR products were analyzed on an ABI 377 automated sequencer using the GS500 Tamra internal size standard. Band sizes were determined using GENESCAN software (PE Applied Biosystems).

Data Analysis - The web-based version of GENEPOP (v. 3.1c; Raymond & Rousset 1995) was used to calculate expected and observed heterozygosities, exact tests for departure from Hardy-Weinberg, and tests for linkage. Variance in the size of genets

was examined graphically. Fruit-body locations from circular plots were converted to X/Y coordinates using algebraic transformations and SigmaPlot (v. 2.01; Jandel Scientific 1993) was used to generate a map for each plot. A spatial grid with a resolution of 10 dm was superimposed onto each plot map and used to estimate the maximum distance between fruit-bodies with identical multilocus genotypes. Statistical comparisons of genet length were made using StatMost (Dataxiom Software Inc. 2001).

Results

ITS-RFLP Variability - During 1997, 355 chanterelle fruit-bodies were collected from the nine circular plots used to study genet size. Random searches of forest stands in the HJA during 1998 yielded a sample of 348 yellow chanterelle fruit-bodies. Amplification of the ITS region yielded PCR products of either 1690 bp or 1490 bp. Later sequence analysis showed that the two variants differ in length due to multiple insertion/deletion events in the ITS 1 region (See Genbank Accessions AY041164 – 1690 bp, AY041160 – 1490 bp for representative sequences) rather than a single insertion event as has been shown for other species of EM fungi (Shinohara *et al.* 1996). RFLP analysis of the ITS region consistently produced two RFLP types that correspond to the two ITS PCR product lengths (Table 1).

Table 2.1. Characterization of variability in the internal transcribed spacer (ITS) region of yellow chanterelles collected in this study using four restriction enzymes. Fragment sizes are reported in approximate number of base-pairs.

	Type A	*Type B*
Total ITS Length	1690	1490
*Alu*I Fragments	540, 490, 340, 200, 120	490, 380, 250, 220, 150
*Hinf*I Fragments	970, 500, 220	480, 430, 340, 240
*Dpn*II Fragments	1090, 400, 200	600, 400, 290, 200
*Hae*III Fragments	780, 330, 180, 170, 140, 90	600, 220, 200, 170, 160, 140

ITS-RFLP type A matches the genetic description given for *C. formosus* by Danell (1995) and Redhead *et al.* (1997) and we refer to these samples as *C. formosus* throughout the remainder of the manuscript. The length of the alternate ITS-RFLP type (B, Table 2.1) is similar to that given for Swedish *C. cibarius* (Danell 1995) but confirmation of identity requires further investigation.

Microsatellite Enrichment - Attempts to isolate microsatellites from the *C. formosus* genome resulted in screening two partial libraries containing a total of 3,300 clones. The longest microsatellite identified contained 13 repeats with most less than ten repeats. The first enrichment yielded 15 positive clones containing eight microsatellites long enough to warrant primer development. Of the eight primer sets screened, two loci (Cf642; Cf145; Table 2.2) showed variability. In the second partial library, 97 positive clones were identified. DNA from 37 clones was amplified and sequenced and primers were designed for 15 clones. Three primer sets (Cf339; Cf126; Cf113; Table 2.2) amplified polymorphic repeats.

Table 2.2. Loci, primer sequences, and additional characteristics of 5 *Cantharellus formosus* microsatellites. Summaries are based on a sample of 89 collections, 45 combined from nine circular plots used to study genet size and 44 collected from the HJ Andrews Experimental forest. For each locus characteristics are given for all samples combined and for samples from each ITS-RFLP type (A = 45 & B = 44) separately. F, forward primer; R, reverse primer; H_E, expected heterozygosity; H_O, observed heterozygosity, * indicates significant departure from Hardy-Weinberg equilibrium ($p \leq 0.001$).

Locus	Primer Sequence (5'-3')	Genbank accession number	Repeat motif	Number of Alleles	Allele size range	H_E	H_O
Cf642	F: 6-FAM-cta tgc aaa tcc gcc agc	AY077690	$(gaca)_9$	4	275-279	0.64	0.30*
	R: ggg aca agt gcc aca c			ITS-A: 2	275, 279	0.09	0.09
				ITS-B: 2	276, 277	0.49	0.52
Cf145	F: 6-FAM-gta cga aac tga ttg gat gg	AY077691	$(gaca)_2(ggta)_4N_{120}$	8	342-473	0.83	0.64*
	R: gtg ttt gca tgt ggc tgg		$(gcgc)_4N_{29}(gct)_5$	ITS-A: 3	342-350	0.64	0.69
				ITS-B: 5	465-473	0.67	0.59
Cf339	F: 6-FAM-gaa tcg ggt tcg cag aag	AY077694	$(ggt)_6$	4	173-182	0.54	0.16*
	R: gaa ctt ctt cat ccg act cc			ITS-A: 4	173-182	0.31	0.31
				ITS-B: 1	176	0.0	0.0
Cf126	F: 6-FAM-gct tgt gcg gga tga cgg	AY077692	$(ggt)_6$	3	221-231	0.62	0.23*

Table 2.2. Continued

	R: cca cgt tca ttt cat tct acc c			ITS-A: 2	228, 231	0.45	0.44
				ITS-B: 1	221	0.0	0.0
Cf113	F: HEX-cga tct cgc tgt tat tgg ag	AY077693	$(ggt)_5$	4	96-120	0.69	0.32*
	R: tgc gac caa ctc atc taa tcc			ITS-A: 4	96-120	0.29	0.29
				ITS-B: 2	117, 120	0.50	0.34

Variable Microsatellite Loci - To characterize microsatellite variability, 44 samples representing both ITS-RFLP types were selected from fruit-bodies collected throughout the HJA (Table 2.3).

Table 2.3. Oregon State University (OSC) collection numbers for the 44 yellow chanterelle vouchers taken from areas in and around the HJ Andrews Experimental forest and screened for variability using 5 microsatellite loci. ITS-RFLP types are compiled from patterns generated using four restriction enzymes (*Alu*I, *Dpn*II, *Hinf*I, and *Hae*III), see Table 1 for details.

OSC	ITS RFLP Type	OSC	ITS RFLP Type
75926	A	75907 - 08	B
75930 - 31	A	75916 - 25	B
75981	A	76072	B
76021	A	75979	B
76024 - 25	A	75982	B
76033	A	75984	B
76037 - 41	A	75986	B
76046	A	75990 - 91	B
76048	A	76002	B
76050	A	76006	B
76054	A	76017 - 19	B
76056	A		
76058 - 59	A		

Genetic information from these 44 samples was pooled with information from 45 samples representing unique multilocus genotypes on circular plots. Each primer set consistently amplified a single product (locus) from all collections screened. In the combined sample, polymorphic loci had 3-8 alleles with expected heterozygosities ranging from 0.54–0.83. Observed heterozygosities were 23%-70% lower than heterozygosities expected under Hardy-Weinberg and all departures were significant ($p \leq 0.001$; Table 2.2). The number of alleles per locus, expected heterozygosities and observed heterozygosities changed substantially when the sample was partitioned by ITS-RFLP type and no significant departures ($p \geq 0.05$) from Hardy-Weinberg equilibrium were detected. The 45 collections that represent *C. formosus* possess 2-4 alleles per locus with expected heterozygosities that ranged from 0.09-0.64. The 44 collections that possess ITS-RFLP type B show 1-5 alleles per locus with expected heterozygosities ranging from 0 (for 2 monomorphic loci) to 0.67. For three of the loci (Cf642, Cf145, Cf126) the samples representing the two ITS-RFLP types share no alleles. At a fourth locus (Cf339) the ITS-RFLP type B sample is fixed at an allele present at low frequency in *C. formosus* and at the fifth locus (Cf113) the two ITS-RFLP types share one allele that occurs at different frequencies in the two samples (120 bp allele frequencies; *C. formosus*–0.02; ITS-RFLP type B–0.56).

All pair-wise comparisons of loci were tested for linkage disequilibrium using the 44 fruit-bodies (20 *C. formosus* and 24 type B) drawn from the HJA. When the two ITS types are combined, all pairs of loci were found to have significantly associated combinations of alleles ($p < 0.001$ for all loci pairs). When samples from

each ITS type were analyzed independently no significant linkage was found between pairs of loci within *C. formosus*. In ITS-RFLP type B, two pairs of loci appeared to be linked namely Cf642 with Cf145 (p=0.02) and Cf642 with Cf113 (p=0.08).

Resolution and Size of Chanterelle Genets - To study the size of chanterelle genets, 355 fruit-bodies were collected from nine circular plots placed in YSTDS and adjacent OG stands. The majority (285) of fruit-bodies were *C. formosus*. Screening the total sample with the microsatellites loci yielded 36 unique multilocus genotypes (*C. formosus*=19; type B=17). Eight multilocus genotypes were found on more than one plot with seven genotypes occurring on 2 plots each and one genotype occurring on 3 plots. Plots were separated by substantial distances so identical multilocus genotypes were thought to represent distinct genets when they were found on different plots. The total number of genets resolved was thus 45 (*C. formosus*=25; type B=20; Table 2.4).

Table 2.4. Number of chanterelle fruit-bodies and number of unique genotypes found within nine circular plots used to study genet size of yellow chanterelles. Totals are partitioned by their ITS-RFLP types (A & B).

ITS Type	Old-Growth		YSTDS Control			YSTDS Light Thin			
	Site 1	*Site 2*	*Site 1*	*Site 2*	*Site 2*	*Site 1*	*Site 1*	*Site 2*	*Site 2*
	Plot 1	Plot 1	Plot 1	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Number of Fruit-bodies									
A	0	0	81	61	97	4	0	23 1	9
B	19	8	0	36	0	0	5	2	0
Number of Genets Resolved									
A	0	0	4	4	10	1	0	3	3
B	5	5	0	6	0	0	3	1	0
Total	5	5	4	10	10	1	3	4	3

Maximum genet width was estimated visually by superimposing a 10 dm grid over each plot map and measuring the distance between the two furthest fruit-bodies possessing like multilocus genotypes (Figs. 2.1 & 2.2, maps for plots not shown here are in Appendix A). There was no evidence that the mean maximum width of chanterelle genets differed between the three forest types for the pooled sample or for either ITS-RFLP type. There was statistical evidence that the mean maximum genet width for *C. formosus* (3.2 m±3.6m) was larger than the mean width for ITS-RFLP type B genets (1.5m±1.7m) (t-value=2.02, p=0.05, 43 df), but the variance within both groups was high (Figure 2.3).

The presence of multilocus genotypes repeated on separate plots indicates that marker variability is insufficient to resolve all the genets sampled. A plot of the mean number of genotypes resolved against number of loci scored (Figure 2.4) shows that the curves for the total sample and for each ITS-RFLP type are approaching but have not reached saturation. Allele frequencies (Table 2.2) were used to calculate expected frequencies for all multilocus genotypes encountered on our plots. All eight genotypes found on more than one plot had a greater than 1% chance (range 1.2-6.8%) that two fruit-bodies drawn from the same plot might share that genotype due to chance rather than due to vegetative propagation. In contrast, genotypes not repeated on different plots had expected frequencies <0.1%. Comparison of the maximum genet width between genets with expected genotype frequencies greater than and less than 1% showed that the mean genet width variability was two to three times higher in the group with expected frequencies >1% (expected frequency >1%: standard deviation

for *C. formosus*=4.1 m, type B=2.0 m; expected frequency <1%: standard deviation for *C. formosus*=2.3 m, type B=0.7 m). The increased maximum genet width variability in the group with expected multilocus genotype frequencies >1% indicates that these genotypes encompass unresolved genets on some plots. The largest genet identified provides an example of a single genotype with a high-expected frequency (5.9%) that likely includes several unresolved genets. This genet is 13 m wide (3+ standard deviations from the mean *C. formosus* width) and is represented by 77 fruit-bodies (Figure 2.2).

Chi-square analysis of genet counts (Table 2.4) demonstrated that the two ITS-RFLP types were not evenly distributed across the three disturbance treatments (X^2=152.25, p<0.001, 5 df). Most notably *C. formosus* was absent from both OG plots but only absent from 1 of 7 SG plots. Alternatively ITS-RFLP type B was present in both OG plots but absent from 4 of 7 SG plots.

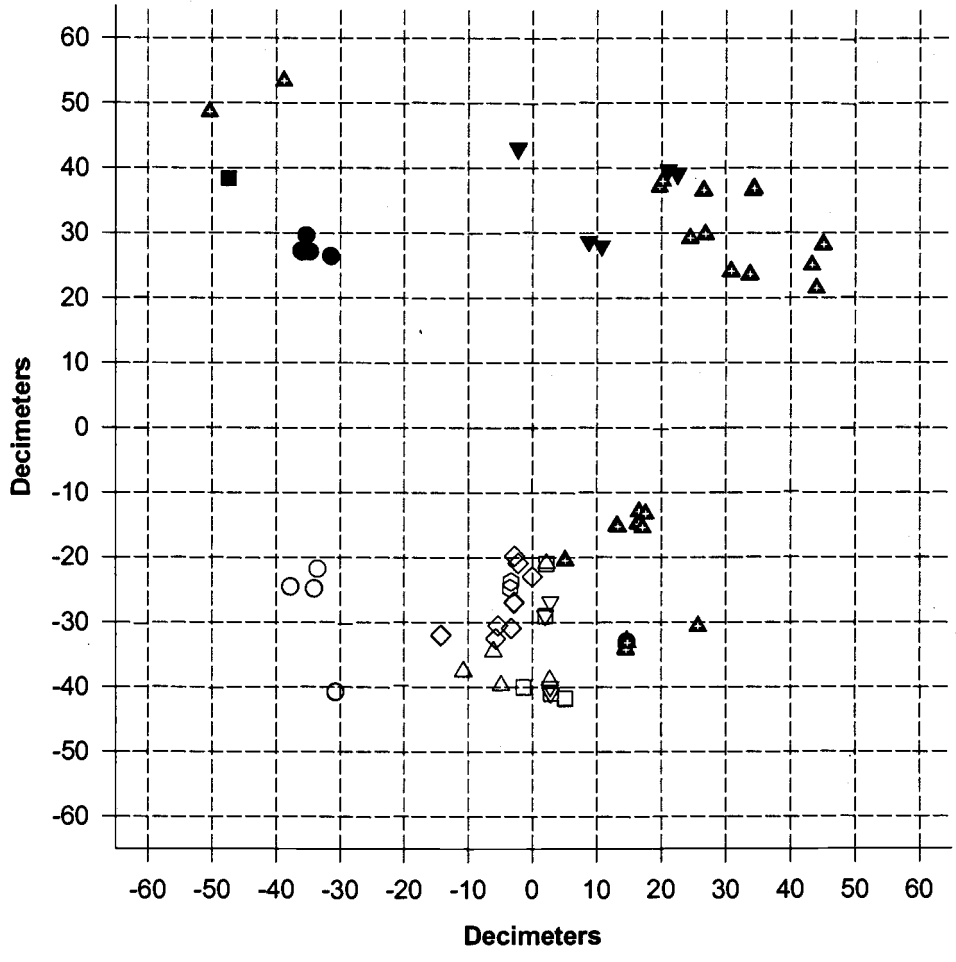


Figure 2.1. A map of plot number one in the YSTDS control SG site number two. Each symbol marks the location of a single chanterelle fruit-body. Filled symbols represent *C. formosus* fruit-bodies and open symbols represent ITS-RFLP type B fruit-bodies. Multiple like symbols delineate the extent of a putative genet measured against a 10 dm grid superimposed on the plot map. Plot sampling boundaries are not shown to improve resolution.

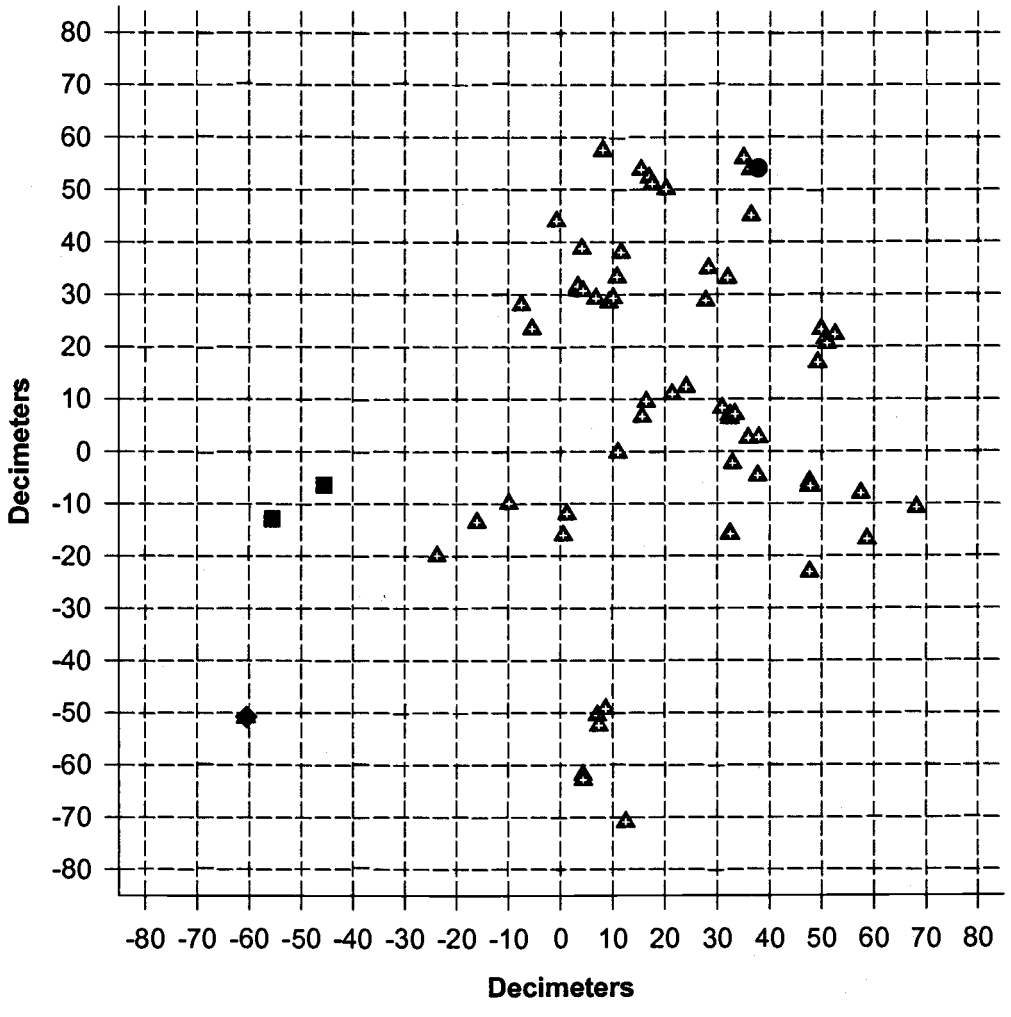


Figure 2.2. A map of plot number one in the YSTDS control SG site number one. Filled symbols represent 81 *C. formosus* fruit-bodies, 77 of which possess identical multilocus genotypes. Plot sampling boundaries are 80 dm from the 0/0 mark that indicates plot center.

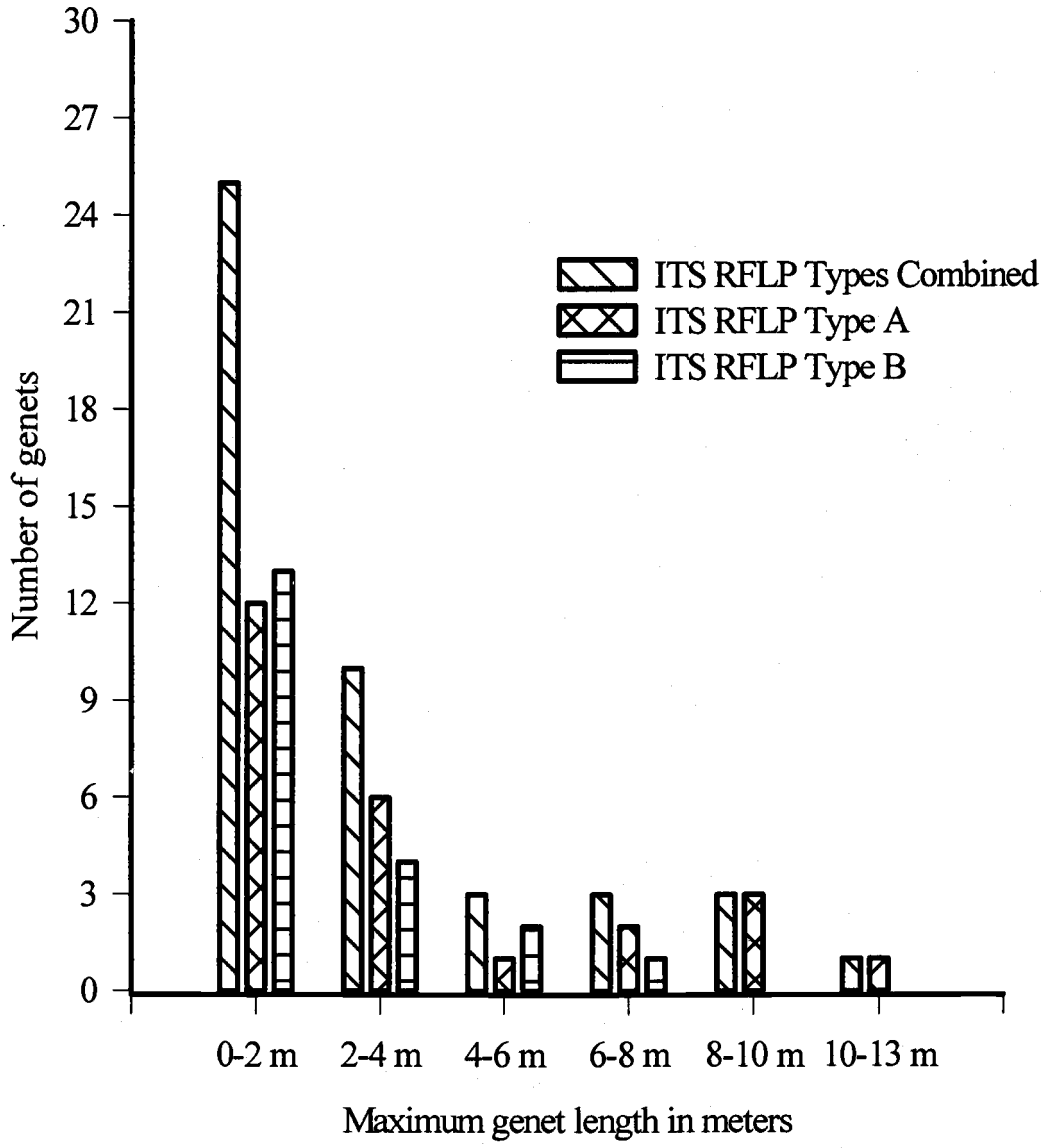


Figure 2.3. Frequency distribution of maximum genet width measured across all plots in three disturbance treatments. Groups of fruit-bodies larger than 8 m (*C. formosus*) or 4 m (ITS-RFLP type B) may encompass several unresolved genets.

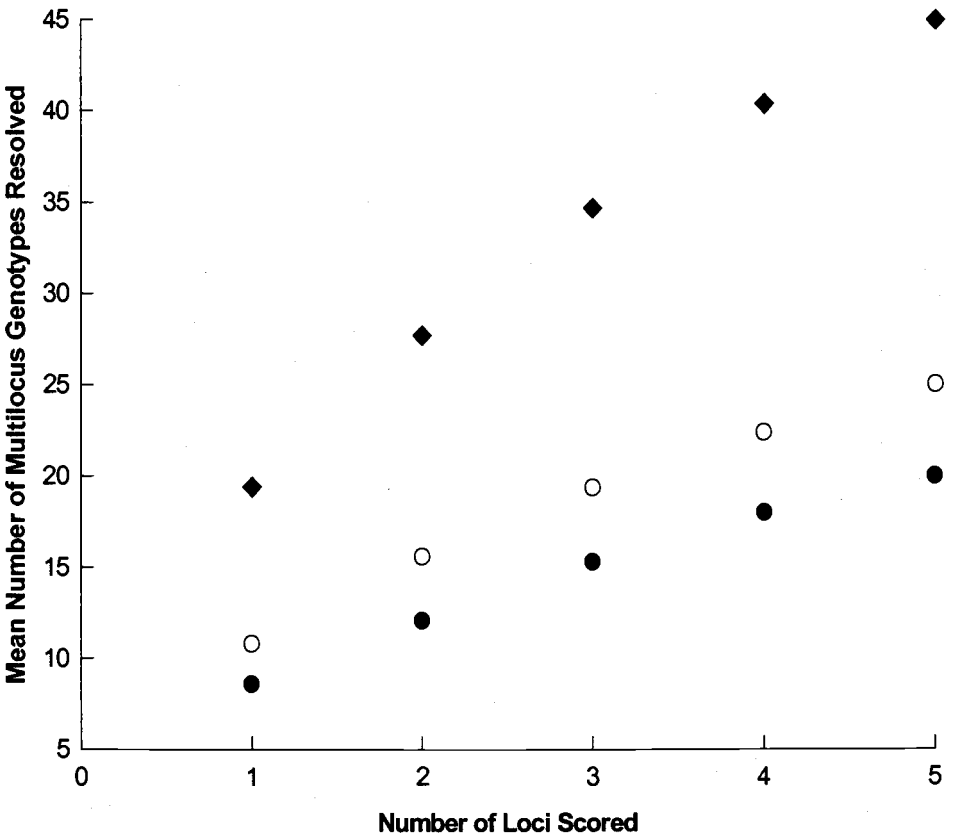


Figure 2.4. Mean number of multilocus genotypes resolved by all possible combinations of 1, 2, 3, 4, and 5 microsatellite loci. Closed diamonds represent the number of genotypes identified in the combined sample (ITS-RFLP types A and B), open circles represent the genotypes identified in *C. formosus* only, closed circles ITS-RFLP type B only.

Discussion

Implications of Intraspecific Variability in the ITS region in Cantharellus -

Throughout the 1900's, mycologists have noted that North American yellow chanterelles are morphologically distinct from European species and exhibit intraspecific variation (Smith & Morse 1947) of unknown evolutionary importance. Delineation of most fungal species relies on morphological characteristics of fruiting structures. Genetic analyses and mating experiments have shown that exclusive use of morphology to determine species boundaries is inadequate for many groups (Petersen & Hughes 1999) because commonly used characters like pileus color can vary at the population level or mask cryptic species when they are conserved (see Redecker *et al.* 2001 for examples from *Russula*).

Genetic studies of the ITS from North American yellow chanterelles have demonstrated length variability in the ITS 1 spacer (Feibelman *et al.* 1994), suggesting that either the ITS 1 varies at the population level or this group is a species complex. Recently researchers have used morphological and genetic data to identify the yellow chanterelle most frequently harvested from Pacific Northwest forests as *C. formosus*, a species once thought to be rare in the region (Redhead *et al.* 1997). A second yellow chanterelle species (*C. cibarius* var. *roseocanus*) apparently restricted to spruce (Redhead *et al.* 1997) and pine forests (T. Horton personal communication) also has been recognized from the region. We have examined several collections of *C. cibarius* var. *roseocanus* that have ITS regions of ~1490 bp and ITS-RFLP patterns that differ from those produced by the fruit-bodies collected from our plots under Douglas-fir

(Dunham unpublished data). Our data indicate the possibility of a third undescribed yellow chanterelle variety or species but it is unclear whether *C. formosus* and ITS-RFLP type B collections are different taxa or locally adapted populations. These two ITS-RFLP types are significantly segregated by habitat and genetic differences may reflect local adaptation within populations of *C. formosus*. Any phenotypic differences between the two yellow chanterelle ITS-RFLP types are subtle and were regarded as environmental variation during collecting for this study. If ITS-RFLP type B does represent an undescribed species it is difficult to distinguish from *C. formosus* in the field.

Species boundaries cannot be discerned using ITS-RFLP data alone, but the heterozygote deficiencies and significant linkage tests present in the combined microsatellite analysis (Table 2.2) both of which are reversed when the two groups are analyzed separately indicate that these ITS-RFLP groups may not be interbreeding. However, Chakraborty & Zhong (1994) have shown that the power of exact tests to detect significant departures from Hardy-Weinberg equilibrium is dependent on both the number of segregating alleles and allele frequencies. Partitioning our sample by ITS-RFLP type reduced both the sample size and the number of segregating alleles present in each group. Due to likely reductions in statistical power, caution must be used in interpreting what these tests mean because we may simply have lost the ability to detect departures from Hardy-Weinberg when the sample was split.

Overall there were several problems with application of microsatellite primers in ITS-RFLP type B that were not observed in *C. formosus*. Patterns of linkage were

not consistent between the two ITS-RFLP types indicating ITS-RFLP type B may have scoring problems, possibly due to the presence of null alleles, in loci Cf145 and Cf113 (Pemberton *et al.* 1995). The presence of null alleles may have resulted in nonrandom associations between the amplifiable alleles at these loci and the alleles at locus Cf642. This hypothesis is supported by low observed heterozygosities calculated for ITS-RFLP type B at these two loci (Table 2.2). Non-random associations between these loci were not detected in *C. formosus* indicating that linkage in ITS-RFLP type B is due either to scoring problems, or that the distance between these loci has been reduced by chromosomal rearrangements. Enrichment protocols were carried out on DNA from a *C. formosus* specimen. If the variation observed is indicative of species level differences then these primers do not cross amplify well between the two species.

Results from our research reveal the need for more molecular data to better define the species boundaries of *Cantharellus* species in the Pacific Northwest. The evolutionary relationships between these taxa will only be resolved with an analysis of DNA sequence data coupled with close scrutiny of morphology. Questions regarding biological species boundaries (sensu Mayr 1963) should be addressed with microsatellite analysis of sympatric broad-scale collections of relevant taxa. We are not able to fully address this question with the collections presented here because they represent a limited number of genets and are confounded by differences in habitat.

Abundance of Microsatellite Repeats in the Cantharellus Genome - The overall efficiency of attempts isolate microsatellites from the *C. formosus* genome was low relative to that observed for non-fungal taxa. The frequencies of different types of microsatellite motifs are known to vary widely between taxa (Hancock 1999; Tóth *et al* 2000). ISSR primers based on $(CAC)_n$ repeats have been used to generate polymorphic fingerprints in other species of fungi (Longato & Bonfante 1997; Anderson *et al.* 1998; Liu *et al.* 1998; Gherbi *et al.* 1999; Sawyer *et al.* 1999; Zhou *et al.* 1999; Kanchanaprayudh *et al.* 2002). Prior to our enrichment attempts we screened several *C. formosus* collections with ISSR primers based on various microsatellite motifs. ISSR fingerprint profiles indicated that the repeat types used in our enrichments were fairly common in our target species. Use of these markers to guide our motif selection may have resulted in poor choices if the primers were able to anneal to imperfect repeats or if ISSR profiles exhibiting discrete bands (rather than smears) are actually indicative of low repeat abundance. Tóth *et al.* (2000) have shown that in the intron and intergenic regions of some fungal genomes, tetranucleotide repeats constitute the least frequent class of microsatellites. How this relates to the abundance of these motifs in other regions of the fungal genome is unknown.

The low efficiency of our enrichments may also reflect the true abundance of microsatellite motifs in the *C. formosus* genome. Several studies have reported a positive correlation between the frequency of microsatellite regions and total genome size (e.g., Field and Wills 1998; Kubis *et al.* 1998; Primmer *et al.* 1997; Hancock 1999). For example the avian genome is roughly one-third the size of the human

genome (~1,200 Mb for the domestic chicken *Gallus gallus*; Bloom *et al.* 1993; Wachtel & Tiersch 1993) and recent research has demonstrated that microsatellite repeats occur less frequently in birds relative to other vertebrates (Primmer *et al.* 1997). The few fungal genomes that have been well characterized are modest in size with high gene density (Goffeau *et al.* 1996) with average basidiomycete genome size ranging around 35-40 Mb (e.g., Ramírez *et al.* 2000), roughly 30 times smaller than the typical avian genome. Microsatellites predominantly occur in noncoding regions, and if the proportion of noncoding DNA in the genome acts as the main constraint for the evolution of simple repeats, both the relative and absolute numbers of repeats in fungal genomes may naturally be low. Further research on the abundance of microsatellites in fungi is needed to clarify if the low number of loci characterized in this study result from factors involved in the generation of simple repeats, selection against "superfluous" repetitive DNA, or low abundance of the repeat motifs we enriched for in the *C. formosus* genome.

Genet Size and Resolution of Fungal Genets - The largest EM fungal genets measured to date cover areas approximately 300 m^2 (*Suillus* sp., Dahlberg & Stenlid 1994; Dahlberg 1997; Bonello *et al.* 1998). Small genets measured for species thought to proliferate by repetitive establishment via basidiospores rather than vegetative growth range from < 1 m^2 for *Laccaria amethystina* (Gherbi *et al.* 1999) and *Russula cremoricolor* (Redecker *et al.* 2001) to 12.5 m^2 for *L. bicolor* (Baar *et al.* 1994). Widths for most of the chanterelle genets we measured are small compared to other

EM species with less than four meters between most fruit-bodies with identical multilocus genotypes (Figure 2.3). Genets of *C. formosus* were significantly larger than those of ITS-RFLP type B but a few large genets interrupted by fruit-bodies with unlike genotypes drive this difference (Figure 2.1). This indicates that the statistical difference between genet sizes of the two ITS-RFLP types may be due to unresolved or fragmented genets.

Estimating genet size using fruit-bodies introduces error from several sources. Fruit-bodies are connected to mycelia that, in order to acquire nutrients, grow through soil and colonize EM root tips (Smith & Read 1997). Fruit-body production occurs during a limited season dependent on environmental conditions and species-specific biological cycles. Guidot *et al.* (2001) demonstrated that the spatial and temporal correspondence between the presence of fruit-bodies and EM root tips was high for *Hebeloma cylindrosporum* but how well these results extend to other EM taxa is unknown. Even if spatial locations of fruit-bodies are correlated with EM root tip locations, fruit-body sampling may not reflect the distribution of mycelia involved in capture of soil nutrients. In addition, some genets may continuously produce fruit-bodies in different locations over the course of a fruiting season while others fruit inconsistently (e.g., *Laccaria* sp. Selosse *et al.* 2001). This makes it difficult to estimate genet size without taking replicate samples throughout the entire fruiting season. Finally, mycelia can persist throughout the year and genet size and shape likely vary between years. When fruit-bodies are used to represent the distribution of mycelia, these sources of error likely result in genet size underestimates. Fruit-bodies,

however, are frequently sampled in ecological studies so having an estimate of the mean distance between fruit-bodies produced by individual genets is extremely useful prior to initiating research on EM species.

The maximum width for *C. formosus* genets ranged <1 m up to 13 m in diameter and this high variance may have occurred for several reasons. An important source of error in this study was that the five microsatellite loci characterized were not variable enough to resolve all the genets on our study plots (Figure 2.4) and this likely lead to overestimates of genet size. EM fungi do not produce mitotic (asexual) spores (Hutchinson 1989) that could allow long-distance dispersal of multilocus genotypes so the presence of multilocus genotypes repeated on plots separated by kilometers supports this conclusion. However, because chanterelles frequently are eaten and collected by various animals, an alternative explanation for the existence of identical multilocus genotypes several kilometers apart is the long-distance transport of mycelium fragments. To our knowledge, this mode of dispersal has not been identified in EM fungi. A related explanation for the high size variance within plots is genet fragmentation. Long or short distance dispersal of EM fungi is assumed to occur via movement of sexually produced basidiospores, but short distance dispersal could occur by vegetative growth of mycelia that subsequently become disjointed. On our study plots fruit-bodies of larger genets were dispersed and interrupted by different multilocus genotypes (Figure 2.1) indicating that fragmentation might occur in chanterelles.

Some larger genets may have been more difficult to resolve due to inbreeding among established genets (Gryta *et al.* 2000). Every fruit-body produces millions of basidiospores, most of which fall in the immediate vicinity of their source. Although EM fungi possess mating-type genes that reduce inbreeding, basidiospores from the same genet can germinate and fuse to form a dikaryotic mycelium capable of fruit-body formation. The mating system for *C. formosus* is unknown but even small amounts of inbreeding coupled with the low numbers of alleles present at our loci (Table 2.2) would exacerbate the difficulty in resolving some genets. For example, the multilocus genotype of the largest genet observed (13 m in diameter) was heterozygous at only one of five loci scored. Genets established by basidiospores from a genet with this genotype could only be detected with a 50% probability. Our study plot locations also were biased towards patches with high fruit-body abundance and this may have contributed to the difficulty in resolving genets because close proximity might facilitate inbreeding. On the other hand, frequent inbreeding or selfing should convert heterozygous loci to a homoallelic state, but no significant heterozygote deficit was detected in samples subdivided by ITS-RFLP type (but see discussion on power of test in subdivided samples).

How EM fungi propagate under different forest stand conditions has been the focus of several previous studies characterizing fungal genets (Dahlberg & Stenlid 1990; De La Bastide *et al.* 1994; Gryta *et al.* 1997, 2000; Bonello *et al.* 1998; Selosse *et al.* 1998, 1999; Gherbi *et al.* 1999; Sawyer *et al.* 1999; Zhou *et al.* 1999; Fiore-Donno & Martin 2001; Guidot *et al.* 2001, 2002; Redecker *et al.* 2001). Uniformly

small genets generally are thought to be indicative of continual establishment via sexual basidiospores (Dahlberg 2001; Guidot *et al.* 2002). The numerous, small genets of *C. formosus* and ITS-RFLP type B in all forest types indicate that genets may be frequently established by basidiospore dispersal and that sexual reproduction is a more prominent component in the life history of chanterelles compared to vegetative growth. In OG forests this might be facilitated by high turnover rates of fine roots (Vogt *et al.* 1986) continually providing new opportunities for genet colonization. Our study design lacks the temporal sampling needed to address persistence of genotypes, so we cannot eliminate the possibility that *C. formosus* genets are slow growing or simply do not produce fruit-bodies across the entire spatial distribution of their mycelium each year.

The broader-scale life history strategy of *C. formosus* is still in question because it is not clear that we actually sampled genets from this species on our OG plots. ITS-RFLP type B dominated the OG sites and since there is a strong possibility that this sample represents an undescribed species, comparisons of genet size among all three treatments are suspect. If ITS type B represents an OG adapted population of *C. formosus* then genet size may actually be smaller in OG than in SG. If ITS type B represents a different species, then we cannot address how *C. formosus* genet establishment and growth patterns are affected by environmental differences associated with stand age. This will require another genet study aided by better chanterelle species concepts.

Conclusions

Our knowledge of patterns in fungal genet propagation has increased substantially over the past ten years and with this knowledge of pattern has come some understanding of the processes that shape genetic diversity in fungal populations (Dahlberg 2001). Each study of genet size has produced unique and sometimes unexpected estimates for different species. These results indicate that investigating genet size is a prerequisite for subsequent studies of genetic diversity at larger spatial scales. Here we describe five co-dominant markers that demonstrate a high degree of utility in the characterization of chanterelle genets. Our investigation of genet size in *C. formosus* in Douglas-fir forests has pointed to the possible existence of a cryptic and undescribed species of yellow chanterelle in the Pacific Northwest that may be associated with OG habitats. Regardless of the evolutionary status of these genetic entities, our data shows that mean maximum width of chanterelle genets is below four meters indicating that genet propagation is more likely the result of basidiospore dispersal rather than vegetative spread

References

Anderson IC, Chambers SM, Cairney JWG (1998) Use of molecular methods to estimate the size and distribution of mycelial individuals of the ectomycorrhizal basidomycete *Pisolithus tinctorius*. *Mycological Research*, **102**, 295-300.

Avise JC (1994) *Molecular Markers, Natural History, and Evolution*. Chapman and Hall New York.

Baar J, Ozinga WA, Kuyper TW (1994) Spatial distribution of *Laccaria bicolor*

genets reflected by sporocarps after removal of litter and humus layers in a *Pinus sylvestris* forest. *Mycological Research,* **98**, 726-728.

Bloom SE, Delany ME, Muscarella DE (1993) Constant and variable features of avian chromosomes. In *Manipulation of the avian genome* (ed. Etches RJ, Verrinder Gibbins AM), pp. 39–60. CRC Press, Boca Raton, Florida.

Bonello P, Bruns TD, Gardes M (1998) Genetic structure of a natural population of the ectomycorrhizal fungus *Suillus pungens. New Phytologist,* **138**, 533-542.

Chakraborty R, Zhong Y (1994) Statistical power of an exact test of Hardy-Weinberg proportions of genotypic data at a multiallelic locus. *Human Heredity*, **44**, 1-9.

Dahlberg A (1997) Population ecology of *Suillus variegatus* in old Swedish Scots pine forests. *Mycological Research,* **101**, 47-54.

Dahlberg A (2001) Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. *New Phytologist,* **150**, 555-562.

Dahlberg A, Stenlid J (1990) Population structure and dynamics in *Suillus bovinus* as indicated by spatial distribution of fungal clones. *New Phytologist,* **115**, 487-493.

Dahlberg A, Stenlid J (1994) Population ecology of *Suillus variegatus* in old Swedish Scots pine forests. *Mycological Research,* **101**, 47-54.

Dahlberg A, Stenlid J (1995) Spatiotemporal patterns in ectomycorrhizal populations. *Canadian Journal of Botany,* **73**, S1222-S1230.

Danell E (1995) Comparisons between Swedish *Cantharellus cibarius* and *Cantharellus* spp. in the Pacific Northwest, based on differences in RFLP patterns of the ITS region. Abstract in *Inoculum,* **46**, 10.

Dataxiom Software Inc. (2001) *StatMost: Statistical analysis and graphics, 4th edition.* Los Angeles, CA.

De La Bastide PY, Kropp BR, Piché Y (1994) Spatial distribution and temporal persistence of discrete genotypes of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton. *New Phytologist,* **127**, 547-556.

Feibelman T, Bayman P, Cibula WG (1994) Length variation in the internal transcribed spacer of ribosomal DNA in chanterelles. *Mycological Research,* **98**, 614-618.

Field D, Wills C (1998) Abundant microsatellite polymorphism in *Saccharomyces cerevisiae*, and the different distributions of microsatellites in eight prokaryotes and *S. cerevisiae*, result from strong mutation pressures and a variety of selective forces. *Proceedings of the National Academy of Science* U.S.A., **95**, 1647–1652.

Fiore-Donno A-M, Martin F (2001) Populations of ectomycorrhizal *Laccaria amethystina* and *Xerocomus* spp. show contrasting colonization patterns in a mixed forest. *New Phytologist,* **152**, 533-542.

Fleischer RC, Loew S (1995) Construction and screening of microsatellite-enriched genomic libraries. In: *Molecular Zoology: Advances, Strategies, and Protocols* (eds. Ferraris J, Palumbi S) pp. 459-468. Wiley-Liss, New York.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes applications to the identification of mycorrhizae and rusts. *Molecular Ecology,* **2**, 113-118.

Gardes M, Bruns TD (1996) ITS-RFLP matching for identification of fungi. *Methods in Molecular Biology,* **50**, 177-186.

Gherbi H, Delaruelle C, Selosse M-A, Martin F (1999) High genetic diversity in a population of the ectomycorrhizal basidiomycete *Laccaria amethystina* in a 150 year-old beech forest. *Molecular Ecology,* **8**, 2003-2013.

Goffeau A, Barrell BG, Bussey H, *et al.* (1996) Life with 6000 genes. *Science,* **274**, 563-567.

Gryta H, Debaud J-C, Effosse A, Gay G, Marmeisse R (1997) Fine-scale structure of populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum* in coastal sand dune forest ecosystems. *Molecular Ecology,* **6**, 353-364.

Gryta H, Debaud J-C, Marmeisse R (2000) Population dynamics of the symbiotic mushroom *Hebeloma cylindrosporum*: mycelial persistence and inbreeding. *Heredity,* **84**, 294-302.

Guidot A, Debaud JC, Marmeisse R (2001) Correspondence between genet diversity and spatial distribution of above-and below-ground populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum. Molecular Ecology,* **10**, 1121-1131.

Guidot A, Gryta H, Gourbière F, Debaud J, Marmeisse R. (2002) Forest habitat

characteristics affect balance between sexual reproduction and clonal propagation of the ectomycorrhizal mushroom *Hebeloma cylindrosporum*. *Oikos,* **99**, 25-36.

Hancock JM (1999) Microsatellites and other simple sequences: genomic context and mutational mechanisms. In *Microsatellites: evolution and applications*. (ed. Goldstein DB, Schlötterer C. Oxford University Press, Oxford. pp. 1–9.

Horton TR & Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black box. *Molecular Ecology,* **10**, 1855-1871.

Hutchinson A (1989) Absence of conidia as a morphological character in ectomycorrhizal fungi. *Mycologia,* **81**, 587-594.

Jandel Scientific (1993) *SigmaPlot Version 2.01 Scientific Graphing Software*: User's Manual. San Rafael, CA.

Kanchanaprayudh J, Lian C, Zhou Z, Hogetsu T, Sihanonth P. (2002) Polymorphic microsatellite markers of a *Pisolithus* sp. from Eucalyptus plantation. *Molecular Ecology Notes,* **2**, 263-264.

Kubis S, Schmidt T, Heslop-Harrison JS (1998) Repetitive DNA elements as a major component of plant genomes. *Annals of Botany,* **82**(Suppl. A), 45–55.

Liu G, Chambers SM, Cairney JWG (1998) Molecular diversity of ericoid mycorrhizal endophytes isolated from *Woollsia pungens*. *New Phytologist,* **140**, 145-153.

Longato S, Bonfante P (1997) Molecular identification of mycorrhizal fungi by direct amplification of microsatellite regions. *Mycological Research,* **101**, 425-432.

Mayr, E. (1963) *Populations, Species, and Evolution*. Harvard University Press, Cambridge, Massachusetts.

Molina R, Pilz D, Smith J *et al.* (2001) Conservation and management of forest fungi in the Pacific Northwestern United States: An integrated ecosystem approach. In: *Fungal Conservation*. (eds. Moore D, Nauta M, Rotheroe M) Cambridge University Press.

Molina R, O'Dell T, Luoma D *et al.* (1993) Biology, ecology, and social aspects of wild edible mushrooms in the forests of the Pacific Northwest: a preface to managing commercial harvest. General Technical Report PNW-GTR-309. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 42p.

Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology*, **4**, 249-252.

Primmer CR, Raudsepp T, Chowdhary BP, Møller AP, and Ellegren H (1997) Low frequency of microsatellites in the avian genome. *Genome Research,* **7**, 471–482.

Petersen RH, Hughes KW (1999) Species and speciation in mushrooms: Development of a species concept poses difficulties. *Bioscience,* **49**, 440-452.

Redecker D, Szaro TM, Bowman RJ, Bruns TD (2001) Small genets of *Lactarius xanthogalactus, Russula cremoricolor* and *Amanita francheti* in late-stage ectomycorrhizal successions. *Molecular Ecology,* **10**, 1025-1034.

Ramírez L, Larraya LM, Pisabarro AG (2000) Molecular tools for breeding basidiomycetes. *International Microbiology,* **3**, 147-152.

Raymond M, and Rousset F (1995) GENEPOP v. 3.1c: Population genetics software for exact tests and ecumenicism. *Journal of Heredity,* **86**, 248-249.

Redhead SA, Norvell LL, Danell E (1997) *Cantharellus formosus* and the Pacific Golden Chanterelle harvest in western North America. *Mycotaxon,* **65**, 285-322.

Sambrook J, Fritsh EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual* 2nd edn. Cold Springs Harbor Laboratory Press, New-York.

Sawyer NA, Chambers SM, Cairney JWG (1999) Molecular investigation of genet distribution and genetic variation of *Cortinarius rotundisporus* in eastern Australian sclerophyll forests. *New Phytologist,* **142**, 561-568.

Selosse M-A, Jacquot D, Bouchard D, Martin F, Le Tacon F (1998) Temporal persistence and spatial distribution of an American inoculant strain of the ectomycorrhizal basidiomycete *Laccaria bicolor* in European forest plantations. *Molecular Ecology,* **7**, 561-573.

Selosse M-A, Martin F, Bouchard D, Le Tacon F (1999) Structure and dynamics of experimentally introduced and naturally occurring *Laccaria* sp. discrete genotypes in a Douglas fir plantation. *Applied and Environmental Microbiology,* **65**, 2006-2014.

Selosse M-A, Martin F, Le Tacon F (2001) Intraspecific variation in fruiting phenology in an ectomycorrhizal *Laccaria* population under Douglas fir. *Mycological Research,* **105**, 524-531.

Shinohara ML, LoBuglio KF, Rogers SO (1996) Group-1 intron family in the nuclear ribosomal RNA small subunit genes of *Cenococcum geophilum* isolates. *Current Genetics,* **29**, 377-387.

Smith AH, Morse EE (1947) The genus *Cantharellus* in the Western United States. *Mycologia,* **39**, 379-495.

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd ed. Academic Press.

Todd NK, Rayner ADM (1980) Fungal Individualism. *Scientific Program at Oxford,* **66**, 331-354.

Tóth G, Gáspári Z, Jurka J (2000) Microsatellites in Different Eukaryotic Genomes: Survey and Analysis. *Genome Research,* **10**, 967-981.

Vogt KA, Grier CC, Vogt DJ (1986) Production, turnover and nutrient dynamics of above- and belowground detritus of world forests. *Advances in Ecological Research,* **15**, 303-377.

Wachtel SS, Tiersch TR (1993) Variations in genome mass. *Comparative Biochemistry and Physiology,* **104B**, 207–213.

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications* (ed. Innis M), pp. 315-322. Academic Press, CA.

Wiens JA (1989) Spatial scaling in ecology. *Functional Ecology,* **3**, 385-397.

Zhou Z, Miwa M, Hogetsu T (1999) Analysis of genetic structure of a *Suillus grevillei* population in a *Larix kaempferi* stand by polymorphism of inter-simple sequence repeat (ISSR). *New Phytologist,* **144**, 55-63.

ANALYSIS OF NRDNA SEQUENCES AND MICROSATELLITE ALLELE FREQUENCIES REVEALS A CRYPTIC CHANTERELLE SPECIES *CANTHARELLUS CASCADENSIS* NOM. PROV. FROM THE PACIFIC NORTHWEST

Susie M. Dunham

Oregon State University, Dept. of Forest Science, 3200 SW Jefferson Way, Corvallis, OR 97331, U. S. A.[1]

Thomas E. O'Dell

Current Address: Grand Staircase-Escalante National Monument, 190 E. Center, Kanab, UT 84741.

Randolph J. Molina

USDA Forest Service, Pacific Northwest Research Station, 3200 Jefferson Way, Corvallis, OR 97331, U.S.A

Mycological Research

In Review

Abstract

In the Pacific Northwest, yellow chanterelles have long been referred to as *Cantharellus cibarius*, synonymous with the European yellow chanterelle. Broad scale genetic surveys of North American chanterelles with *C. cibarius*-like morphology have demonstrated that the nrDNA internal transcribed spacer exhibits length variability, suggesting that this common morphology masks a species complex. Researchers recently have used morphological and genetic data to identify the yellow chanterelle most frequently harvested from the Pacific Northwest forests as *C. formosus*, a species once thought to be rare in the region. We present three genetic data sets and one morphological data set that characterize a previously undescribed, species of yellow chanterelle from the central Cascade Mountains of Oregon. Phylogenetic analyses of the nrDNA large subunit and ITS regions show that *C. cascadensis* nom. prov., along with two other yellow chanterelle taxa (*C. cibarius* var. *roseocanus* and European *C. cibarius*), are more closely related to white chanterelles (*C. subalbidus*) than they are to *C. formosus*. Data from five microsatellite loci provide evidence that *C. formosus*, *C. subalbidus*, and *C. cascadensis* nom. prov. do not interbreed when they co-occur spatially and temporally in Douglas fir-western hemlock forests. This demonstrates that these three sympatric chanterelles are biological species with boundaries congruent with those delineated by nrDNA phylogenetic clades. Morphological data indicate that the color of the pileus and shape of the stipe can be used to separate fresh collections of the two yellow species now known to co-occur in Douglas fir-western hemlock forests in Oregon.

Introduction

Chanterelles are popular edible fungi commercially and recreationally harvested from wild populations in Europe, Africa, Asia, and the western United States (Danell 1999). Species in the genus *Cantharellus* also are ectomycorrhizal with commercially harvested conifers and their economic and ecological importance has inspired research into the evolution and ecology of the genus (Danell 1999; Molina *et al.* 2001). Until recently, morphologically similar yellow chanterelles throughout North America were lumped under a single Latin binomen and considered synonymous with the European yellow chanterelle (*Cantharellus cibarius* Fr.). In contrast, throughout the 1900's, mycologists have noted that yellow chanterelles found in North America not only are morphologically distinct from European species (Murrill 1912; Smith & Morse 1947; Corner 1966; Petersen 1979) but also exhibit intra-specific variation across North America (Feibelman *et al.* 1997) and at regional scales (Smith & Morse 1947; Redhead *et al.* 1997).

Genetic studies of North America chanterelles with *C. cibarius*-like morphology have demonstrated significant length variability in the nuclear ribosomal internal transcribed spacer (nrDNA ITS), which suggests that this common morphology masks a species complex (Feibelman *et al.* 1994). Recently researchers have used morphological and genetic data to identify the yellow chanterelle most frequently harvested from the PNW forests as *C. formosus* Corner, a species once thought to be rare in the region (Redhead *et al.* 1997). A second yellow chanterelle thought to be a variety of the European chanterelle (*C. cibarius* var. *roseocanus*) also has been

recognized from the region but is less common and may be restricted to coastal and high elevation spruce forests (Redhead *et al.* 1997).

We have studied genetic variability within putative *C. formosus* populations using RFLP analysis of the ITS region (ITS 1, ITS 2, and the 5.8s gene) and co-dominant microsatellite markers (Dunham *et al.* in press). Putative *C. formosus* collections taken from 16 m diameter circular plots under Douglas fir (*Pseudotsuga menziesii*) exhibit two genotypes that vary in the total length of the ITS region and in RFLP patterns. One ITS genotype from these collections is similar to that described for *C. formosus* (~1,690 base pairs; Danell 1995; Redhead *et al.*1997), the other, a ~1,490 base pair (bp) ITS region, is more similar in length to the ITS from both white chanterelles (*C. subalbidus*) and European *C. cibarius* (Danell 1995).

Techniques employing RFLP analysis of the ITS region commonly are used to characterize below-ground fungal species diversity in various ecological systems dominated by ectomycorrhizal plants (Horton & Bruns 2001). ITS length variability similar to that described above for *C. formosus* has been observed in the ectomycorrhizal fungus *Cenococcum geophilum* and was thought to delineate several cryptic species (LoBuglio *et al.* 1991) until subsequent analyses showed that the length polymorphism is due to the presence of a family of group-I introns inserted near the 3' end of the nrDNA small subunit. Hence, *C. geophilum* collections with ITS regions of different length actually represent a single species (Shinohara *et al.* 1996, 1999). In contrast, studies of the ITS region in *Armillaria* (Anderson & Stasovski 1992) and *Hebeloma* (Aanen *et al.* 2000) have characterized situations where

variability in ITS sequences is low even among genetically isolated species. Clearly, more precise molecular and morphological comparisons are needed to better understand the evolutionary implications of ITS length and RFLP variability within *Cantharellus*.

A re-examination of species concepts in PNW chanterelles must precede further population genetic studies. Contemporary delineation of most chanterelle species relies on morphological characteristics of fruiting structures, a technique thought to be inadequate for fully differentiating species in many genera of fungi (Petersen & Hughes 1999; Taylor *et al.* 2000). Species in the genus *Cantharellus* exhibit a limited number of conserved morphological characters (Smith & Morse 1947; Thiers 1985), thus application of molecular techniques to a much larger sample of fruit bodies should prove useful in defining genetic lineages that can be closely examined for previously undetected phenotypic differences (e.g., Fisher *et al.* 2002).

Sequence analysis of the ITS region is a popular method often used to characterize the boundaries of genera and species in fungi (e.g., Shinohara *et al.* 1999; Høiland & Holst-Jensen 2000). Homogenization of the multiple copies of this gene family due to concerted evolution is an important consideration in their use in phylogenetic analysis. Studies at lower taxonomic levels (within species and among populations) are at risk for error due to introgression of alternate nrDNA copies at rates faster than they can be homogenized within the genome (Martin 1990; O'Donnell 1992; Sanderson & Doyle 1992; Hartmann *et al.* 2001; Horton 2002). Because every copy might not represent the entire gene family within a population of

interbreeding individuals, nrDNA sequence variation can imply the presence of multiple species when none exist.

Mapping species boundaries onto the hierarchical structure of a nrDNA phylogeny requires an objective method of recognizing the level at which dichotomous branching represents divergence of lineages on independent evolutionary paths (i.e., evolutionary species *sensu* Simpson 1951, 1961; Wiley 1978). Methods proposed for recognizing evolutionarily independent lineages include mapping morphological data onto a topology to identify minimum aggregations that share distinct, diagnosable phenotypic characters (Harrington & Rizzo 1999) or generating multiple phylogenies from independent loci and identifying concordant lineages (Taylor *et al.* 2000). These methods require either observable variability in morphology, which may be difficult for groups with reduced morphology or sequencing multiple genes for each taxon, which is expensive and limits number of collections that can be examined. A third option exists when the taxa in question are sympatric in distribution. Variable co-dominant loci can be used to test for significant allele frequency differences among sympatric collections. This sampling strategy eliminates local adaptation or independent population histories as an alternate explanation for genetic differentiation (Fisher *et al.* 2000) while greatly reducing expense and allowing characterization of large samples prohibited by the expense of sequencing based studies.

This investigation describes evolutionary relationships among PNW chanterelle species and uses this information to determine if observed ITS and

microsatellite variability is indicative of biological species boundaries. Specific objectives of the present study are to (1) analyze nrDNA sequences to determine the phylogenetic relationships among PNW chanterelle collections with alternate ITS genotypes and European *C. cibarius*, (2) analyze microsatellite allele frequencies from sympatric chanterelle collections that represent multiple ITS genotypes to test for gene flow among ITS genotypes, and (3) identify morphological characters useful in differentiating cryptic species within the *C. formosus* morphology. Finally, we describe a new species of *Cantharellus* based on these genetic and morphological characters.

Materials and Methods

Material Examined - To better characterize the total genetic diversity present in the ITS region within western chanterelles, samples for RFLP analysis were taken from several locations in Washington, Oregon, and California. A large proportion of the collecting effort was centered in the H. J. Andrews Experimental Forest (HJA, lat. 44°15′N, long. 122°10′W) and surrounding areas in the central Oregon Cascades during 1997-98 with additional collecting efforts made opportunistically in more broadly distributed localities during 1997-2000. The broad host range of *Cantharellus* allowed sample collection in forests containing a variety of host trees (i.e., *Pseudotsuga, Tsuga, Picea*, and *Querqus*) at elevations ranging from sea level to approximately 1,700 m. Collections of European *C. cibarius, C. cibarius* var. *roseocanus, C. cinnabarinus* and *C. persicinus* were obtained from mycologists also

familiar with these species (Table 1). nrDNA large subunit sequences for *Clavulina cristata, Hydnum umbilicatum, Craterellus odoratus*, and *Craterellus tubaeformis* used here as outgroup taxa were taken from Dahlman *et al.* (2000). Dried voucher materials from all analyzed collections are deposited in the Oregon State University herbarium (OSC, Table 3.1).

Table 3.1. Collection numbers, geographical origins, habitat, and genbank accession numbers associated with samples used in molecular and morphological analyses.

OSC	Species	State/ Country	County	Land Owner	General Area	Dominant Overstory	Collector	ITS Type	Genbank Accession No.
67713	*Cantharellus. formosus*	OR, USA	Lincoln	SNF	CPSA	P	SD	A	
67714	*Ca. formosus*	OR, USA	Lincoln	SNF	CPSA	P	SD	A	
75906	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]	
75909-11	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]	
75926	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[34]	
75930	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[134]	[1]AY041164
75931	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[134]	[1]AY041166
75941-42	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]	
75945-56	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]	
75957-59	*Ca. formosus*	OR, USA	Benton	SNF	MPRA	PT	SD	A	
75960	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A	

Table 3.1. Continued

75961	*Ca. formosus*	OR, USA	Lane	UNF	MJ	PT	SD	A
75962-67	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A
75972	*Ca. formosus*	OR, USA	Linn	WNF	BRRD	PT	SD	A[4]
75973-74	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]
75980	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]
75981	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[34]
75987-89	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]
75992-94	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]
75996	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]
75997-99	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]
76000-01	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]
76003-05	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]
76008	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]

Table 3.1. Continued

76009-11	*Ca. formosus*	OR, USA	Tillamook	OSP	CLSP	P	SD	A
76012-13	*Ca. formosus*	OR, USA	Lincoln	OSP	FCSP	P	SD	A
76014	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A^{4}
76015	*Ca. formosus*	OR, USA	Tillamook	OSP	CLSP	P	SD	A
76016	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A^{4}
76021	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A^{3}
76024-25	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A^{34}
76033	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A^{34}
76037-41	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A^{34}
76046	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A^{34}
76048	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A^{34}
76050	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A^{3}
76054	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A^{1234}

[1]AY041165

Table 3.1. Continued

									[2]AY041184
76056	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[34]	
76058	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[3]	
76059	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[34]	
76060	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A[4]	
76065-70	*Ca. formosus*	OR, USA	Lane	WNF	BRRD	PT	SD	A	
76057	*Cantharellus. sp.*	CA, USA	Santa Barb.	LPNF	SR	Q	HE	F[1]	[1]AY041167
75902-05	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	
75912	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C	
75927-29	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	

75932-33	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	

Table 3.1. Continued

75935-36	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	
75937	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[123]	[1]AY041149 [2]AY041179
75968-71	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C	
75983	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[13]	[1]AY041146
75995	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C	
76007	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C	
76020	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	
76023	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	
76026	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	
76028	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[13]	[1]AY041150
76032	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[13]	

[1]AY041148

76034	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[123]	[1]AY041147

Table 3.1. Continued

[2]AY041178

76035-36	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	
76042-43	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	
76047	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	
76049	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	
76051	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	
76053	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	
76055	*Ca. subalbidus*	OR, USA	Lane	WNF	BRRD	PT	SD	C[3]	
76061-62	*Ca. subalbidus*	OR, USA	Linn	WNF	BRRD	PT	SD	C	
76063-64	*Ca. subalbidus*	OR, USA	Linn	WNF	IM	PT	SD	C	

75907	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B^{34}	
75908	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B^{123}	^{1}AY041160

Table 3.1. Continued

									^{2}AY041181
75913-15	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B^{4}	
75916	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B^{3}	
75917	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B^{1234}	^{1}AY041158
									^{2}AY041180
75918-20	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B^{3}	
75921	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B^{34}	
75922	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B^{3}	
75923-25	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B^{34}	
75934	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B^{4}	
75938	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B^{4}	

75975	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[124]	[1]AY041163 [2]AY041183

Table 3.1. Continued

75976-78	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[4]	
75979	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[13]	[1]AY041159
75982	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[134]	[1]AY041161
75984	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[3]	
75985	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[124]	[1]AY041162 [2]AY041182
75986	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[3]	
75990	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[34]	
75991	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[3]	
76002	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[34]	
76006	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[3]	

76017	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[3]	
76018-19	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[34]	

Table 3.1. Continued

76072	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[34]	
76073	*Ca. cascadensis* nom. prov.	OR, USA	Lane	WNF	BRRD	PT	SD	B[4]	
70397	*Ca. cascadensis* nom. prov.	OR, USA	Josephine	SKNF	PRD	PL	TO	B	
70396	*Ca. cascadensis* nom. prov.	OR, USA	Jackson	MBLM	BFRA	PT	TO	B	
75940	*Ca. cibarius* var. *cibarius*	Sweden	---	---	---	B	ED	E[12]	[1]AY041157
									[2]AY041177
76027	*Ca. cibarius* var. *cibarius*	Sweden	---	---	---	B	ED	E[12]	[1]AY041155
									[2]AY041175
76031	*Ca. cibarius* var. *cibarius*	Sweden	---	---	---	Conifers	ED	E[12]	[1]AY041156

[2]AY041176

Table 3.1. Continued

66326	*Ca. cibarius* var. *roseocanus*	OR, USA	Lincoln	SNF	CPSA	P	TO	D[1]	[1]AY041151
67634	*Ca. cibarius* var. *roseocanus*	WA, USA	Skamania	GPNF	SRRNA	P	JEL	D[12]	[1]AY041154
									[2]AY041174
67711	*Ca. cibarius* var. *roseocanus*	OR, USA	Lincoln	SNF	CPSA	P	TO	D	
67712	*Ca. cibarius* var. *roseocanus*	OR, USA	Lincoln	OSP	PSP	P	SD	D[12]	[1]AY041152
									[2]AY041172
67715	*Ca. cibarius* var. *roseocanus*	OR, USA	Lincoln	OSP	PSP	P	SD	D	
75939	*Ca. cibarius* var. *roseocanus*	OR, USA	Lincoln	OSP	FCSP	P	SD	D	
75943-44	*Ca. cibarius* var. *roseocanus*	OR, USA	Linn	WNF	SHRD	P	SD	D	
76022	*Ca. cibarius* var. *roseocanus*	OR, USA	Linn	WNF	SHRD	P	SD	D	

76029	*Ca. cibarius* var. *roseocanus*	OR, USA	Linn	WNF	SHRD	P	SD	D	
76030	*Ca. cibarius* var. *roseocanus*	OR, USA	Tillamook	OSP	CLSP	P	SD	D	
76044	*Ca. cibarius* var. *roseocanus*	OR, USA	Linn	WNF	SHRD	P	SD	D	
76045	*Ca. cibarius* var. *roseocanus*	OR, USA	Lincoln	OSP	PSP	P	SD	D[12]	[1]AY041153

Table 3.1. Continued

									[2]AY041173
76052	*Ca. cibarius* var. *roseocanus*	OR, USA	Lincoln	OSP	PSP	P	SD	D	
76071	*Ca. cibarius* var. *roseocanus*	OR, USA	Lincoln	OSP	PSP	P	SD	D	
69197	*Ca. cinnabarinus*	SC, USA	Oconee	SUNF	BFT	MH	JS	N/A[1]	[1]AY041168
69195	*Ca. persicinus*	SC, USA	Oconee	SUNF	BFT	MH	JS	N/A[1]	[1]AY041169

Outgroup Taxa

---	*Craterellus odoratus*[a]	MS, USA	---	---	---	--	ED	N/A[1] [1]AF105306
---	*Cr./Ca. tubaeformis*[a]	Sweden	---	---	Uppsala	--	ED	N/A[1] [1]AF105307
49915	*Cr./Ca. tubaeformis*[a]	WA, USA	---	---	---	--	TO	N/A[1] [1]AF105309

Table 3.1. Continued

---	*Hydnum umbilicatum*[a]	OR, USA	---	---	---	--	JS	N/A[1] [1]AY041170
---	*Clavulina cristata*[a]	OR, USA	---	---	---	--	JS	N/A[1] [1]AY041171

OSC: Oregon State University Herbarium Collection Number
Land Owner: WNF – Willamette National Forest, SNF – Siuslaw National Forest, GPNF – Gifford Pinchot National Forest, UNF – Umpqua National Forest, SKNF – Siskiyou National Forest, OSP – Oregon State Parks, SUNF – Sumter National Forest, LPNF – Los Padres National Forest, MBLM – Medford District Bureau of Land Management.
General Area: BRRD – Blue River Ranger District, CPSA – Cape Perpetua Scenic Area, SRRNA – Steamboat River Resource Natural Area, PRD – Powers Ranger District, PSP – Patterson State Park, FCSP – Fogarty Creek State Park, SHRD – Sweet Home Ranger District, CLSP – Cape Lookout State Park, MPRA – Mary's Peak Recreation Area, MJ – Mount June, IM – Iron Mountain, BFT – Burrell's Ford Trail, SR – Sedgwick Reserve, University of California Natural Reserve System, BFRA – Butte Falls Resource Area.
Dominant Overstory: PT – *Pseudotsuga menziesii* and *Tsuga heterophylla*, PL – *Pseudotsuga menziesii* and *Lithocarpus densifolia*, P – *Picea sp.*, B – *Betula sp.*, Q – *Quercus sp.*, MH – Mixed Hardwoods.
Collector: SD – Susie Dunham, TO – Thomas O'Dell, JEL – Janet E. Lindgren, HE – Helmut Ehrenspeck, JS – Joey Spatafora, ED – Eric Danell.
ITS Type: RFLP types are compiled from data collected using four restriction enzymes (*Alu*, *Dpn II*, *Hinf I*, and *Hae III*).

Chanterelle collections used to define phylogenetic lineages, test for gene flow among ITS variants, and to describe morphological variation were drawn primarily from the HJA. Elevations within the HJA range from 420 to 1630 m and the area is typical of the central portion of the western Cascade Mountains in Oregon (Franklin *et al.* 1990). The primary overstory species of HJA study areas are Douglas fir, western hemlock (*Tsuga heterophylla*), and Pacific silver fir (*Abies amabilis*) and are in the western hemlock and Pacific silver fir zones of Franklin & Dyrness (1973). Collections made within the HJA came from 18 stands dominated by Douglas fir and western hemlock trees either 350+ years old or stands that had been clear cut during 1950-1961 and regenerated naturally. Second growth stands ranged from 10-15 ha and the area searched within old growth stands roughly approximated this range. Two collectors searched each stand for 1.5 hours once in October and once in November and samples from these two time periods were pooled to derive data for microsatellite analyses. To avoid re-sampling individuals a minimum of five meters (based on preliminary genet size estimates, Dunham *et al.* in press) was kept between any chanterelle collections of the same color (white or yellow) and collection locations were flagged to avoid re-sampling individuals encountered by collectors at different times. A minimum of five meters also was kept between collections made outside of the HJA but in these areas random search methods were not employed and most collecting took place along established hiking trails.

DNA Extraction and Amplification - After grinding an small amount of fresh or dried tissue in 1 ml of lysis buffer (100 mM Tris, 10mM EDTA, 2% sodium dodecyl sulfate, 1mg/ml proteinase-K, pH 8.0), each sample was incubated for 1.5 hours at 55 °C and DNA was extracted using a standard phenol/chloroform emulsification and precipitation (Maniatis *et al.* 1982). Resulting pellets were vacuum-dried and re-suspended in 50 to 200 µl of sterile TE buffer (10mM Tris, 1mM EDTA). Unquantified DNA samples were diluted 10 to 1,000-fold prior to use and the polymerase chain reaction (PCR) was attempted on successive ten-fold dilutions until amplification of the desired nrDNA region was achieved. The entire ITS region spanning the 3' end of the 18S, ITS-1, 5.8S, ITS-2, and 5' end of the 28S was amplified with the fungal specific primer ITS1-F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). The 5' end of the nuclear large subunit was amplified with ITS4R (White *et al.* 1990) and LR5 (Vilgalys & Hester 1990). All amplifications of nrDNA regions were carried out in 40 µl volumes that contained 5 µl of diluted template. Final concentrations of PCR mix components were 200 µM of each dNTP, 0.2 µM of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, and 0.75 units of DNA polymerase. Following an initial denaturation period at 95 °C for 3 min., reactions were subject to 35 PCR cycles with the following profile, denaturation at 95 °C for 1 min., primer annealing at 50 °C for 1 min., and primer extension at 72 °C for 90 s. Following the 35th cycle, samples were subjected to a final extension of 72 °C for 10 min.

Amplification of microsatellite loci were carried out using primer sequences developed for *C. formosus* described in Dunham *et al.* (in press). PCR reactions were carried out in 20 µl volumes and contained 2-3 µl diluted genomic DNA, 200 µM dNTPs, 0.25 µM of each locus specific primer, 1 U Taq DNA polymerase and 1X Assay Buffer A (Fisher Scientific). The PCR profile consisted of initial denaturation at 95° C for 3 min. followed by 35 cycles (95° C, 45s; 55° C, 1 min.; 72° C, 1 min.) in a PTC-100 Programmable Thermal Controller (MJ Research Inc.). Following the 35th cycle, samples were subjected to a final 72° C extension of 60 min. to maximize the proportion of complete PCR products prior to analysis. PCR products were analyzed on an ABI 377 automated sequencer using the GS500 Tamra internal size standard. Band sizes were determined using GENESCAN software (PE Applied Biosystems).

Restriction Fragment Length Polymorphisms (RFLP's) - Unpurified PCR products amplified with ITS1-F and ITS4 were digested separately with four restriction enzymes, *Alu*, *Hinf I*, *DPN* II, and *HaeIII*, according to the manufacturer's recommendations (GIBCO BRL, Grand Island, NY). Restriction fragments were electrophoretically separated on 3% agarose gels (2% Nu-Sieve agarose, 1% SeaKem LE agarose; FMC BioProducts), stained with ethidium bromide, and scored against a 100 bp ladder using the Alpha Imager 2000 documentation and analysis system V 3.2 (Alpha Innotech Corp.). Each collection sampled in this study was assigned to an ITS type that summarised information from the four restriction enzyme profiles. Thus, collections with the same ITS type designation share identical restriction fragment

patterns across all four enzymes. Information including RFLP types, fruit-body morphology, and geographic location were used to identify the groups of interest in subsequent sequence and microsatellite comparisons.

DNA Sequencing - Prior to sequencing PCR products were purified using QIAquick PCR purification kit (Qiagen, Chatsworth, CA) and diluted 2-fold. Sequencing followed standard direct dye-terminator automated florescence methods. Both large subunit and ITS sequencing was performed using primers employed in PCR reactions. The length of the large subunit products permitted complementary strands to be compared for the 24 novel sequences produced in this study. Internal ITS primers (2 and 3; White *et al.* 1990) do not have high homology in *C. formosus* preventing their use as internal sequencing primers. The length of the ITS region from *Cantharellus* (1,400 – 1,600 bp) does not permit the production of overlapping sequences from opposite directions. Because complementary strands could not be obtained for either end of the ITS region, sequences were truncated at 500 bp from either direction. In our general experience with the ABI sequencing system 500 bp is within the range of maximum sequence accuracy, thus reducing the need for complementary sequences. Sequences were edited and manually aligned by eye using Sequence Navigator v. 1.0.1 (Applied Biosystems, Inc.).

Sequence Data Analysis - nrDNA large subunit and ITS sequences were aligned to minimize the number of inferred gaps and portions were re-coded from the DNA

format to the symbols format in order to use selected gaps as informative characters (*sensu* Bruns *et al.* 1992). Taxa used to root this analysis were selected based on phylogenetic relationships presented in Feibelman *et al.* (1997), Pine *et al.* (1999), and Dahlman *et al.* (2000). Phylogenetic analyses on the resulting ITS alignment were conducted with the unweighted maximum parsimony algorithm and branch and bound search option available in PAUP* v 4.0b2 (Swofford 1999). The large subunit alignment was analyzed using the stepwise random sequence addition heuristic search option. Nonparametric bootstrapping (Felsenstein 1985; Sanderson 1989) was used to assess the reliability of individual nodes in the resulting trees. For the ITS bootstrap analysis, branch and bound search options were employed with 1000 bootstrap replicates, while for the large subunit analyses heuristic search parameters included ten replicates of random addition sequences for each of the 1000 bootstrap replicates.

Microsatellite Data Analysis - After RFLP analysis, samples for microsatellite analyses were selected from forest stands on the HJA where the two RFLP types found within yellow chanterelles were sympatric with each other and with *C. subalbidus*. Sympatric as used here means that fruit bodies with alternate ITS genotypes were found growing in close proximity in the same 10 – 15 ha stand. *C. subalbidus* was used as a reference to determine how microsatellite differentiation between the two yellow types compared to the differentiation between *C. formosus* and *C. subalbidus*. Prior to tests for genetic differentiation between the sympatric ITS genotypes, each pair of loci was tested for linkage disequilibrium and each locus was

tested for departure from Hardy-Weinberg equilibrium (Weir 1990; Guo & Thompson 1992) within each ITS 'population' across all 18 stands. Allele frequency differences within and among ITS genotypes were analyzed using Fisher exact tests (Raymond & Rousset 1995) and F-statistics (Weir & Cockerham 1984). All analyses were carried out using the web-based version of GENEPOP (v. 3.1c; Raymond & Rousset 1995).

Morphology - Morphological and color data were taken from fresh collections to determine if yellow chanterelles with different ITS genotypes could be differentiated in the field. Chanterelles were spread out side by side after each day of collecting and 4 to 5 mushrooms representing the full range in color variation (hue and intensity) and fruit body shape were selected for detailed description. To avoid damaging valuable color standards with fresh material, colors of fresh fruit bodies were compared to a set of Lucite paint chips selected to represent a full range of yellow, pink, orange, red, and brown colors. These chips were subsequently keyed out under the same lighting in color standards commonly used in chanterelle species descriptions (Ridgway 1912; Munsell 1966) and given the nearest ISCC-NBS color-name equivalent (Kelly & Judd 1976). For each fruit body, we determined the dominant color of the pileus, hymenium, stipe, and immediate staining reactions in each of these locations. Prior to warm air drying voucher material a sample of context was taken for later genetic analyses. Microscopic observations of spores and tissues were made from dried material mounted in 5% KOH at 1000X magnification using the methods of Smith & Smith (1973) and Largent *et al.* (1977). RFLP data were generated after collection of

morphological data so that more subjective measurements (e.g., color determination) were not biased by knowledge of the ITS genotypes.

Results

Restriction Enzyme Analysis - PCR products amplified using ITS1-F and ITS4 were either ~1490 or ~1690 bp in length. The number of distinct RFLP profiles from the 180 collections examined (Table 3.1) differed between the four enzymes used. *HaeIII*, the most variable enzyme produced six distinct RFLP profiles followed by *HinfI* (five), *Alu* (four), and *DPNII* (three). In several profiles, particularly those generated with *HaeIII*, the lengths of individual restriction fragments (Table 3.2) sum to less than the 1490 or 1690 bp of the original ITS product. This is due to the high number of recognition sites for this enzyme in the ITS of *Cantharellus* and our inability to detect and accurately size RFLP fragments smaller than 90 bp. *HaeIII* digests typically exhibited several small fragments below this size cut off which were ignored during RFLP comparisons.

Table 3.2. Pooled restriction fragment profiles used to define ITS types assigned to 180 *Cantharellus* collections included in this study. Values identify restriction fragment profiles for each enzyme. In some cases the fragment sizes in an RFLP profile do not sum to the size of the original ITS product (1490 or 1690 bp) because the sizes of fragments below 100 bp were difficult to accurately estimate. Restriction fragment sizes (bp) for each profile are as follows: *Alu* (1) 540-490-340-200-120, (2) 490-380-250-220-150, (3) 490-350-250-220-150, (4) 740-490-340-120; *DPN II* (1) 1090-400-200, (2) 600-400-290-200, (3) 800-400-290-200; *Hinf I* (1) 970-500-220, (2) 480-430-340-240, (3) 690-500-300, (4) 670-500-320, (5) 750-500-250; *HaeIII* (1) 780-330-180-170-140-90, (2) 600-220-200-170-160-140, (3) 690-170-160-140-100, (4) 690-310-220-180, (5) 690-180-150, (6) 690-300-180.

	Restriction Fragment Profile				
RFLP type	*Alu*	*DPN II*	*Hinf I*	*HaeIII*	ITS size (bp)
A	1	1	1	1	~1690
B	2	2	2	2	~1490
C	2	2	3	3	~1490
D	2	2	3	4	~1490
E	3	2	4	5	~1490
F	4	3	5	6	~1690

Information pooled from individual restriction fragment profiles was used to characterize six unique RFLP types assigned across the 180 collections analyzed (Table 3.2). Eighty-eight yellow chanterelle collections possessed a ~1690 bp ITS region and this group was further differentiated into two RFLP types (A and F) that differed in profile for all four enzymes. The 87 fruit bodies that exhibited RFLP type A resemble both morphological and genetic descriptions given for *C. formosus* (Corner 1966; Danell 1995; Redhead *et al.* 1997) while the one collection exhibiting RFLP type F (taken from under *Quercus* in southern California) possibly represents an undescribed relative of *C. formosus.* The remaining 92 fruit bodies exhibited a total ITS length of ~1490 bp and were partitioned into four distinct RFLP types (B-E). Within this group *DPNII* RFLP profiles were monomorphic and consequently, RFLP types are based on differences found at only two or three enzymes out of the four analyzed. White chanterelles (*C. subalbidus*; Smith & Morse 1947), also differentiated by their unique color, were represented by 39 collections with a single unique RFLP type (C). European *C. cibarius* var. *cibarius* collections, characterized by a single RFLP type (E), were differentiated from 15 *C. cibarius* var. *roseocanus* (type D; Redhead *et al.* 1997) collections by three restriction enzymes. The remaining 35 yellow chanterelle collections characterized by RFLP type B represent a currently undescribed chanterelle species and have been given the Latin binomen *C. cascadensis* nom. prov. in all tables and figures based on genetic analyses described below.

Host Associations of ITS RFLP Types - Our sampling design was not specifically intended to define ectomycorrhizal host associations for chanterelles but we did detect some interesting associations between the dominant overstory and the distribution of RFLP types (species) throughout the study area that warrant future investigation. Two RFLP types (D & F) identified in the broader sampling effort were not found at the HJA possibly because they are ectomycorrhizal host specialists. *C. cibarius* var. *roseocanus* (D) collections were found only in coastal and high elevation forests containing spruce, a characteristic also mentioned by Redhead *et al.* (1997) and have recently been collected from pure stands of lodgepole pine (*Pinus contorta*; T. Horton pers. comm.) along the Oregon coast. RFLP type F (*Cantharellus* sp.) was not detected in any other forest type sampled other than oak. These forest types were not present in our sampling areas on the HJA. Similarly, *C. subalbidus* (C) and *C. cascadensis* nom. prov. (B) were collected only in forests dominated by Douglas fir despite efforts to locate these species in other forest types. Data from genet size research indicates that the occurrence of *C. cascadensis* nom. prov. may also be correlated with stand age within this forest type (Dunham *et al.* in press). *C. subalbidus*, *C. cascadensis* nom. prov., and *C. cibarius* var. *roseocanus* also have the potential to extend into in stands dominated by *Abies* and the extent to which this occurs is unknown because stands of this type were not represented in our sampling efforts. *C. formosus* (A) collections were present in forests containing Douglas fir and or spruce and probably extend into forests containing *Abies,* indicating that it may be more of a generalist than other chanterelle species.

DNA Sequence Characteristics - PCR amplification with the primers ITS4R and LR5 produced ~950 bp fragments from the 5' end of the nrDNA large subunit gene. The 24 new sequences generated by this study were relatively free of length variation but proper alignment of the 29 analyzed sequences required the introduction of gaps one to 32 nucleotides long resulting in a total of 982 recoded characters that were included in maximum parsimony analyses. With gaps included as a character state the large subunit contained 420 variable sites, of which 322 were parsimony informative and 103 of these parsimony informative sites included information from gaps. Large subunit sequences from *Cantharellus* species (ingroup) contained 27 parsimony informative sites, 10 of which included information from gaps. Levels of sequence divergence ranged from 0.0 % within species represented by a single RFLP type, up to 33.2% between ingroup and outgroup taxa. The highest pair-wise divergence values observed within *Cantharellus* were between *C. cinnabarinus* and other taxa (~ 7.0%). Divergences among other *Cantharellus* species were below 3%. Sequence variability was extremely low (<1%) between the four species that possess ITS regions of ~1490 bp mirroring the lower RFLP variability seen within this group.

Combined sequences generated from the ITS contained 431 nucleotide bp from the 5' end of the ITS 1 and 558 nucleotide residues from the 3' end of the ITS 2 including the 5.8s nrDNA gene. These sequences also include 30 nucleotides each from the flanking nrDNA small (18s) and large (28s) subunits. Due to pronounced length variation in the ITS 1, alignment of the 13 analyzed sequences required the introduction of gaps one to 74 nucleotides long resulting in a total of 989 recoded

characters available for maximum parsimony analyses. With gaps included as a character state the alignment contained 95 variable sites, of which 32 were parsimony informative and 15 of these parsimony informative sites included information from gaps. Within these partial ITS sequences, pair-wise divergence values ranged from 0.0% - 0.5% within single RFLP types, up to 8.1% between *C. formosus* and other *Cantharellus* taxa. The lowest divergence values observed between two ingroup taxa with different RFLP types were between *C. cibarius* var. *cibarius* and *C. cibarius* var. *roseocanus* (0.7 - 0.8%). Sequence divergence values for all other ingroup comparisons were at least double this (1.4 – 3.0%).

Maximum Parsimony Analyses - Unweighted maximum parsimony analysis of the nrDNA large subunit resulted in 34 equally parsimonious trees, 479 steps in length (strict consensus see Fig. 3.1). The relative amount of genetic difference (number of steps) between taxa are visually summarized in one of the equally parsimonious trees (Fig. 3.2) selected based on its congruence with the strict consensus tree. Bootstrap consensus shows strong support for splits between *C. formosus*, the oak associated chanterelle (~1690 bp ITS region), and a poorly resolved clade containing European *C. cibarius* var. *cibarius*, *C. cascadensis* nom. prov., *C. cibarius* var. *roseocanus*, and *C. subalbidus*. These splits represent on average 1.8% total sequence divergence in the large subunit between *C. formosus* collections and the oak associated chanterelle and an average of 2.3 % between these two species and the collections that fall within the larger unresolved clade (Fig. 3.2).

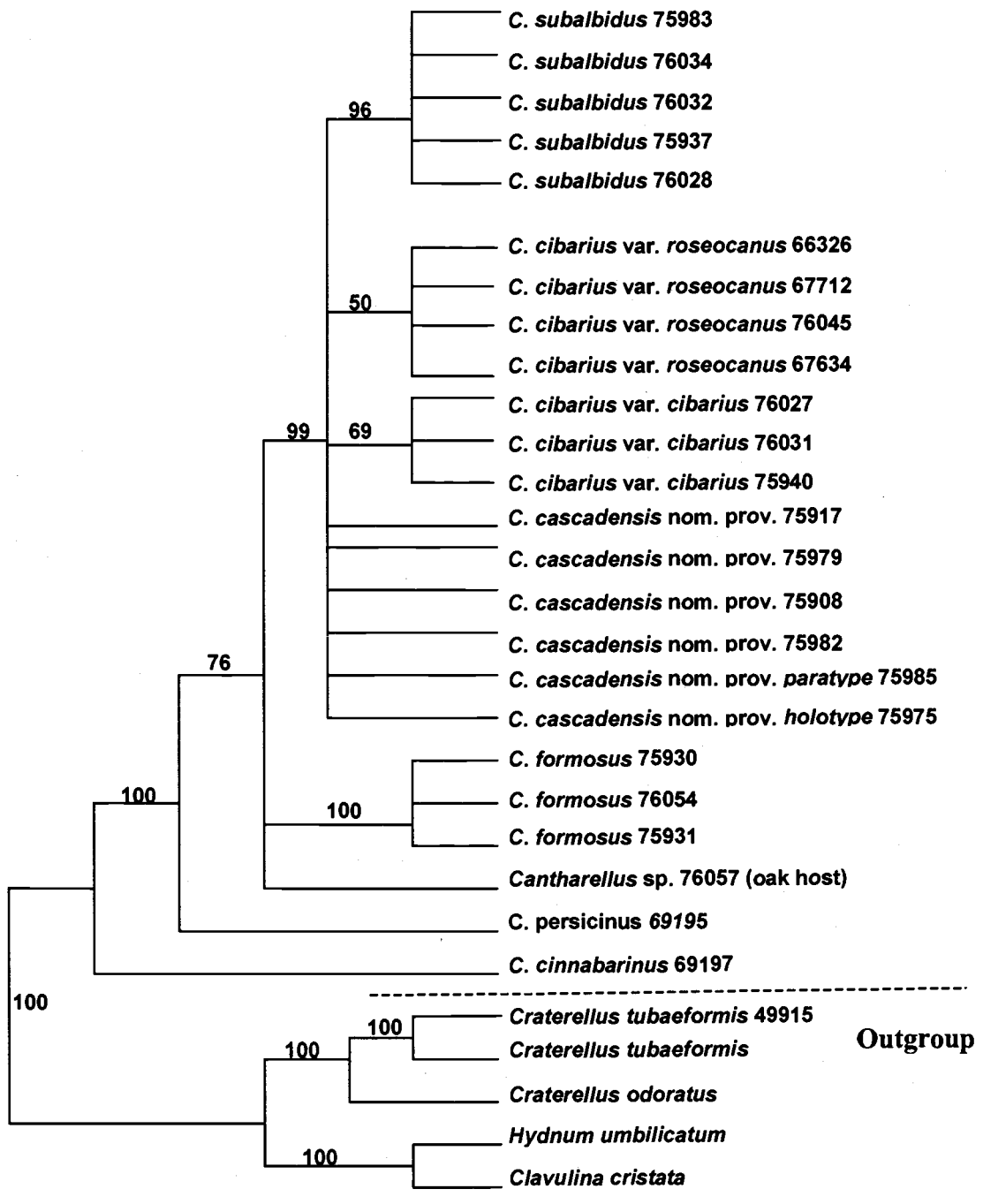


Figure 3.1. Majority rule consensus of 34 equally parsimonious trees, with consistency and homoplasy indices of 0.871 and 0.129 respectively, found using heuristic stepwise random addition analysis of the nrDNA large subunit alignment. Above each branch are heuristic bootstrap values summarized from 1000 pseudo-replicates with 10 random stepwise addition searches per replicate. Numbers after each name correspond to OSC collection numbers presented in Table 1 and letters correspond to the RFLP type determined for each collection defined in Table 2.

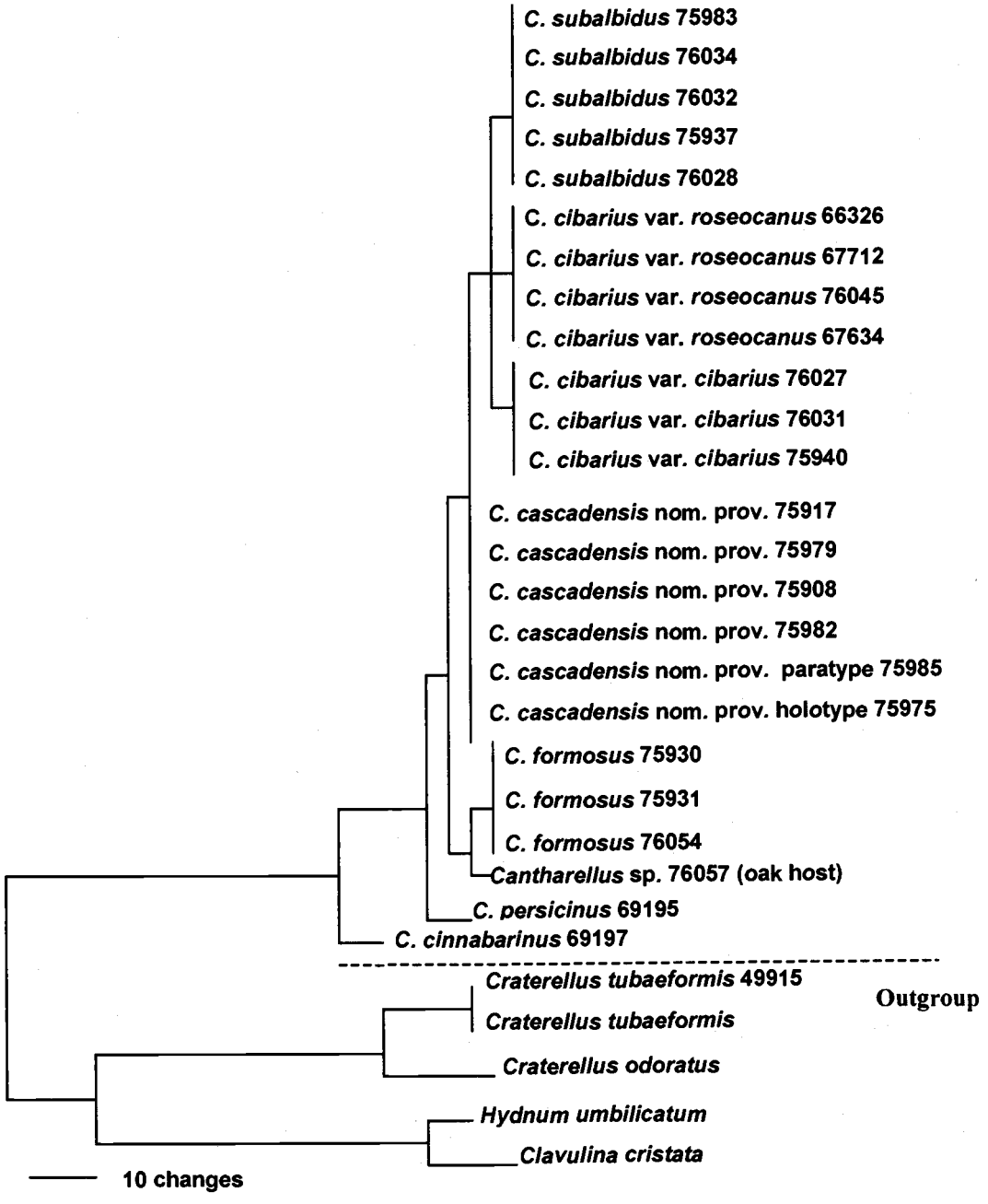


Figure 3.2. A phylogram of a single tree from the 34 equally parsimonious trees found in the heuristic analysis of the nrDNA large subunit alignment. This tree was selected based on its congruence with Figure 1 and is only intended to visually summarize the genetic distances between taxa.

The analysis of ITS sequences used *C. formosus* to root the large poorly resolved clade identified in the large subunit tree. Unweighted analysis of the ITS alignment produced 3 equally parsimonious trees for which the strict consensus (Fig. 3.3) and a single tree that visually summarizes the relative amounts of genetic differentiation (Fig. 3.4) are shown. Monophyletic groups with greater than 80 % bootstrap support are consistent with those identified by RFLP analysis. Bootstrap analysis supports a close relationship between *C. cascadensis* nom. prov. and white chanterelles (*C. subalbidus*). Monophyly of *C. cibarius* var. *roseocanus* collections is strongly supported, as is its close association with *C. cibarius* var. *cibarius* (Redhead *et al.* 1997).

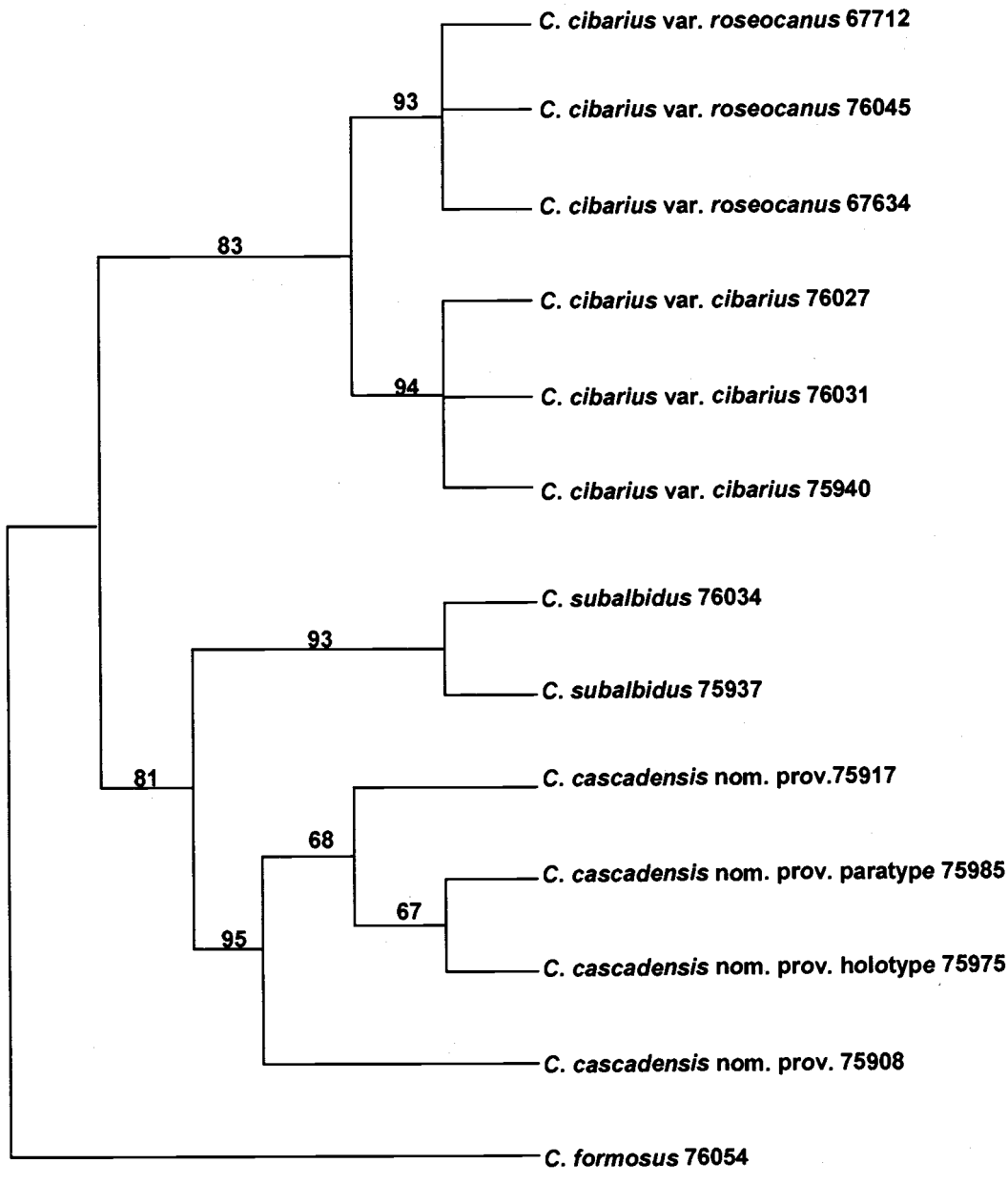


Figure 3.3. Majority rule consensus of 3 equally parsimonious trees, with consistency and homoplasy indices of 0.902 and 0.098 respectively, found using a branch and bound analysis of the nrDNA ITS alignment. Above each branch are bootstrap values summarized from 1000 branch and bound pseudo-replicates.

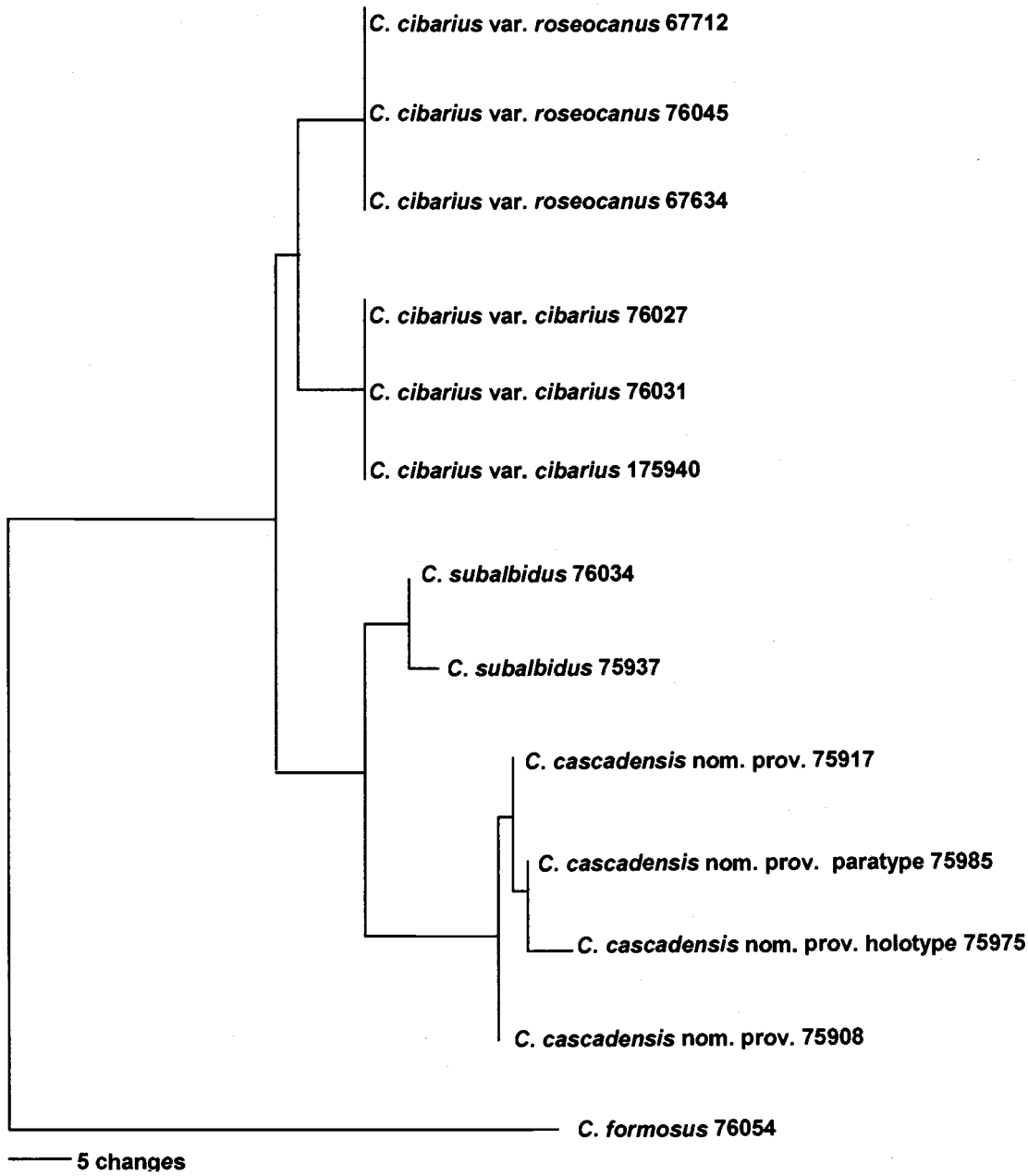


Figure 3.4. A phylogram of a single tree from the 3 equally parsimonious trees found in the branch and bound of the nrDNA ITS alignment. This tree was selected based on its congruence with Figure 3 and is only intended to visually summarize the genetic distances between taxa.

Microsatellite Analysis - Multilocus genotypes for 72 sympatric collections (Table 3.1) of *C. formosus* (A), *C. cascadensis* nom. prov. (B), and *C. subalbidus* (C) were determined using five microsatellite loci recently developed for *C. formosus*. The number of alleles within each species ranged from one to five with an average number of 2.4 alleles per locus. Expected heterozygosities ranged from 0.05 – 0.61 in *C. formosus*, 0 (for two momomorphic loci) to 0.58 in C. *cascadensis* nom. prov., and 0 – 0.67 in *C. subalbidus* which exhibited no amplification at locus Cf145 and was monomorphic at another locus. Across the five loci, each species had observed heterozygosities that matched those expected under the assumptions of Hardy-Weinberg equilibrium ($p > 0.05$) and no convincing evidence of linkage between pairs of loci was observed.

Fisher's exact tests of population independence, performed for all pair-wise species comparisons at each locus indicated that allelic distributions differ significantly among the three species ($p \leq 0.05$; Table 3.3). Fixation indices are measures of differentiation that combine information on both allele frequency and identity. Assuming an infinite allele mutation model, F_{st} values calculated for pair-wise comparisons between *C. formosus*, *C. subalbidus*, and *C. cascadensis* nom. prov. at individual loci ranged from 0.22 to 1.0 (100% genetic differentiation) and mean F_{st} values calculated across all loci ranged from 0.66 – 0.68 (Table 3.3). Sympatric sampling excludes isolation by distance as an explanation for the extreme genetic differentiation. Results from these comparisons provide strong support for the hypothesis that *C. formosus*, *C. subalbidus*, and *C. cascadensis* nom. prov. are not

interbreeding and represent biological species on independent evolutionary trajectories.

Table 3.3. Summary of allele identities and frequencies at five microsatellite loci. Identification of species associated with each allele and sample sizes used to generate summaries: *C. cascadensis* nom. prov. (n=24) / *C. formosus* (n=20) / ***C. subalbidus*** (n=28)

	Locus				
Allele	Cf642	Cf145	Cf339	Cf126	Cf113
Allele 1	*0.95*	*0.27*	*0.15*	***0.02***	*0.97*
Allele 2	*0.29*	*0.55*	*0.12 / 1.0*	***0.66***	***0.96*** */ 0.42*
Allele 3	*0.71*	*0.18*	***0.73 / 1.0***	***0.32*** */ 1.0*	*0.03 /* ***0.04*** */ 0.58*
Allele 4	*0.05*	*0.60*		*0.72*	
Allele 5	***0.46***	*0.02*		*0.28*	
Allele 6	***0.32***	*0.19*			
Allele 7	***0.11***	*0.17*			
Allele 8	***0.11***	*0.02*			

F_{st} and Fisher Exact test probabilities (Null hypothesis = random union of gametes)

C. formosus vs *C. cascadensis* nom. prov.

F_{st}	0.73	0.41	0.76	0.81	0.71	(Global = 0.68)

Table 3.3. Continued

p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
C. subalbidus vs *C. cascadensis* nom. prov.						
F_{st}	0.45	---	1.0	0.63	0.52	(Global = 0.67)
p	<0.0001	---	<0.0001	<0.0001	<0.0001	
C. subalbidus vs *C. formosus*						
F_{st}	0.59	---	0.22	0.56	0.94	(Global = 0.66)
p	<0.0001	---	<0.0001	<0.0001	<0.0001	

Table 3.4. Pileus and hymenium colors in mature basidiocarps of the *C. formosus* and *C. cascadensis* nom. prov.

Color Name			Number of Basidiocarps with Observed Color	
[a]ISSC –NBS Color/No.	[b]Munsell Renotation	[c]Ridgway Color	*C. formosus*	*C. cascadensis* nom. prov.
Dominant Pileus Colors				
Vivid Orange Yellow/66	8.6YR 7.3/15.2	Cadmium Yellow	1	19
Vivid Yellow/82	3.3Y 8.0/14.3	Light Cadmium Yellow	1	1
Light Orange Yellow/70	9.4YR 8.3/6.8	Pale Orange Yellow	1	1
Brilliant Orange Yellow/67	0.1Y 8.1/10.5	Antimony Yellow	7	2
Light Yellowish Brown/76	8.7YR 6.5/5.0	Yellow Ocher	21	0
Mod. Yellowish Brown/77	9.5YR 4.4/3.9	Buckthorn Brown	2	0
Deep Orange Yellow/69	8.6YR 6.0/12.1	Chamois	24	0
Mod. Orange Yellow/71	8.7YR 7.2/8.3	Cinnamon Buff	3	0
Dominant Hymenium Colors				
Light Orange Yellow/70	9.4YR 8.3/6.8	Pale Orange Yellow	20	5

Pale Orange Yellow/73	9.2YR 8.7/4.4	Pale Ochraceous Salmon	37	12

Table 3.4. Continued

Pale Yellow/89	4.7Y 9.0/3.8	Massicot Yellow	1	6

[a]Kelly and Judd (1976); [b]Munsell Color Co. (1966); [c]Ridgway (1912)

Morpholgical Comparisons - Future chanterelle research will benefit from knowledge of morphological characters useful in differentiating fresh collections of species described using genetic data. Past taxonomic work on PNW chanterelles has identified variability in color and morphology thought to be caused both by seasonal changes in environmental conditions and genetic differentiation (Smith & Morse 1947; Corner 1966; Thiers 1985; Redhead *et al.* 1997). The analyses presented here can be used to expedite the search for taxonomically useful morphological characters by partitioning the large range of morphological variation into genetically distinct groups. Morphological comparisons made are primarily intended to differentiate *C. formosus* from *C. cascadensis* nom. prov. because the two co-occur extensively in Douglas fir-western hemlock forests.

In a small subset of samples, microscopic characteristics were examined for taxonomic characters potentially useful in differentiating dried collections of the two species. These characters include spore shape, spore dimensions (length, width, length/width ratio), clamp connection characteristics, sterigmata shape, appearance, arrangement, and dimensions of hyphae. Only spore dimensions showed any potential for differentiating dried collections and were examined in more detail. Microscopic examination of spores from seven *C. formosus* and six *C. cascadensis* nom. prov. collections revealed little information useful in differentiating the two species. Thirty spores were examined from each collection and mean spore length and width measurements [9.53 ± 0.19 μm (7-13 μm) X 6.55 ± 0.15 μm (5-8 μm) for *C. formosus* and 9.3 ± 0.15 μm (7-13 μm) X 6.3 ± 0.23 μm (5-8 μm) for *C. cascadensis* nom.

prov.] and spore length to width ratios [1.46 ± 0.04 (1.14-2) for *C. formosus* and 1.49 ± 0.05 (1.13-2.2) for *C. cascadensis* nom. prov.] overlapped completely between the two species. In collections of both species, spores were subglobous to ellipsoid and smooth with large oil droplets, clamp connections were abundant and diverse in type, and hyphae from the context and trama appeared thin-walled and hyaline. These observations are comparable to those reported in other chanterelle species descriptions (Smith & Morse 1947; Corner 1966; Smith 1968; Petersen 1979; Redhead *et al.* 1997) indicating that molecular evolution within the genus is proceeding at a more rapid pace than changes in microscopic morphology.

Analysis of macroscopic morphology yielded a few characters more useful in separating field collections of the two species. Colors were most variable on the pileus and far less so on the hymenium, stipe, or in bruising reactions. A summary of color notes (Table 3.4) taken from 81 fresh collections of yellow chanterelles (58 *C. formosus*; 23 *C. cascadensis* nom. prov.) shows that, relatively consistent differences in fresh pileus color can be used to differentiate these two species. In general, *C. cascadensis* nom. prov. tends toward a intensely bright pure yellow while *C. formosus* shows orange-yellow to brownish-yellow hues. The center of the pileus of *C. cascadensis* nom. prov. also tends to fade to white in wet weather giving the appearance of concentric circles of yellow increasing in intensity toward the outer edge. Particularly old or water logged fruit bodies may only show yellow on the outer most edge and can resemble *C. subalbidus*. The differences in the hue and intensity of

yellow observed in pileus color are subtle and should be used in combination with other molecular and morphological characters to increase confidence in identification.

The maximum width of the pileus ranged from 2.5 – 15 cm in *C. formosus* and 4-12 cm in *C. cascadensis* nom. prov. but the distributions of these measurements were skewed in opposite directions with *C. formosus* (mean = 6.1 ± 2.45 cm) tending toward smaller pileus widths and *C. cascadensis* nom. prov. (mean = 8.6 ± 2.13 cm) tending toward larger pileus widths. A non-parametric analysis of maximum pileus width measurements shows that the means differ significantly (Mann- Whitney Z = 3.85; p=0.0001). Given that collections were made randomly under similar environmental conditions throughout the fall of 1998, it is reasonable to conclude that maximum width of the pileus is a moderately useful character in differentiating these two species. Fruit body size can vary widely from year to year in the same location so this character is likely useful only when collections are made at the same time and place.

The stipe of *C. cascadensis* nom. prov. is only occasionally equal in width throughout its length and is more often clavate to ventricose or bulbose. This contrasts with the stipe of *C. formosus* which is sometimes equal but more often tapered. All of the characters discussed show a minor degree of overlap between *C. formosus* and *C. cascadensis* nom. prov. but when taken in combination show promise in their usefulness in differentiating field collections of the two species. It should also be noted that, within the HJA, the relative sample sizes used to make macroscopic morphological comparisons reflect the relative encounter rate with each species, that is

C. cascadensis nom. prov. appears to occur in lower frequency relative to *C. formosus* in Douglas fir forests.

Taxonomy

Cantharellus cascadensis Dunham, O'Dell & R. Molina, nom. prov.

Diagnosis: A *Cantharellus formosus* Corner ob conjunctione pilei vivide armeniaci vel pallidule armeniaci, in caelo humido a disco radialiter extrinsecus albescentis vel luteolescentis, in caelo sicco rimas profundas lateribus obscure brunneoaurantiis effectescentis, et stipitis plani, clavati, ventricosi, vel basi bulbosi differt. Typus hic designatus. OSC 75975.

Basidiocarps solitary to gregarious or occasionally cespitose, 4-10 cm tall. *Pileus* 4 - 12 cm broad, planoconvex to slightly umbonate, becoming depressed to deeply depressed with age. Surface dry and finely tomentose, when developing in dry conditions, toward margin tomentum forming radial fibrils on some specimens. Surface bumpy or with occasional warts of hymenial tissue, often showing dark brownish orange discoloration along cracks with exposure to sun or very dry conditions. Most specimens are vivid orange yellow (8.6YR 7.3/15.2) to vivid yellow (3.3Y 8.0/14.3) or light orange yellow (9.4YR 8.3/6.8). Central disk occasionally fades to white or very pale yellow (5Y 9/4) when water soaked with fading progressing toward margin. *Margin* incurved at first (never inrolled) soon becoming uplifted with undulating or crisped margins. Tending to retain vivid yellow coloration during wet conditions. *Context* firm and yellowish white (2.5Y 9/2). Odor and taste

not distinctive. *Hymenium* with ridges to 2mm deep, close and narrow, long and strongly decurrent, variously forked or anastomosing. Color ranging from light orange yellow (9.4YR 8.3/6.8) to pale yellow (4.7Y 9.0/3.8). *Stipe* occasionally even but more often clavate to ventricose or bulbose at base, flaring upward and indistinct from pileus. Solid, 2-4.5 cm tall X 1-2 cm wide at apex (below hymenium), 1.5-3 cm at widest spot near base. *Staining* reactions when bruised similar in all tissues with intensity increasing as fruit body dries. Color ranges from deep orange yellow (10YR 7/10) to brownish red (10R 5/6). *Spores* white to yellowish white (2.5Y 9/2) in deposits, 9.3 ± 0.15 µm (7-13 µm) long X 6.3 ± 0.23 µm (5-8 µm) wide, ellipsoid to subglobous and smooth with variable number of oil droplets. *Basidia* 85-100 X 7-9 µm with incurved sterigmata and 4-8 spores per basidia. *Hyphae* hyaline, thin-walled, wavy to interwoven in both context and trama, variable in width, 5-12 µ in diameter, regular and diverse clamp connections at cross walls. *Habitat* in Douglas fir – western hemlock forests of variable age and elevation. *Etymology* This species was first encountered in the Mill Creek drainage along the SE boundary of the H. J. Andrews Experimental Forest. The epithet, *cascadensis*, refers to the Cascade mountains of Oregon. *Known distribution* is throughout the HJ Andrews Experimental Forest, forest stands along the eastern boundary of the HJA, along the west shore of Cougar reservoir, Willamette National Forest, Blue River ranger district, and the Powers Ranger District and Butte Falls Resource Area in southern Oregon.

Collections examined: HOLOTYPE: *United States, Oregon*. Lane County, Mill Creek, southern boundary of H. J. Andrews Experimental Forest, south of intersection of

forest roads 1501 and 2633, T 16S, R5E, section 5 south, elevation 730 m, collectors S. Dunham and T. E. O'Dell, 10 October, 1999. PARATYPE: *United States, Oregon.* Lane County, boundary of Mill Creek and Florence Creek drainage, south of forest road 700 one mile from intersection with forest road 1501, T 16S, R5E, section 9, south east, elevation 480 m, collectors S. Dunham and T. E. O'Dell, 10 October, 1999.

Taxonomic Discussion - Genetic data have proven extremely useful in delineating PNW chanterelle species, but in order for these species to function as operationally useful units in ecological studies and conservation efforts they need to be recognizable as distinct entities. Several yellow chanterelles were included in our analyses but only *C. formosus* overlaps in habitat with the previously undescribed taxon (*C. cascadensis* nom. prov.) within our study area. Despite their genetic distinctiveness, *C. formosus* and *C. cascadensis* nom. prov. are difficult to differentiate in the field because both species display a high degree of morphological plasticity caused by environmental variability. Because microscopic characters are uninformative and overlap exists in the few useful macroscopic characters, several factors (pileus size, color, shape of the stipe) must be considered simultaneously to increase confidence in identification.

Carotenoid pigments produce the yellow colors observed in many chanterelle species and the expression of color produced by carotenoids can be altered by changes in environmental factors (e.g., carbon and nitrogen sources, pH, light, and temperature) that alter the physiology of fungal individuals. Alternatively, studies on *Neurospora* have shown that single genetic mutations also can significantly alter the

expression of color produced by carotenoids (Bramley & Mackenzie 1992). Our analysis has partitioned environmental and genetic sources of color variability such that the range of color variation can be used to differentiate two very similar species. Although *C. formosus* and *C. cascadensis* nom. prov. exhibited a wide range of yellowish pileus colors most fresh collections can be separated with careful examination of the pileus color. These subtle differences in yellow hues are likely due to unique combinations of various carotenoid pigments, as has been demonstrated for other morphologically similar chanterelle species (Mui *et al.* 1998). The pattern of fading to white from the center of the pileus outward is also a characteristic of *C. cascadensis* nom. prov. not observed in collections of *C. formosus*. *C. cascadensis* nom. prov. has a general size and shape more similar to *C. subalbidus* but tends toward a more bulbous stipe. Both *C. subalbidus* and *C. cascadensis* nom. prov. develop large cracks in the pileus flanked by dark orange brown discoloration during dry weather. Discolored cracking of this nature was not observed in *C. formosus*, which also is more likely to develop warts containing hymenial tissue regardless of environmental conditions.

Discussion

Species Diversity in PNW Cantharellus - A suite of phenotypic characters that exhibit variability resulting from both evolutionary divergence and environmental plasticity confuse the morphological delineation of PNW chanterelle species. The lack of clear patterns in gross or microscopic morphological variation has led to a taxonomy that

inadequately characterizes the species diversity within *Cantharellus.* Achieving a taxonomy based on fully differentiated chanterelle species boundaries requires characters that can demonstrate the genetically based discontinuities between evolutionarily independent groups. Our molecular and morphological data suggest there are at least four distinct clades within the genus *Cantharellus* inhabiting Douglas fir and spruce forests of Oregon and possibly a fifth distinct clade inhabiting oak forests in California. We propose that these clades represent distinct evolutionary lineages that should be elevated to species status based on genetic, morphological, and ecological evidence.

The magnitudes of genetic divergence among clades supported by bootstrap analyses do not overlap with those within poorly resolved clades. In the nrDNA large subunit analysis (Figs. 3.1 & 3.2), no sequence variability is observed within clades characterized by a single RFLP type. Divergence between collections in the clade containing *C. formosus* (RFLP type A) and *C.* sp. (oak host; RFLP type F) and taxa characterized by RFLP types B-E are on average ten times higher than divergence values among collections with RFLP types B-E. This disjunction in large subunit divergence indicates that length difference in the total ITS region is a good preliminary genetic character for detecting new species in *Cantharellus.* With the exception of differences observed between *C. cibarius* var. *cibarius* and *C. cibarius* var. *roseocanus,* genetic distances in the ITS sequence analysis (Figs. 3 & 4) follows a pattern identical to the nrDNA large subunit analysis where divergences values among well supported clades are on average 10 times higher than those within clades. This

disjunction in sequence similarities indicates that ITS RFLP profiles generated using at least three restriction enzymes represent reliable characters for differentiating species in *Cantharellus* and that ITS length variability alone does not represent total species diversity. The close association and high sequence similarity between *C. cibarius* var. *cibarius* and *C. cibarius* var. *roseocanus* and lack of data from more variable markers make genetic delimitation of these two taxa as separate species more problematic. High bootstrap values generated in the ITS analysis result from seven fixed differences between these taxa and support full differentiation of *C. cibarius* var. *roseocanus* from *C. cibarius* var. *cibarius*. These fixed sequence differences provide weak support for raising *C. cibaris* var. *roseocanus* to full species status as it likely represents an early but independent evolutionary lineage derived from *C. cibarius* var. *cibarius*. A larger sample of both European and North American *C. cibarius* var. *cibarius* collections compared to *C. cibarius* var. *roseocanus* using more variable loci is required to completely understand the genetic boundaries between these taxa.

Of the five lineages defined using phylogenetic analysis of nrDNA sequences two show some evidence of restriction in ectomycorrhizal host associations. Collections of *C. cibarius* var. *roseocanus* have been found only in association with costal and high elevation spruce forests (Redhead *et al.*1997) and in pure stands of lodgepole pine (*Pinus contorta*) along the Oregon coast (T. Horton, Pers. Comm.). In costal spruce habitats (at least) *C. cibarius* var. *roseocanus* overlaps in distribution with *C. formosus* and differentiating field collections of the two requires attention to details of hymenium and pileus color as described by Readhead *et al.* (1997)

particularly under dry conditions during the early fall (Dunham personal observation). Our analysis also includes a single chanterelle collection with a unique nrDNA characteristics, found growing in association with oak but not identified in other sampled forest types. This collection (RFLP type F) may represent the variety *C. cibarius* var. *pallidifolius* described from Michigan by Smith (1968) and described by Thiers (1985) as mycorrhizally associated with tanbark oak (*Lithocarpus densiflora*) in California. Unfortunately the identity of this collection could not be confirmed using genetics because DNA extracted from available *C. cibarius* var. *pallidifolius* type material was highly degraded, preventing PCR amplification of nrDNA regions. The distribution of our sampling locations is heavily biased toward forests dominated by Douglas fir, and while our limited sampling in other forest types detected *C. cibarius* var. *roseocanus* (RFLP type D) and *C. sp* (RFLP type F), they were never observed in forests where spruce or pine (type D), or oak (type F) were absent. Three additional lineages (*C. formosus, C. subalbidus*, & *C. cascadensis* nom. prov.) have sympatric distributions in Douglas fir forests and provide an opportunity to extend our phylogenetic analysis from the identification of genetically isolated clades to the detection of interbreeding individuals and biological species boundaries.

A comparison of numerous sporocarps of *C. formosus* (RFLP type A), *C. cascadensis* nom. prov. (RFLP type B), and *C. subalbidus* (RFLP type C) revealed that most microsatellite loci show fixed allelic differences between at least two taxa (4 loci; Table 3.3) if not all three (2 loci). These qualitative differences at a large proportion of variable loci are characteristic of separate species, rather than

conspecific populations (Avise & Ball 1990). Although the frequencies of alleles may change through time, the allelic identities within populations are expected to be far more stable and fixed differences in allelic identities (replicated across unlinked loci) are indicative of a complete lack of gene flow. The diagnosis of cryptic species in difficult groups of fungi currently is moving toward the use of multiple gene genealogies where the transition from conflict to concordance among separate gene trees delimits species boundaries (Genealogical Concordance Phylogenetic Species Recognition, Taylor *et al.* 2000). When applicable, the use of microsatellite markers and sympatric sampling to test for gene flow among lineages defined by a single gene genealogy can greatly reduce the expense and effort of confidently defining species compared to generating multiple gene genealogies. Both techniques recently have been used in combination to define cryptic biological species within the pathogenic fungus *Coccidioides immitis* (Burt *et al.* 1996, 1997; Koufopanou *et al.* 1997, 2001; Fisher *et al.* 2000, 2002). Concerns with using microsatellites to define species include limits on measures of genetic distance created by constraints on allele sizes (Garza *et al.* 1995; Lehmann *et al.* 1996) and high levels of homoplasy due to high mutation rates regenerating identical alleles in genetically isolated species. Both phenomena can create the appearance of gene flow when none exists. These issues are of minor concern in our comparisons because even though we did observe identical alleles in some pair-wise comparisons of species, their frequencies differed substantially and their presence did not cloud the obvious genetic differentiation among these three taxa.

These data shed some light on the relationship between nrDNA sequence similarity and levels of gene flow. Global F_{st} values indicate that *C. cascadensis* nom. prov. is differentiated from *C. subalbidus* and *C. formosus* at similar levels even though nrDNA sequences divergence values differ drastically in these pairwise comparisons (Figs. 3.2 & 3.4, Table 3.3). *C. cascadensis* nom. prov. differs from *C. subalbidus* at only 1.5-2.2% of the 989 ITS nucleotide sites examined while it differs from *C. formosus* at 7.5-8.0% of nucleotide sites. The observed level of differentiation between *C. formosus* and the other two taxa may be limited by the constraints on microsatellite evolution discussed above. The substantial sorting of microsatellite alleles within *C. cascadensis* nom. prov. and *C. subalbidus* indicates that the 0.7-0.8% divergence observed between *C. cibarius* var. *roseocanus* and *C. cibarius* var. *cibarius* may be large enough to indicate species level differentiation. The 1.5-2.2% divergence observed between *C. cascadensis* nom. prov. and *C. subalbidus* is within the range of intraspecific ITS sequence variability reported for *Cenococcum geophilum* (0.0-4.0%; Shinohara *et al.* 1999), *Marasmius quercophilus* (1.11-1.25%; Farnet *et al.* 1999), *Galerina marginata* (0.3-2.7%; Gulden *et al.* 2001), and *Phialophora americana* (up to 2%; Yan *et al.* 1995), and similar to that observed between closely related species in *Hebeloma* (Aanen *et al.* 2000), *Dermocybe* (Liu *et al.* 1997), *Sarcodon* (Johannesson *et al.* 1999), and *Cortinarius* (Høiland & Holst-Jensen 2000). In addition, other studies have reported identical ITS sequences for morphologically distinguishable ascomycete species (Harrington & Potter 1997; Seifert *et al.* 1995) including those demonstrated to differ in ecology and isozyme variability (Harrington & McNew 1998; Harrington

et al. 1996; Witthuhn *et al.* 1998). While ITS sequences are often used to delineate relationships among fungal species within genera (e.g., Yan *et al.* 1995; Aanen *et al.* 2000; Høiland & Holst-Jensen 2000), expense often precludes these studies from including numbers of individuals adequate to characterize intraspecific sequence variability (Harrington & Rizzo 1999). These and our results demonstrate that, when working at the interface between population level variation and species boundaries, the meaning of sequence variability in these regions requires case specific interpretation.

Collections of *C. formosus, C. subalbidus*, and *C. cascadensis* nom. prov. used to compare microsatellite allele frequencies overlap completely in the time frame of collection and in their spatial distributions. We selected roughly equal numbers of each species from the random collections made in 18 separate stands at the HJA, and in many cases fruit bodies of each species were found growing within inches of each other. The maintenance of substantial genetic differentiation in the face of such close geographic proximity demonstrates that these three species have unique evolutionary histories that are not simply a result of isolation by distance. Sympatric, outcrossing species maintain species cohesion via pre or post-zytogic barriers to gene flow. If there is no gene flow between these three sympatric species then it is logical to assume that they have differing ecological adaptations (Harrington & Rizzo 1999) that either reduce hybrid fitness or inhibit sexual reproduction.

Systematics and Conservation - A solid taxonomic underpinning is also critical for conservation, because we must first know the abundance and distribution of species before we can ascertain that a species or community is in need of protection. Since most conservation efforts focus on maintaining α-level biodiversity, it is important for taxonomy to accurately reflect species diversity (Avise 1989). Accurate delineation of fungal species is required for the preservation of biodiversity and ecosystem function because many regions are rich in ectomycorrhizal diversity. PNW forest ecosystems are dependent on ectomycorrhizal symbioses, and we do not understand which taxa are most important to the maintenance of ecosystem health. Our molecular analyses represent the most rigorous estimation of species boundaries in *Cantharellus* to date and we suggest they be used to guide conservation efforts presently underway in the PNW. The presence of a new chanterelle species within the area covered by the Northwest Forest Plan (USDA & USDI 1994a) has obvious conservation implications, as this taxon currently is known only from a restricted range in and adjacent to the HJA with single collections from two sites in southern Oregon. Further investigation is now required to estimate its abundance and distribution as the species may have old growth associations (Dunham *et al.* in press). The Northwest Forest Plan is designed to allow for sustainable timber harvest while ensuring the persistence of old-growth dependent species. Species listed within the Record of Decision (USDA & USDI 1994b) are afforded official protection under survey and manage guidelines that define the plans implementation. The initial list of fungal taxa within in the Record of Decision included *C. formosus*, which has subsequently been removed, and *C.*

subalbidus, which is currently under consideration for removal. Given the potential rarity and old growth association of *C. cascadensis* nom. prov. it might qualify for protection under survey and manage guidelines outlined in the Northwest Forest Plan.

Our findings have larger implications for management of fungi under the Northwest Forest Plan. If our knowledge of *Cantharellus* species concepts is similar to those for other major genera of ectomycorrhizal fungi in the PNW, these species-based conservation efforts will likely miss many rare, cryptic species of unknown ecological importance. There currently is no way to determine if the conservation of other well described but rare taxa will protect this unknown diversity in PNW forests.

We use both phylogenetic species recognition criteria and biological species recognition criteria (Taylor *et al.* 2000) to diagnose a cryptic species within *Cantharellus* in the PNW. Molecular markers (e.g., RFLPs, RAPDs, AFLPs, SSCPs, microsatellites) have been used in other genera to distinguish fungal taxa difficult to characterize by traditional morphological means (Anderson *et al.* 1987; Bruns *et al.* 1991; Fukuda *et al.* 1994; Harrington & Wingfield 1995; Kohn 1992). The RFLP and phylogenetic analyses presented were used both to define genetically distinct lineages of chanterelles within the PNW and to identify multiple lineages within a single forest type to facilitate tests for interbreeding using microsatellites. These analyses are intended simply to differentiate species rather than attempt to determine their evolutionary relationships to other *C. cibarius* 'like' chanterelles. Our sampling was inadequate for this purpose as the relationships among the taxa analyzed may change with the addition closely related taxa from several other regions in North America,

particularly the southeast (Mui *et al.* 1998), which are proving to be very rich sources of new *Cantharellus* species. Until the species boundaries of eastern North American taxa are better clarified the construction of a comprehensive phylogeny of the genus is not practical. For example, our initial phylogenetic analyses included three eastern collections accessioned into OSC (#'s 69198, 69199, & 69258) as *C. cibarius*. The sequences from these three collections were highly divergent from each other and from sequences reported here. To avoid problems with long-branch attraction in parsimony analyses (Felsenstein 1978; Huelsenbeck 1997) these collections were excluded from our analyses and we narrowed our focus to only collections from the PNW and Europe.

Phylogenetic analyses of the nrDNA large subunit and ITS regions show that *C. cascadensis* nom. prov., along with two other yellow chanterelle taxa (*C. cibarius* var. *roseocanus* and European *C. cibarius* var. *cibarius*), are more closely related to white chanterelles (*C. subalbidus*) than they are to the most common yellow species in the PNW, *C. formosus*. Data from five microsatellite loci provide evidence that *C. formosus*, *C. subalbidus*, and *C. cascadensis* nom. prov. do not interbreed when they co-occur spatially and temporally in Douglas fir - western hemlock forests. This demonstrates that the three co -occurring species fit into biological species concepts congruent with nrDNA phylogenetic species concepts.

References

Aanen DK, Kuyper TW, Boekhout T, Hoekstra RF (2000). Phylogenetic relationships in the genus *Hebeloma* based on ITS 1 and 2 sequences, with special emphasis on the *Hebeloma crustuliniforme* complex. *Mycologia,* **92**, 269-281.

Anderson JB, Petsche DM, Smith ML (1987) Restriction fragment polymorphisms in biological species of *Armillaria mellea. Mycologia,* **79**, 69-76.

Anderson JB, Stasovski E (1992) Molecular phylogeny of northern hemisphere species of *Armillaria* species. *Mycologia,* **84**, 505-516.

Avise JC (1989) A role for molecular genetics in the recognition and conservation of endangered species. *Trends in Ecology and Evolution,* **4**, 279-281.

Avise JC, Ball Jr. RM (1990) Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Survey of Evolutionary Biology,* **7**, 45-67.

Bramley PM, Mackenzie A (1992) Carotenoid biosynthesis and its regulation in fungi. Pgs. 401-444 *in* Aroroa D, Elander R, Mukerji K (eds.) *Hanbook of applied mycology*. Vol. 4. Fungal biotechnology. Marcel Dekker, New York.

Bruns TD, White TJ, Taylor JW (1991) Fungal molecular systematics. *Annual Review of Ecology and Systematics,* **22**, 525-564.

Bruns TD, Vilgalys R, Barns SM, Gonzales D, Hibbett DS, Lane DJ, Simon L, Stickel S, Szaro TM, Weisberg WG, Sogin ML (1992) Evolutionary relationships within the fungi: Analyses of nuclear small subunit rRNA sequences. *Molecular Phylogenetics and Evolution,* **1**, 231-241.

Burt A, Carter DA, Koenig GL, White TJ, Taylor JW (1996) Molecular markers reveal cryptic sex in the human pathogen *Coccidioides immitis. Proceedings of the National Academy of Science, USA,* **93**, 770-773.

Burt A, Dechairo BM, Koenig GL, Carter DA, White TJ, Taylor JW (1997) Molecular markers reveal differentiation among isolates of *Coccidioides immitis* from California, Arizona, and Texas. *Molecular Ecology,* **6**, 781-786.

Corner EJH. (1966) *A monograph of Cantharelloid fungi*. Oxford University Press, London 255 pgs.

Dahlman M, Danell E, Spatafora JW (2000) Molecular systematics of *Craterellus*:

cladistic analysis of nuclear LSU rDNA sequence data. *Mycological Research,* **104**, 388-394.

Danell E (1995) Comparisons between Swedish *Cantharellus cibarius* and *Cantharellus* spp. in the Pacific Northwest, based on differences in RFLP patterns of the ITS region. Abstract in *Inoculum,* **46(3)**, 10.

Danell E (1999) *Cantharellus*. In Cairney JWG, Chambers SM (eds), *Ectomycorrhizal Fungi: Key Genera in Profile*. Springer-Verlag, pp 253-267.

Dunham SM, Kretzer A, Pfrender ME (In Press) Characterization of the extent and distribution of Pacific golden chanterelle (*Cantharellus formosus*) individuals using co-dominant microsatellite loci. *Molecular Ecology.*

Farnet AM, Roux M, Le Petit J (1999) Genotypic variations among isolates of *Marasmius quercophilus*, a white-rot fungus isolated from evergreen oak litter. *Canadian Journal of Botany,* **77**, 884-890.

Feibelman T, Bayman P, Cibula WG (1994) Length variation in the internal transcribed spacer of ribosomal DNA in chanterelles. *Mycological Research,* **98**, 614-618.

Feibelman TP, Doudrick RL, Cibula WG, Bennett JW (1997) Phylogenetic relationships within the Cantharellaceae inferred from sequence analysis of the nuclear large subunit rDNA. *Mycological Research,* **101**, 1423-1430.

Felsenstein J (1978) Cases in which parsimony or compatibility methods will be positively misleading. *Systematic Zoology,* **27**, 401-410.

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* **39,** 783-791.

Fisher MC, Koenig GL, White TJ, Taylor JW (2000) A test for concordance between the multilocus genealogies of genes and microsatellites in the pathogenic fungus *Coccidioides immitis. Molecular Biology and Evolution,* **17**, 1164-1174.

Fisher MC, Koenig GL, White TJ, Taylor JW (2002) Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognized as the non-California population of *Coccidioides immitis*. *Mycologia,* **94**, 73-84.

Franklin JF, Bledsoe CS, Callahan JT (1990) Contributions of the Long-Term Ecological Research Program. *Bioscience,* **40**, 509-523.

Franklin JF, Dyrness CT (1973) *Natural vegetation of Oregon and Washington.* USDA Forest Service Gen. Tec. Rep. PNW-8. Pacific Northwest Forest and Range Exp. Station, Portland, Oregon.

Fukuda M, Nakai YF, Hibbett DS, Matsumoto T, Hayashi Y (1994) Mitochondrial DNA restriction fragment length polymorphisms in natural populations of *Lentinula edodes. Mycological Research,* **98**, 169-175.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes applications to the identification of mycorrhizae and rusts. *Molecular Ecology,* **2**, 113-118.

Garza JC, Slatkin M, Freimer NB (1995) Microsatellite allele frequencies in humans and chimpanzees, with implications for constraints on allele size. *Molecular Biology and Evolution,* **12**, 594-603.

Gulden G, Dunham S, Stockman J (2001) DNA studies in the *Galerian marginata* complex. *Mycological Research,* **105**, 432-440.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics,* **48**, 361-372.

Harrington FA, Potter D (1997) Phylogenetic relationships within *Sarcoscypha* based on nucleotide sequences of the internal transcribed spacer of nuclear ribosomal DNA *Mycologia,* **89**, 258-267.

Harrington FA, McNew DL (1998) Partial interfertility among the *Ceratocystis* species on conifers. *Fungal Genetics and Biology,* **25**, 44-53.

Harrington TC, Steimel J, Wingfield MJ, Kile GA (1996) Isozyme variation and species delimitation in the *Ceratocystis coerulescens* complex. *Mycologia,* **88**, 104-113.

Harrington TC, Rizzo DM (1999) Defining species in the fungi. pages 43-71 *In*: Worrall JJ (ed.) *Structure and Dynamics of Fungal Populations*. Kluwer Press. Dordrecht, The Netherlands.

HarringtonTC, Wingfield BD (1995) A PCR-based identification method for species of *Armillaria. Mycologia,* **87**, 280-288.

Hartmann S, Nason JD, Bhattacharya D (2001) Extensive ribosomal DNA genic variation in the columnar cactus *Lophocereus. Journal of Molecular Evolution,* **53**, 124-134.

Høiland K, Holst-Jensen A (2000) *Cortinarius* phylogeny and possible taxonomic implications of ITS rDNA sequences. *Mycologia,* **92**, 694-710.

Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black box. *Molecular Ecology,* **10**, 1855-1871.

Horton TR (2002) Molecular approaches to ectomycorrhizal diversity studies: Variation in ITS at a local scale. *Plant and Soil* **244**, 29-39.

Huelsenbeck JP (1997) Is the Felsenstein zone a fly trap? *Systematic Biology,* **46**, 69-74.

Johannesson H, Ryman S, Lundmark H, Danell E (1999) *Sarcodon imbricatus* and *S. squamosus* – two confused species. *Mycological Research,* **103**, 1447-1452.

Kelly KL, Judd DB (1976) Color-universal language and dictionary of names. US Department of Commerce, National Bureau of Standards, NBS Special Publication 440. US Government Printing Office, Washington, DC.

Kohn LM (1992) Developing new characters for fungal systematics: An experimental approach for determining the rank of resolution. *Mycologia,* **84**, 139-153.

Koufopanou V, Burt A, Taylor JW (1997) Concordance of gene genealogies reveals reproductive isolation in the pathogenic fungus *Coccidioides immitis. Proceeding of the National Academy of Science, USA,* **94**, 5478-5482.

Koufopanou V, Burt A, Szaro T, Taylor JW (2001) Gene genealogies, cryptic species, and molecular evolution in the human pathogen *Coccidioides immitis* and relatives (Ascomycota, Onygenales). *Molecular Biology and Evolution,* **18**, 1246-1258.

Largent DL, Johnson D, Watling R (1977) *How to identify mushrooms to genus III. Microscopic features.* Mad River Press Inc., Eureka, California. 86 pp.

Lehmann T, Hawley WA, Collins FH (1996) An evaluation of evolutionary constraints on microsatellite loci using null alleles. *Genetics,* **144**, 1155-1163.

Liu Y, Ammirati JF, Rogers SO (1997) Phylogenetic relationships in *Dermocybe* and related *Cortinarius* taxa based on nuclear ribosomal RNA internal transcribed spacers. *Canadian Journal of Botany,* **75**, 519-532.

LoBuglio KF, Rogers SO, Wang CJK (1991) Variation in ribosomal DNA among isolates of the mycorrhizal fungus *Cenococcum geophilum. Canadian Journal of Botany,* **69**, 2331-2343.

Maniatis T, Fritsch EF, Sambrook J (1982) *Molecular cloning. A laboratory manual.* Cold Spring Harbor Laboratory, Cold Springs: Harbor, New York.

Martin FN (1990) Variation in the ribosomal DNA repeat unit within single-oospore isolates of the genus *Pythium*. *Genome,* **33**, 585-591.

Mayr E (1940) Speciation phenomena in birds. *American Naturalist,* **74**, 249-278.

Mayr E (1963) *Populations, Species, and Evolution*. Harvard University Press, Cambridge, Massachusetts.

Molina R, Pilz D, Smith J, Dunham S, Dreisbach T, O'Dell T, Castellano M (2001) Conservation and management of forest fungi in the Pacific Northwestern United States: an integrated ecosystem approach. In Moore D, Nauta MM, Evans SE, Rotheroe M *Fungal Conservation: Issues and Solutions*. Cambridge University Press.

Mui D, Feibelman T, Bennett JW (1998) A preliminary study of the carotenoids of some North American species of Cantharellus. *International Journal of Plant Science,* **159**, 244-248.

Munsell Color Company Inc. (1966) *Munsell book of color*, Munsell Color Company, Baltimore, Maryland. 22pp. with approximately 1,500 color standards.

Murrill WA (1912) The Agaricaceae of the Pacific Coast – I. *Mycologia,* **4**, 205-217.

O'Donnell K (1992) Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). *Current Genetics,* **22**, 213-220.

Petersen RH (1979) Notes on cantharelloid fungi. IX. Illustrations of newly or poorly understood taxa. *Nova Hedwigia,* **31**, 1-23.

Petersen RH, Hughes KW (1999) Species and speciation in mushrooms: Development of a species concept poses difficulties. *Bioscience,* **49**, 440-452.

Pine EM, Hibbett DS, Donoghue MJ (1999) Phylogenetic relationships of cantharelloid and clavarioid Homobasidiomycetes based on mitochondrial and nuclear rDNA sequences. *Mycologia,* **91**, 944-963.

Raymond M, Rousset F (1995) GENEPOP v. 3.1c: Population genetics software for exact tests and ecumenicism. *Journal of Heredity,* **86**, 248-249.

Redhead SA, Norvell LL, Danell E (1997) *Cantharellus formosus* and the Pacific Golden Chanterelle harvest in Western North America. *Mycotaxon,* **65**, 285-322.

Ridgway R (1912) *Colour standards and colour nomenclature.* Washington DC.

Sanderson MJ (1989) Confidence limits on phylogenies: The bootstrap revisited. *Cladistics,* **5**, 113-129.

Sanderson MJ, Doyle JJ (1992) Reconstruction of organismal and gene phylogenies from data on multigene families concerted evolution homophasy and confidence. *Systematic Biology,* **41**, 4-17.

Seifert KA, Wingfield BD, Wingfield MJ (1995) A critique of DNA sequence analysis in the taxonomy of filamentous Ascomycetes and ascomycetous anamorphs. *Canadian Journal of Botany,* **73** (Suppl. 1), 760-767.

Shinohara ML, Lobuglio KF, Rogers SO (1996) Group-I intron family in the nuclear ribosomal RNA small subunit genes of *Cenococcum geophilum* isolates. *Current Genetics,* **29**, 377-387.

Shinohara ML, Lobuglio KF, Rogers SO (1999) Comparison of ribosomal DNA ITS regions among geographic isolates of *Cenococcum geophilum. Current Genetics,* **35**, 527-535.

Simpson GG (1951) The species concept. *Evolution,* **5**, 285-298.

Simpson GG (1961) *Principles of Animal Taxonomy.* Columbia University Press, New York.

Smith AH (1968) The Cantharellaceae of Michigan. *Michigan Botanist,* **7**, 143-183.

Smith AH, Morse EE (1947) The genus *Cantharellus* in the western United States. *Mycologia,* **39**, 497-534.

Smith HV, Smith AH (1973) *How to know the non-gilled fleshy fungi.* Wm. C. Brown Co., Dubuque. 402 pp.

Swofford DL (1999) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).* Version 4. Sinauer Associates: Sunderland, Massachusetts.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology,* **31**, 21-32.

Thiers HD (1985) *The Agaricales (Gill Fungi) of California. 2. Cantharellaceae.* 34 pgs. Mad River Press, Eureka.

US Department of Agriculture, Forest Service; US Department of the Interior, Bureau of Land Management (1994a) *Final Supplemental Environmental Impact Statement on Management of Habitat for Late-successional and Old-growth Forest Related Species Within the Range of the Spotted Owl.* Vol. 1, US Department of Agriculture, Forest Service; US Department of the Interior, Bureau of Land Management: Washington, DC.

US Department of Agriculture, Forest Service; US Department of the Interior, Bureau of Land Management (1994b) *Record of Decision for Amendments to Forest Service and Bureau of Land Management Planning Documents Within the Range of the Northern Spotted Owl.* US Department of Agriculture, Forest Service; US Department of the Interior, Bureau of Land Management: Washington, DC.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA form several *Cryptococcus* species. *Journal of Bacteriology,* **172**, 4238-4246.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution,* **38**, 1358-1370.

Weir BS (1990) *Genetic data analysis.* Sinauer Publ., Sunderland, MA

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols, a Guide to Methods and Applications* (MA Innis, DH Gelfand, JJ Sninsky, TJ White, eds.), pp. 315-322. Academic Press: New York.

Wiley EO (1978) The evolutionary species concept reconsidered. *Systematic Zoology,* **27**, 17-26.

Witthuhn RC, Wingfield BD, Wingfield MJ, Wolfaard M, Harrington TC (1998) Monophyly of the conifer species in the *Ceratocystis coerulescens* complex using DNA sequence data. *Mycologia,* **90**, 96-101.

Yan ZH, Rogers SO, Wang CJK (1995) Assessment of *Phialophora* species based on ribosomal DNA internal transcribed spacers and morphology. *Mycologia,* **87**, 72-83.

WATERSHED-SCALE HABITAT ASSOCIATIONS OF THREE CHANTERELLE (CANTHARELLUS) SPECIES IN OREGON'S CENTRAL CASCADE MOUNTAINS

Susie M. Dunham

Oregon State University, Dept. of Forest Science, 3200 SW Jefferson Way, Corvallis, OR 97331, U. S. A.

Thomas E. O'Dell

Current Address: Grand Staircase-Escalante National Monument, 190 E. Center, Kanab, UT 84741.

Kelly P. Collins

Oregon State University, Dept. of Forest Science, 3200 SW Jefferson Way, Corvallis, OR 97331, U. S. A.

Randolph J. Molina

USDA Forest Service, Pacific Northwest Research Station, 3200 Jefferson Way, Corvallis, OR 97331, U.S.A

Abstract

We describe the habitat associations of three chanterelle species at the watershed scale with respect to stand age and harvesting disturbance. Samples used to characterize habitat associations were collected during the fruiting seasons of 1998 and 2000, from 18 forest stands located in and adjacent to the HJ Andrews Experimental Forest in the central Cascade Mountains of Oregon. Stands included in our analysis fell into two broad habitat categories, old growth approximately 350+ years old and 40 to 60 year old second growth stands naturally regenerated from clear-cut harvest. Each old growth stand was spatially paired with a second growth stand to reduce the chance of spurious abundance / environment relationships caused by correlations between stand age and associated habitat variables. Changes in precipitation reduced chanterelle productivity by 52 % in 2000 compared to 1998, with larger reductions occurring in old growth stands. At the watershed scale where stands of 10-20 ha are the sampling units stand age is a good predictor of the distribution of *C. subalbidus* and *C. formosus*, but is probably only marginally important for *C. cascadensis* nom. prov. The odds that a randomly located mushroom will be *C. subalbidus* compared to *C. formosus* are 2.92 to 30.83 times higher in OG than in SG. Conversely, the odds are only 3.2-34% that a randomly located mushroom will be *C. formosus* compared to *C. subalbidus* in OG. The habitat associations of *C. cascadensis* nom. prov. were difficult to model using our study design because this species is rare throughout the study area. Abundance of *C. cascadensis* nom. prov. individuals increased substantially with

decreasing elevation in both study years indicating that landscape features other than stand age may be more important in modeling its distribution.

Introduction

The mutualistic association formed between ectomycorrhizal (EM) fungi and forest trees is one of the most common forms of symbioses in western forests (Molina *et al.* 2001). The benefits received by host plant partners in the EM association are well documented and include increased nutrient uptake efficiency, drought resistance, and protection from root pathogens (Perry *et al.* 1987, 1989; Smith & Read 1997). Ectomycorrhizal fungi also create below ground linkages between plants and strongly influence community development and resiliency by mediating inter- and intra-specific competition (Read *et al.* 1985; Perry *et al.* 1987, 1989, 1992; Molina *et al.* 1992; Simard *et al.* 1997). Ectomycorrhizal host species in the western United States include all species in the commercially important Pinaceae family (*Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, and *Tsuga*), as well as the Fagaceae (*Castinopsis*, *Lithocarpus*, *Quercus*), Salicaceae (*Populus*, *Salix*), and Betulaceae (*Alnus*, *Betula*, *Corylus*). These species dominate western forests (especially Pinaceae) and are associated with thousands of EM fungal symbionts. Douglas-fir (*Pseudotsuga menziesii*) alone hosts nearly 2,000 species of EM fungi (Trappe 1977).

Ecological studies of EM fungi have identified both species with broad and narrow host ranges (Molina & Trappe 1982; Molina *et al.* 1992). For example, numerous EM fungi with narrow host ranges associate with Douglas-fir and this group differs in composition from the fungi typically associated with the trees in the genus *Pinus*. Conversely, many species of fungi form associations with both Douglas-fir and *Pinus* species and are considered to have broad host ranges. Because of the intimate

link between EM fungi and their host associates, plant community composition is an important factor for predicting EM species occurrence at regional scales. At the watershed scale, forest stand age and disturbances of various types and severity are known to influence the composition of EM communities (Molina *et al.* 1992; O'Dell *et al.* 1992; Vogt *et al.* 1992; Waters *et al.* 1997; Baar *et al.* 1999; Colgan *et al.* 1999; Taylor & Bruns 1999). For example, fire is a primary disturbance mechanism in Pacific Northwest forests (Morrison & Swanson 1990; Agee 1993) and several studies have demonstrated impacts of this perturbation on formation of the EM symbiosis and fungal productivity (Visser 1995; Horton *et al.* 1998; Jonsson *et al.* 1999). Disturbance associated with forest harvest can also affect the persistence of EM fungi (Pilz & Perry 1984; Amaranthus & Perry 1987). In Sweden, where 95% of forests are used for commercial purposes (Berg *et al.* 1994), the high proportion of threatened macrofungi in spruce forests demonstrates the negative impact of forestry management practices (Rydin *et al.* 1997). The combined effects of clear-cut harvesting of old growth forests and replacement of native forests with tree plantations have been implicated in the decline of EM fungi throughout Europe (Arnolds 2001). In western North America the impacts of forest management activities on EM fungal communities have been characterized by few studies, many of which are descriptive (Miller & Lodge 1997). Broadcast burning following clear-cut (Harvey *et al.* 1980a) and partial cuts (Harvey *et al.* 1980b; Waters *et al.* 1994) have been found to decrease the abundance of active ectomycorrhizae in the forest floor.

Habitat suitability at finer spatial scales is likely controlled by several

characteristics of the forest floor that are frequently altered by disturbance. An important forest floor component for many species of EM fungi is coarse woody debris because it serves as an important water reservoir available to fungi during periods of drought (Harvey *et al.* 1978; Amaranthus *et al.* 1989; Harmon & Sexton 1995) and is also a substrate used for growth (Harvey *et al.* 1976). Gradients in soil chemistry have been shown to effect EM community structure with nitrogen availability emerging as an important factor controlling the distribution of various EM species (Johansson 2002). Soil pH may influence the enzymatic capabilities of some EM fungi and affect their competitiveness (Leake & Read 1990) and therefore may effect their distribution at fine spatial scales. These factors likely interact to create complex patterns in soil decomposition, nutrient availability, and soil moisture retention that contribute to the high diversity of EM fungi (Dickie *et al.* 2002).

In the Pacific Northwest, forest harvest patterns over the last 50 years have significantly reduced (~17% remaining, Spies & Franklin 1988) and fragmented the volume of old growth forests. As a result, pre-settlement forests dominated by old growth (primarily Douglas-fir) have been converted to a mosaic of mature and old growth remnants in a matrix of younger, managed forest (Franklin & Forman 1987; Morrison 1988). Several ecological characteristics, important to the biology of EM fungi, that distinguish old growth (~200 – 1000 year of age) forests from young forests include high photosynthetic productivity that is largely invested in respiration and maintenance rather than new growth, and high nutrient retention. These distinctive functional characteristics are products of a complex set of structural characteristics

(diverse tree age and size, multi-layered canopies, and abundant dead wood) that also differentiate of old growth from managed forests (Franklin & Spies 1991). As a result old growth forests support a taxonomically diverse set of organisms that have specialized this long-lived habitat that historically has dominated Pacific Northwest landscapes (Lehmkuhl & Ruggiero 1991; Thomas *et al.* 1993).

EM fungi have long been neglected in forest management plans, but because of their prominent place in forest community dynamics and their beneficial symbiosis with most economically important forest trees, the value of including EM fungi such plans has recently been acknowledged. The Northwest Forest Plan (NFP) is a document that serves as a comprehensive policy statement for the management of public forests within the range of the Northern Spotted Owl (*Strix occidentalis*) which includes substantial forest areas of Oregon, Washington, and northern California. Within the NFP the Record of Decision (ROD) lists all taxa thought to require management attention in order to protect long-term population viability. Of current ROD listed taxa 200 are mycorrhizal and saprobic fungi (USDA & USDI 1994). Because detection of fungal individuals usually depends on the production of reproductive structures, we only poorly understand the specific habitat requirements of a handful of species (Dreisbach *et al.* 2002). This lack of information makes it difficult to include fungi in land management schemes that provide for persistence of habitat and therefore populations and species. Survey and manage guidelines within the ROD outline four alternative strategies for protecting viable populations of listed species. Although the objectives of these four strategies differ each requires information on the

distribution and abundance of listed species with high priority placed on site-specific habitat information.

Chanterelles are popular edible EM fungi commercially and recreationally harvested from wild populations in the western United States (Danell 1999). Timber harvests have been reduced on Federal lands over the last decade and in response the demand on nontimber forest products has dramatically increased. As a result chanterelles (primarily *Cantharellus formosus*, Redhead *et al.* 1997) have become one of the most important edible mushrooms harvested from PNW forests (Pilz & Molina 2002). Because they are both economically and ecologically important in the Pacific Northwest region species in the genus *Cantharellus* are of particular interest to forestland managers. In this study we characterize the relative abundances and habitat associations of the Pacific Golden Chanterelle (*Cantharellus formosus*), the ROD listed White Chanterelle (*C. subalbidus*), and a recently described species (*C. cascadensis* nom. prov.) in old growth and young second growth forests.

Methods

Study Areas - The HJ Andrews Experimental Forest (HJA) has been administered as a long-term ecological research site on the Willamette National Forest since 1948 (Franklin *et al.* 1990). The HJA (lat. 44°15′N, long. 122°10′W) is a 6,400 ha watershed that contains Lookout Creek, a tributary to the McKenzie River. Elevations within the HJA range from 420 to 1630 m and the area is typical of the central portion of the western Cascade Mountains in Oregon (Franklin *et al.* 1990). About 90% of the

annual precipitation falls from October to April with the wettest period in December and peak drought conditions occurring in July. The primary overstory species are Douglas-fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), and Pacific silver fir (*Abies amabilis*) and are in the western hemlock and Pacific silver fir zones of Franklin & Dyrness (1973).

During the fall of 1997, we conducted genetic studies to determine the size of chanterelle individuals and to assess if variation in individual size is related to forest disturbance. The species under study were not evenly distributed across the sampled disturbance treatments with *C. cascadensis* nom. prov. occurring primarily on old growth (OG) plots and *C. formosus* dominating second growth (SG) plots (Dunham *et al.* in press). The primary goal of this research was to expand sampling of OG and SG stands to the watershed scale to characterize the relative abundances and habitat associations of *C. formosus*, *C. subalbidus*, and *C. cascadensis* nom. prov. Within the HJA, stands containing trees at least 350 years old that have not experienced recent (100 years or less) fire events and stands that had been clear cut during 1950-1960 were identified and scouted prior to the fall 1998 chanterelle fruiting season. Seven OG and seven SG stands were selected based on their accessibility and spatial arrangement. To reduce the chance of spurious abundance / environment relationships caused by correlations between stand age and associated habitat variables (slope, aspect, elevation, etc.) each OG stand was spatially paired with a nearby SG stand. Two additional pairs of OG and SG stands were selected in the Mill Creek area along the southeast boundary of the HJA in order to increase the total number of replicate

stands. The post cutting history of stands in the Mill Creek area is not well documented but the seral zone (Franklin & Dyrness 1973) and habitat associations (Franklin 1979) are similar to the HJA. A total of nine groups of paired stands (18 stands total) were selected with two pairs in the western end of the HJA, three pairs in the central eastern end of the HJA, two pairs in the northeast corner of the HJA, and two pairs in the Mill Creek area (Figure 4.1).

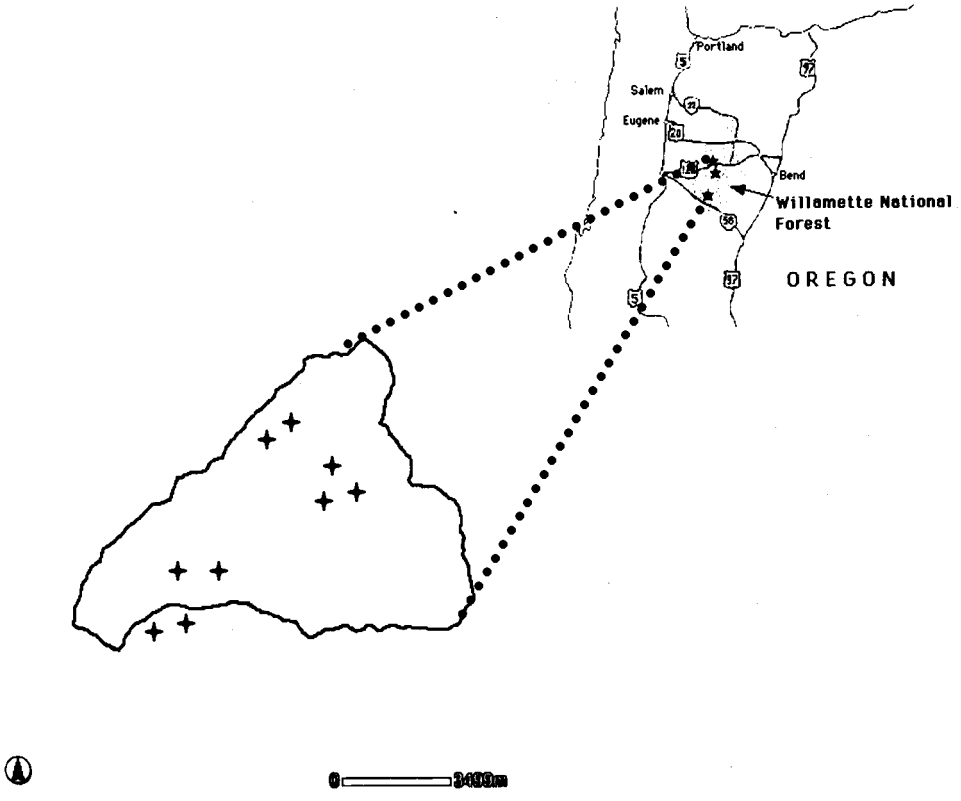


Figure 4.1. Study site locations. Each star marks the location of a set of spatially paired OG and SG stands

Stands selected for this study were in the ranged from 420 to 1100 m in elevation, had north, south, southwest, or southeast aspects, and ranged from 20-35% in average slope. All stands fall within the western hemlock community type patterns of Franklin (1979) and areas known to contain Pacific silver fir were avoided during sampling to minimize confounding effects of additional EM host species. Within stands, understory vascular plants include *Polystichum munitum/Oxalis oregana* in wet sites, *Berberis nervosa/Rhododendron macrophyllum* in mesic sites, *Berberis nervosa/Gautheria shallon* in drier sites, and *Linnaea borealis* in cooler, drier sites.

Field Sampling Methods - Collecting began two weeks after the first substantial fall rain (October) of 1998 and 2000 and ended after the first winter snows covered the area (December). Two collectors conducted searches in each stand for 1.5 hours once in October and once in November and data from these two time periods were pooled for analysis within each year. Random sampling was initiated in each stand by walking to a tree that could be seen from a road but for which the ground at the base was not visible. Random compass bearings were used to sample a continuous series of 150 – 200 m transects. The width of each transect included the entire visual field of the collector and changed depending on the understory characteristics in the immediate area. Second growth stands ranged from 10 to 20 ha in total area and the area searched within larger old growth stands roughly approximated this range. When chanterelles were located, a minimum of five meters (based individual size estimates, Dunham *et al.* in press) was kept between any two collections of the same species to

avoid re-sampling individuals. Chanterelle locations were marked to avoid re-sampling patches encountered by the two collectors at different times within each year. Collections taken in 2000 represent a temporal replicate of the area sample collected in 1998. Genetic samples and vouchers were taken from each fruit-body collected and the species identification confirmed using molecular genetic techniques.

DNA Extraction and Amplification - After grinding a small amount of fresh or dried tissue in 1 ml of lysis buffer (100 mM Tris, 10mM EDTA, 2% sodium dodecyl sulfate, 1mg/ml proteinase-K, pH 8.0), each sample was incubated for 1.5 hours at 55 °C and DNA was extracted using a standard phenol/chloroform emulsification and precipitation (Maniatis et al. 1982). Resulting pellets were vacuum-dried and re-suspended in 50 to 100 µl of sterile Tris-EDTA. One microliter of the unquantified DNA extracts were used in 40ul polymerase chain reaction (PCR) amplifications of the Internal Transcribed Spacer (ITS) region. The ITS region, spanning the 3' end of the 18S, ITS-1, 5.8S, ITS-2, and 5' end of the 28S was amplified with the fungal specific primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). Final concentrations of PCR mix components were 0.2 mM of each dNTP, 0.2 µM of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, and 0.75 units of DNA polymerase. Reactions were subject to 35 PCR cycles with the following protocol: initial denaturation at 94 °C for 3 min, cycling denaturation at 94 °C for 50 s primer annealing at 55 °C for 50 s, and primer extension at 72 °C for 1.5 min. Samples were subjected to a final extension of 10 min following the 35th cycle.

Restriction Fragment Length Polymorphisms (RFLP's) - According to the manufacturer's recommendations (GIBCO BRL, Grand Island, NY), unpurified PCR products amplified with ITS1-F and ITS4 were digested separately with two restriction enzymes (*Hinf*I and *Hae*III) known to differentiate the three species of interest. Restriction fragments were electrophoresed through 3% agarose gels (2% Nu-Sieve agarose, 1% SeaKem LE agarose; FMC BioProducts), stained with ethidium bromide, and scored against a 100 base-pair molecular weight standard using the Alpha Imager 2000 documentation and analysis system V 3.2 (Alpha Innotech Corp.). Each collection sampled in this study was assigned to an ITS type that summarized information from the two restriction enzyme profiles. Collections with the same ITS type designation share identical restriction fragment patterns across both enzymes and, based on previous experience with ITS variability in *Cantharellus*, are considered to represent a single species.

Statistical Analysis - Because sampling efforts in 1998 and 2000 likely included fruit bodies from the same fungal individuals, analyses of habitat associations were conducted separately for each year. Given that the response variable in this analysis (*Cantharellus* species) is categorical with three possible outcomes maximum likelihood multinomial regression was used to test if the odds of finding the three species of interest are equal in the OG and SG treatments. The goal of this analysis is to determine if, relative to two other species, the odds of occurrence for each species is equal in OG and SG.

The following general model assumes a multinomial probability distribution for the response variable:

$$\text{Log}\,(p_{ij} / p_{iJ}) = \beta_j x_i$$

Where p_{ij} is the probability that habitat category (i) contains species (j) and (j) = 1,..., J-1 (Allison 1999). Multinomial logit models are built from the set of binary logit equations possible for different levels of the response variable and can be estimated using a binary logit procedure. The GENMOD procedure available in SAS v.8 (SAS Institute Inc., Allison 1999) was used to estimate intercept and slope parameters for three binary logit models with each equation corresponding to a comparison between two species. For example, we used a single binary analysis that included *C. subalbidus* and *C. formosus* as alternate response levels to model the relative odds of randomly encountering each species in OG habitat. For each binary model observations that fall into the third category (species) for the response variable were excluded. This technique is slightly less efficient than analysis with a single model statement and inflates standard errors resulting in more conservative significance tests (Allison 1999). Drop-in deviance F-tests were used to test the significance of regression slopes that were used to calculate the relative odds of the three chanterelle species occurring in OG and SG stands. Additional categorical predictive variables included in initial regression models were time (2 levels; 1998 & 2000), to test whether the overall patterns of species occurrence differed between 1998 and 2000 and location of paired OG / SG stands (9 levels), to test if model predictions were equal across the study area.

Results

Distribution and Abundance of Chanterelle Species - A total of 596 and 308 chanterelle fruit-bodies were collected across the 18 OG / SG stands during the fall fruiting seasons of 1998 and 2000 respectively (Table 4.1). In each year RFLP analysis detected only the three genotypes expected from the three species of interest, *C. formosus, C. subalbidus,* and *C. cascadensis* nom. prov. The total number of fruit bodies detected in 2000 was 52% of the number collected in 1998 but the reduction in fruiting was not uniform across the three species or the two habitats sampled. The reduction in fruiting abundance in 2000 was most evident for *C. subalbidus* with the number of fruit bodies collected declining by 69% compared to 1998. For this species, reductions in abundance were most prevalent in OG with all nine stands showing at least a 2-fold reduction in abundance in 2000 compared to only four SG stands showing reductions of similar magnitude. In contrast, *C. formosus* numbers in 2000 were only 31% lower that those in 1998 with five OG and four SG stands showing 2-fold or greater abundance reductions. *C. cascadensis* nom. prov. was intermediate between the two with a 48% reduction in 2000 collections compared to 1998. Similar to *C. subalbidus* all 2-fold or greater reductions in 2000 fruiting abundance occurred in OG stands. Despite these overall reductions in fruiting abundance, there was no significant effect of time (1998 vs. 2000) or the time / age interaction in any of the binary logit models.

Table 4.1. Abundance (fruit-body number) of the three study species pooled across 18 study stands by year and stand age.

Age	Species					
	C. cascadensis nom. prov.		*C. formosus*		*C. subalbidus* n = 248	
	1998 (n=67)	2000 (n=35)	1998 (n=281)	2000 (n=196)	1998 (n=248)	2000 (n=77)
OG	33	10	68	24	190	43
SG	34	25	213	172	58	34

In both years *C. formosus* and *C. subalbidus* collections were evenly dispersed across the entire study area with *C. formosus* consistently frequent in eight of nine SG stands and infrequent or not detected in all OG stands. The reverse was true for *C. subalbidus,* which was consistently frequent in eight of nine OG stands and infrequent or not detected in all SG stands. Stand pair effects had no significant influence in the binary logit model that included *C. formosus* and *C. subalbidus* as the response. In other words, the location of paired OG / SG stands did not affect the response (species). The total number of *C. cascadensis* nom. prov. collections was much lower than those made for *C. formosus* and *C. subalbidus* in both years (Table 4.1) and the abundance of collections made across the 18 stands was also more variable from 1998 to 2000. The distribution of *C. cascadensis* nom. prov. collections was uneven across the study area and its occurrence within stands appeared particularly patchy. In the logit models that included *C. formosus* and *C. cascadensis* nom. prov. as the response, the p-values associated with stand effects were low (0.1; 0.06) but not significant in 1998 or 2000 respectively. *C. cascadensis* nom. prov. was not detected in four SG and two OG stands in 1998 and four SG and five OG stands in 2000. In five of these stands (three SG; two OG) *C. cascadensis* nom. prov. was not detected in either year.

Twenty-six of 34 (1998) and 17 of 25 (2000) *C. cascadensis* nom. prov. SG collections (Table 4.1) were taken from a single SG stand in the Mill Creek area, and this stand also has the lowest elevation (426 m) of all the stands sampled. An OG stand, also in the Mill Creek area, with the second lowest elevation (490 m) had the second highest abundance of *C. cascadensis* nom. prov. with 12 collections in 1998

and six in 2000. All other stands sampled are 600 m or higher in elevation and contained eight or fewer *C. cascadensis* nom. prov. collections in 1998 (four or fewer in 2000; Figure 4.2). Elevation appeared also to be associated with *C. cascadensis* nom. prov. distribution, but since elevation was not a factor of interest when this study was designed it was not sampled well enough to include it in the multinomial regression analysis as an explanatory variable. To better understand the distribution of this species in stands above 600 m and to examine the influence of stands below 600 m on this analysis multinomial analyses were performed both with and without stands with mean elevations below 600 m.

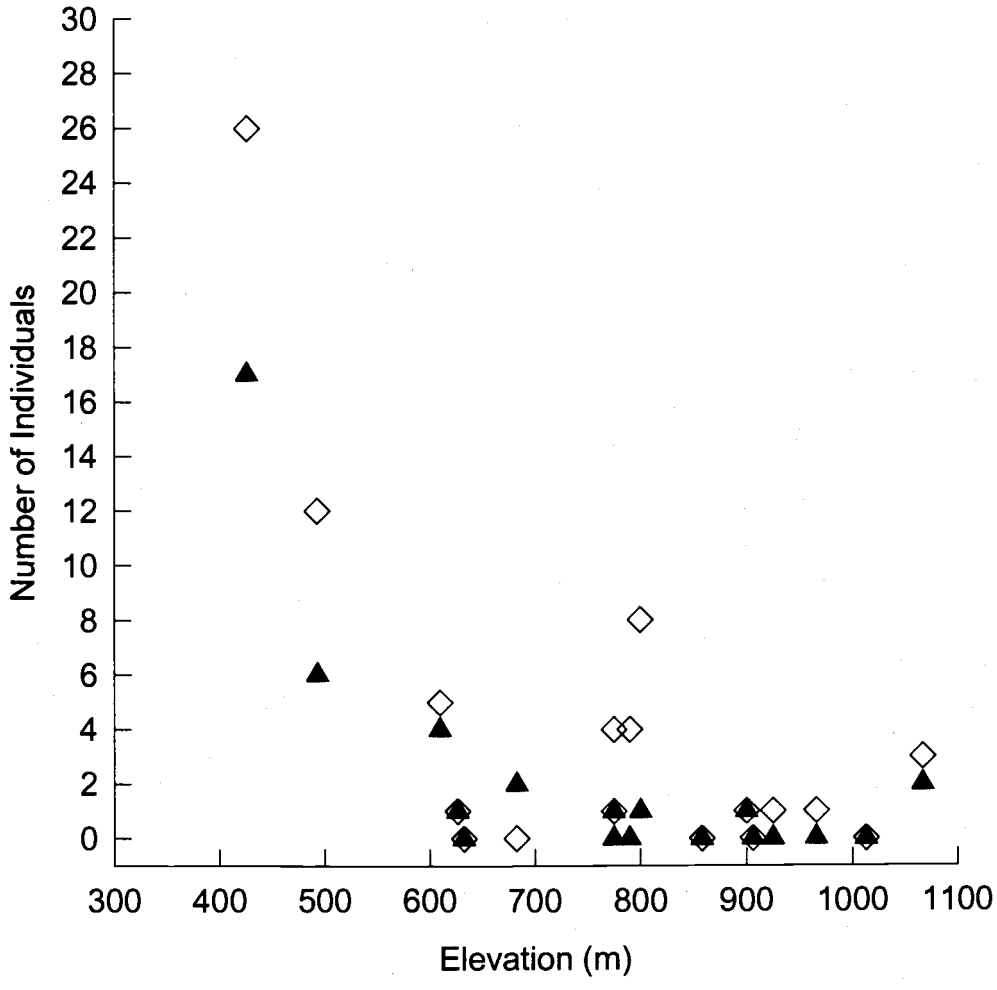


Figure 4.2. Abundance of *C. cascadensis* nom. prov. plotted against the mean elevation of the stand where collections were made. Open diamonds represent 1998 collections and closed triangles represent 2000 collections

Habitat Associations - Intercept and slope parameters were estimated for both an analysis including all stands and an analysis excluding stands below 600 m (Tables 4.2 and 4.3 respectively). When all the observations are considered, *C. subalbidus* was more likely to occur in OG stands relative to (Table 4.2) in both 1998 and 2000. Conversely, *C. formosus* was more likely to occur in SG relative to *C. subalbidus*. The odds that a randomly located mushroom will be *C. subalbidus* compared to *C. formosus* are 2.92 to 30.83 (95% CI, P=0.002; 2000 comparison) times higher in OG than in SG and this odd ratio interval narrows slightly in the 1998 comparison due to the increased sample size.

When stands with mean elevations below 600 m are deleted from the analysis the strong association between *C. subalbidus* and OG habitats remains statistically significant with only a minor shift in the 95% confidence interval for the odds ratio (Table 4.3). In this comparison the contrast between *C. cascadensis* nom. prov. and *C. formosus* reveals a significant OG association for *C. cascadensis* nom. prov. in stands above 600 m where the odds that a randomly located mushroom will be *C. cascadensis* nom. prov. relative to *C. formosus* is 1.95 to 49.9 (95% CI, P=0.01) times higher in OG. This association is only significant in 1998 due to reduced statistical power in the 2000 comparison caused by reductions in fruiting which disproportionately affected old growth stands.

Table 4.2. Multinomial parameter estimates for the relationship between the response variable (species type) and the predictive variable (stand age) using three binary logit models to estimate the multinomial response for all replicate stands sampled. Degrees of freedom change for some comparisons when neither species involved occurred in a stand replicate.

	C. cascadensis vs. *C. formosus*		*C. cascadensis* vs. *C. subalbidus*		*C. subalbidus* vs. *C. formosus*	
Estimate	1998	2000	1998	2000	1998	2000
Intercept	-1.84	-1.93	-0.53	-0.31	-1.30	-1.62
Standard Error	0.60	0.37	0.66	0.64	0.38	0.35
95% CI**	-3.22, -0.79	-2.73, -1.26	-1.96, 0.74	-1.65, 0.95	-2.09, -0.61	-2.38, -0.96
Stand Age	1.11	1.05	-1.22	-1.15	2.33	2.20
Standard Error	0.91	0.75	0.88	1.07	0.52	0.60
95% CI	-0.73, 2.97	-0.53, 2.49	-3.01, 0.53	-3.54, 0.85	1.35, 3.40	1.07, 3.43
P-value*	$F_{1,16}$=1.46	$F_{1,15}$=1.80	$F_{1,15}$=1.90	$F_{1,14}$=1.24	$F_{1,16}$=23.68	$F_{1,15}$=14.92
	p=0.24	p=0.20	p=0.19	p=0.28	p=0.0002	p=0.0015
Odds Ratio	3.04	2.87	0.30	0.32	10.26	9.06
95% CI	0.48, 19.45	0.59, 12.07	0.05, 1.70	0.03, 2.35	3.86, 29.90	2.92, 30.83

*From drop in deviance liklihood ratio tests, **Maximum liklihood ratio estimates.

Table 4.3. Multinomial parameter estimates for the relationship between the response variable (species type) and the predictive variable (stand age) using three binary logit models to estimate the multinomial response. Observations from stands below 600 m in elevation (2 stands) have been deleted from this analysis. Degrees of freedom change for some comparisons when neither species involved occurred in a stand replicate.

	C. cascadensis vs. *C. formosus*		*C. cascadensis* vs. *C. subalbidus*		*C. subalbidus* vs. *C. formosus*	
Estimate	1998	2000	1998	2000	1998	2000
Intercept	-3.26	-2.76	-1.98	-1.39	-1.28	-1.37
Standard Error	0.65	0.45	0.78	0.64	0.39	0.35
95% CI**	-4.86, -2.19	-3.76, -1.98	-3.92, -0.67	-2.86, -0.24	-2.12, -0.56	-2.11, -0.73
Stand Age	2.13	1.05	-0.12	-0.99	2.25	2.04
Standard Error	0.79	0.80	0.92	1.07	0.55	0.58
95% CI	0.67, 3.91	-0.69, 2.57	-1.81, 1.98	-3.44, 1.04	1.21, 3.39	0.94, 3.23
P-value*	$F_{1,14}$=8.28	$F_{1,13}$=1.54	$F_{1,15}$=0.02	$F_{1,12}$=0.91	$F_{1,14}$=19.30	$F_{1,13}$=13.71
	p=0.01	p=0.24	p=0.90	p=0.36	p=0.0006	p=0.0027
Odds Ratio	8.44	2.86	0.89	0.37	9.48	7.70
95% CI	1.95, 49.97	0.05, 13.12	0.16, 7.25	0.03, 2.83	3.35, 29.62	2.57, 25.23

Discussion

Long-term viability is dependent on both the size and geographic distributions of populations. For EM fungi, these population characteristics are dependent on the status of small and large-scale landscape features. Here we describe the habitat associations of three chanterelle species with respect to stand age and harvesting disturbance. Results from this study are potentially of use in management plans giving the logistic model certain advantages relative to other types of analysis appropriate for count data. The odds ratio and its associated confidence intervals are of particular interest because they allow for definition of a biologically meaningful response. At the watershed scale where stands of 10-20 ha are the sampling units, stand age is a good predictor of the distribution of *C. subalbidus* and *C. formosus*, but is probably only marginally important for *C. cascadensis* nom. prov. Other processes or patterns not captured by the grain of this study may explain the distribution of *C. cascadensis* nom. prov. better or its distribution on the HJA may not be stable. Our sampling scale was too coarse to tease out the biological reasons why these relationships exist but clearly stand age and possibly elevation are correlated with the spatial arrangement of finer scale habitat variables important to chanterelle species. A logical extension of this work would be to study the fine scale soil and host tree characteristics that control the distribution of each species within stands. The overall response of these three chanterelle species to factors such as moisture fluctuations may be more important but are also more difficult to characterize at fine spatial scales as they may depend on the severity and scale of the fluctuations.

Yearly Variation in Chanterelle Abundance - For fungi, biological productivity frequently is defined as the total number of mushrooms produced per unit area over the course of a fruiting season. Moisture and temperature are often cited as the primary factors controlling fungal biomass production (Lodge *et al.* 1994). Soil moisture has been identified as an important variable controlling the diversity and distribution of EM species across stands within the same dominant host species that are variable in age (Luoma *et al.* 1991) and distributed across a moisture gradient (O'Dell *et al.* 1999). Several studies have demonstrated that high annual variability in chanterelle productivity is correlated with weather (Vogt *et al.* 1992; Liegel 1998). Overall fruit body abundance reductions in 2000 may be due to reduced rainfall and lower temperatures in November of 2000 truncating the fruiting season in OG stands. Oregon climate service records show that monthly rainfall totals in the HJA area were two thirds lower in November of 2000 than in 1998 (116 mm vs. 360 mm respectively) and that the mean monthly temperature was 5°C in 1998 compared to 1.5°C in 2000. Without stand level climate data it is difficult to tell if reductions in OG fruiting were truly correlated with differences in moisture, temperature, or an interaction between the two factors.

Habitat Associations of PNW Chanterelle Species - We examined chanterelle habitat associations at the watershed scale for to two extremes in stand age. The SG stands selected are the youngest where abundant chanterelle fruiting can be expected and the OG stands are some of the oldest and least disturbed sites on the HJA. PNW forest

ecosystems are complex and the treatment categories utilized here (OG / SG) encompass multiple ecological characteristics. While we have defined the effect of stand age on chanterelle distributions, the mechanisms (abiotic, biotic, or both) that control chanterelle establishment and persistence in these habitats have not been uncovered. We can speculate on potential causal factors but more research is needed to characterize species distributions in the broad range of intermediate age classes. Now we discuss the potential causes for the patterns observed for each species.

C. subalbidus – Our results demonstrate that the probability of locating *C. subalbidus* fruit bodies increases significantly in OG habitats. On the HJA, OG surrounds all sampled SG stands creating a habitat mosaic that would maximize the chances of *C. subalbidus* colonizing SG stands as they matured. The reduced odds of finding *C. subalbidus* in SG indicate that biological, physical or chemical characteristics of the soil or competition from other newly establishing species are limiting its distribution in young stands. Primeval OG forests exhibit an undisturbed development of vegetation and soil extending over centuries. Characteristic features include the abundance of coarse woody debris in all stages of decay, uneven aged trees, gaps and uneven soil surface caused by large uprooted trees (Spies *et al.* 1988; Spies & Franklin 1989, 1991). Recently the importance of coarse woody debris in predicting the occurrence of threatened macrofungi in late successional conifer forests in Europe has been demonstrated (Berg *et al.* 1994; Rydin *et al.* 1997). Wide confidence intervals for the odds ratio characterizing the association between *C. subalbidus* and OG may result from the retention of coarse woody debris in SG stands

allowing for patchy development of preharvest soil conditions required by this species or from the existence of microclimatic edge effects along OG / SG boarders creating *C. subalbidus* habitat within SG stands. More detailed examination of fine scale habitat variables may produce better predictive models.

The Pacific Northwest (PNW) region provides a large share of the world timber supply (Waddell *et al.* 1989). Clear cut harvesting followed by subsequent even-aged forest management has been ongoing on private lands for well over a century (Robbins 1988) and on federal lands since the 1940's (Harris 1984). During the past few decades, rotation lengths on public lands have generally been in the range of 80-100 years while private landowners generally cut on rotation lengths of 50 years (Spies *et al.* 1994). This intense forest harvest activity has resulted in profound alterations in the age structure of the forest environment since European settlement (Wallin *et al.* 1996). Recent estimates of the percentage of old growth forests > 200 years old remaining from pre-logging time periods range from 17.5% (Haynes 1986) to 13.1% (Spies & Franklin 1988; Booth 1991). Depending on the minimum stand age at which the probability of locating *C. subalbidus* increases as OG characteristics necessary for its establishment and fruiting develop, the conversion of PNW forests from OG to SG may have significantly reduced the distribution of *C. subalbidus* in the western Cascades. This assertion depends on how representative the HJA is of the western Cascades as a whole. The HJA is representative of the winter climate of the northern Cascades and of the Pacific Northwest in general (Greenland 1994) and has been included in an enormous amount of basic and applied research used in the

development of forest management plans for the region (Cissel *et al.* 1999). If these results can be extended to other areas, *C. subalbidus* has likely been largely excluded from private lands with short cutting rotations and possibly also from public lands if 80-100 year rotation cycles are not long enough for it to establish and maintain viable populations. Future research on this species should be directed towards identifying the suite of factors that limit its distribution and determining if optimal conditions for its growth develop as SG stands continue to mature beyond 50 years.

C. formosus – A recent study of niche differentiation of EM fungi demonstrated that there is a wide range of substrate utilization patterns among different EM species (Dickie *et al.* 2002). Binomial logistic regression analyses presented here indicate that, relative to *C. subalbidus*, *C. formosus* is strongly associated with 40-60 year old SG habitats. Large scale harvesting disturbance that has taken place throughout the Pacific Northwest may have increased dispersal capabilities of *C. formosus* giving it an establishment advantage over *C. subalbidus*. Given the proximity of our sampled SG stands to OG (a rare occurrence in the Pacific Northwest), it is more likely that *C. formosus* is better adapted to soils altered by disturbance or physiological differences associated with younger host trees. Buried coarse woody has been identified as a factor important to the ecology of *C. formosus* (Norvell *et al.* 1996; Largent & Sime 1994) but in most productive soils this is not a limiting resource. Alternatively, quantitative work completed by Bergemann & Largent (2000) demonstrated that above ground coarse woody debris is not a significant predictor of *C. formosus* occurrence. Instead, the percent cover of bare

humus unoccupied by moss, moderate duff depth, and low exchangeable acidity, were found to be useful variables for predicting *C. formosus* fruiting (Bergemann & Largent 2000).

Disturbances associated with forest harvest usually result in a loss of soil spatial heterogeneity (Miller & Lodge 1997) that can alter many soil characteristics important to EM fungi (Harvey *et al.* 1980a, 1980b; Pilz & Perry 1984; Amaranthus & Perry 1987). Following such a major disturbance, stress tolerance can allow some fungal species to replace others that are less tolerant (Lodge & Cantrell 1995). Fungi that are favored by disturbance generally exhibit effective dispersal, rapid nutrient uptake, and rapid extension for resource capture (Pugh & Boddy 1988). The age at which stands regenerating from clear-cut harvest are colonized by *C. formosus* is unclear because the fungus may establish the EM symbiosis with young trees early on but delay fruiting until the stand is somewhat recovered from the initial disturbance. Future research on this species should be directed at exploring conditions favorable to both host tree colonization and the onset of fruiting to determine if there is a significant time lag between the two events.

C. cascadensis nom. prov. – Uneven abundances and the patchy distribution of *C. cascadensis* nom. prov. across the study area make delineation of habitat associations difficult given our study design. We found that, in 1998, *C. cascadensis* nom. prov. was significantly associated with OG habitats above 600 m in elevation. The overall reduction in OG fruiting for 2000 reduced the statistical power of comparisons above 600 m but the general fruiting patterns were similar. While, the

association of *C. cascadensis* nom. prov. with OG habitats over 600 m in elevation is statistically significant in 1998, the over all implications of this result for the species are likely of marginal importance. The spatial pairing of stands has allowed us to detect a confounding variable for *C. cascadensis* nom. prov. habitat associations. It is clear that environmental variables correlated with stand age have some control over the distribution of *C. cascadensis* nom. prov. but this relationship is not constant over the range of elevation we sampled. In addition, factors that control the abundance of fungal individuals present in a stand are unclear.

These conflicting results are left with no satisfactory explanation and while distribution is important abundance is of more value in conservation efforts aimed at protecting viable populations. This study clearly did an inadequate job of sampling the range of habitats in which this species occurs. The size of *C. cascadensis* nom. prov. populations may increase drastically at elevations below 600 m and its association with OG characteristics potentially becomes insignificant below 600 m. Conservation issues are slightly different for this species than for *C. subalbidus* because low elevation habitats are threatened both by development and forestry practices. A recent study of forest cover changes demonstrated that timber harvest activities are more frequent on private forests than on public lands (Alig *et al.* 2000). These conclusions are tentative, however, because only one OG and one SG stand in this elevation range was sampled.

It was clear from this sampling that *C. cascadensis* nom. prov. is rare in the study area relative to other chanterelles and much survey work still is required to fully

understand its distribution in Oregon. This analysis indicates that future survey work should focus on lower elevation habitats that will require the cooperation of private landowners. In light of these findings it may be appropriate to include *C. cascadensis* nom. prov.in the ROD until its distribution is better understood.

References

Agee JK (1993) Fire ecology of Pacific Northwest forests. Island Press, Washington, D.C.

Alig RJ, Zheng D, Spies TA, Butler BJ (2000) *Forest cover dynamics in the Pacific Northwest west side: Regional trends and projections.* USDA Forest Service Research Paper PNW-RP-522.

Allison PD (1999*) Logistic Regression using the SAS System: Theory and Application.* SAS Institute Inc., Cary, NC, USA.

Amaranthus MP, Perry DA (1987) Effect of soil transfer on ectomycorrhiza formation and survival and growth of conifer seedlings in disturbed forest sites. *Canadian Journal of Forest Research,* **17**, 944-950.

Amaranthus MP, Parrish DS, Perry DA (1989) Decaying logs as moisture reservoirs after drought and wildfire. In *Stewardship of Soil, Air and Water Resources, Proceedings of Watershed 89* (ed. E Alexander), pp. 191-194. USDA Forest Service. R10-MB-77. USDA Forest Service, Alaska Region: Juneau, AK.

Arnolds E (2001) The future of fungi in Europe: threats, conservation and management. In Moore D, Nauta MM, Evans SE, Rotheroe M (eds.) *Fungal Conservation: Issues and Solutions.* Cambridge University Press.

Baar J, Horton TR, Kretzer AM, Bruns TD (1999) Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. *New Phytologist,* **143**, 409-418.

Berg Å, Ehnström B, Gustafsson L, Hallingbäck T, Jonsell M, Weslien J (1994) Threatened plant, animal, and fungus species in Swedish forests: Distributions and habitat associations. *Conservation Biology,* **8,** 718-731.

Bergemann SE, Largent DL (2000) The site specific variables that correlate with the

distribution of the Pacific Golden Chanterelle, *Cantharellus formosus*. *Forest Ecology and Managemen,t* 130, 99-107.

Booth DE (1991) Estimating prelogging old-growth in the Pacific Northwest. *Journal of Forestry*, **89**, 25-29.

Cissel JH, Swanson FJ, Weisberg PJ (1999) Landscape management using historical fire regimes: Blue River, Oregon. *Ecological Applications,* 9, 1217-1231.

Colgan W, Carey AB, Trappe JM, Molina R, Thysell D (1999) Diversity and productivity of hypogeous fungal sporocarps in a variably thinned Douglar-fir forest. *Canadian Journal of Forest Research,* **29**, 1259-1268.

Danell E (1999) Cantharellus. In Cairney JWG, Chambers SM (eds), *Ectomycorrhizal Fungi: Key Genera in Profile.* Springer-Verlag, pp 253-267.

Dickie IA, Xu B, Koide RT (2002) Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytologist,* **156**, 527-535.

Dreisbach TA, Smith JE, Molina R (2002) Challenges of modeling fungal habitat: when and where do you find chanterelles? In *Predicting Species Occurrence: Issues of Scale and Accuracy* (eds. Scott JM, Heglund PJ, Morrision M, Raphael M, Haufler J, Wall B). Island Press, Covello, CA.

Dunham SM, Kretzer A, Pfrender ME (In Press) Characterization of the extent and distribution of Pacific golden chanterelle (*Cantharellus formosus*) individuals using co-dominant microsatellite loci. *Molecular Ecology.*

Franklin JF (1979) Vegetation of the Douglas-fir region. Pp. 93-112. In: *Forest soils of the Douglas-fir region.* Eds., PE Heilman, HW Anderson, DM Baumgartner. Washington State University, Cooperative Extension, Pullman.

Franklin JF, Bledsoe CS, Callahan JT (1990) Contributions of the Long-Term Ecological Research Program. *Bioscience*, **40**, 509-523.

Franklin JF, Dyrness CT (1973) *Natural vegetation of Oregon and Washington.* USDA Forest Service Gen. Tec. Rep. PNW-8. Pacific Northwest Forest and Range Exp. Station., Portland, Oregon.

Franklin JF; Forman RTT (1987) Creating landscape patterns by forest cutting: ecological consequences and principles. *Landscape Ecology*, **1**, 518.

Franklin JF, Spies TA (1991) *Composition, function, and structure of old-growth*

Douglas-fir forests. In Wildlife and Vegetation of Unmanaged Douglas-Fir Forests. (eds. Ruggiero LF, Aubry KB, AB, Huff MH) USDA Forest Service General Technical Report PNW-GTR-285. 474pp.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for Basidiomycetes: Applications to the identification of mycorrhizae and rusts. *Molecular Ecology,* **2**, 113-118.

Greenland D (1994) The Pacific Northwest regional context of the climate of the HJ Andrews Experimental Forest. *Northwest Science*, **69**, 81-96.

Harmon ME, Sexton J (1995) Water balance of conifer logs in early stages of decompositon. *Plant and Soil,* **172**, 141-152.

Harris LD (1984) *The Fragmented Forest.* University of Chicago Press, Chicago, IL.

Harvey AE, Larsen MJ, Jurgensen MF (1976) Distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. *Forest Science,* **22**, 393-398.

Harvey AE, Jurgensen MF, Larsen MJ (1978) Seasonal distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. *Forest Science,* 24, 203-208.

Harvey AE, Jurgensen MF, Larsen MJ (1980a) Clearcut harvesting and ectomycorrhizae: survival of activity on residual roots and influence on bordering forests stance in western Montana. *Canadian Journal of Forest Research,* **10**, 300-303.

Harvey AE, Larsen MJ, Jurgensen MF (1980b) Early effects of partial cutting on the number and distribution of active ectomycorrhizae in steep, rocky soils of Douglas-fir/larch forests in western Montana. *Canadian Journal of Forest Research,* **10**, 436-440.

Haynes RW (1986) *Inventory and value of old-growth in the Douglas-fir region.* USDA Forest Service Research Note PNW-437, 18p.

Horton TR, Cazares E, Bruns TD (1998) Ectomycorrhizal vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first five months of growth after wildfire. *Mycorrhiza*, 8, 11-18.

Johansson JF (2002) Belowground ectomycorrhizal community structure along a local nutrient gradient in a boreal forest in Northern Sweden. MS Thesis, Swedish University of Agricultural Sciences, Uppsala.

Jonsson L, Dahlberg A, Nilsson M-C, Zackrisson O, Kårén O (1999) Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. *Molecular Ecology*, **8**, 205-215.

Largent DL, Sime AD (1994) A preliminary report on the phenology, sporulation, and lifespan of *Cantharellus cibarius* and *Boletus edulis* basidiomes at Patrick's Point State Park. In: Adams DH, Rios JE, Stoere AJ (eds.) Proc. Symp. Of 43rd Annual Meeting of the California Forest Pest Council, November 16-17 1994. Ranco Cordova, CA pp. Appendix xxxii-xliv.

Leake JR, Read DJ (1990) Proteinase activity in mycorrhizal fungi. I. The effect of extracellular pH on the production and activity of proteinase by ericoid endophytes from soils of contrasting pH. *New Phytologist,* **155**, 243-250.

Lehmkuhl JF, Ruggiero LF (1991) Forest fragmentation in the Pacific Northwest and its potential effects on wildlife. In Wildlife and Vegetation of Unmanaged Douglas-Fir Forests. (eds. Ruggiero LF, Aubry KB, AB, Huff MH) USDA Forest Service General Technical Report PNW-GTR-285. 474pp.

Liegel L, Ed. (1998) *The biological, socio-economic, and managerial aspects of chanterelle mushroom harvesting: the Olympic Peninsula, Washington State, USA.* AMBIO Special Report No. 9, 35 pp.

Lodge DJ, Cantrell S (1995) Fungal communities in wet tropical forests: variation in time and space. *Canadian Journal of Botany,* 73 (Suppl), S1391-S1398.

Lodge DJ, McDowell WH, McSwiney CP (1994) The importance of nutrient pulses in tropical forests. *Trends in Ecology and Evolution,* **9**, 384-387.

Luoma DL, Frenkel R, Trappe JM (1991) Fruiting of hypogeous fungi in Oregon Douglas-fir forests: seasonal and habitat variation. *Mycologia*, **83**, 335-353.

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Springs: Harbor, New York.

Miller RM, Lodge DJ (1997) Fungal Responses to Disturbance: Agriculture and Forestry. In *The Mycota IV: Environmental and Microbial Relationships* (Eds. Wicklow DT, Söderström B) Springer-Verlag, Berlin.

Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In. Allen M (ed.) *Mycorrhizal functioning, and integrative plant-fungal process.* New York, Chapman and Hall. Pp. 357-423.

Molina R, Pilz D, Smith J, Dunham S, Dreisbach T, O'Dell T, Castellano M (2001) Conservation and management of forest fungi in the Pacific Northwestern United States: an integrated ecosystem approach. In Moore D, Nauta MM, Evans SE, Rotheroe M *Fungal Conservation: Issues and Solutions*. Cambridge University Press.

Molina R, Trappe JM (1982) Patterns of ectomycorrhizal host specificity and potential among Pacific Northwest conifers and fungi. *Forest Science,* **28**, 423-458.

Morrison PM (1988) Old growth in the Pacific Northwest: a status report. Washington, DC: *The Wilderness Society*, 46 p.

Morrison P, Swanson FJ (1990) *Fire history and pattern in a Cascade Range landscape.* U.S. Forest Service General Technical Report PNW-GTR-254. Pacific Northwest Research Station, Portland Oregon, USA.

Norvell L, Roger J, Lindgren J, Kopecky F (1996) Oregon Cantharellus Study Project 1986-96. In: Pilz D, Molina R, eds. *Managing Forest Ecosystems to Conserve Fungus Diversity and Sustain Wild Mushroom Harvests*. General Technical Report PNW-GTR-371. US Department of Agriculture, Forest Service. Pacific Northwest Research Station, Portland, OR, pp. 69-72.

O'Dell TE, Luoma DL, Molina RJ (1992) Ectomycorrhizal fungal communities in young, managed, and old-growth Douglas-fir stands. *Northwest Environmental Journal*, **8**, 166-168.

O'Dell TE. Ammirati JF, Schreiner EG (1999) Species richness and abundance of ectomycorrhizal basidiomycete sporocarps on a moisture gradient in the *Tsuga heterophylla* zone. *Canadian Journal of Botany,* **77**, 1699-1711.

Perry DA, Molina R, Amaranthus MP (1987) Mycorrhizae, mycorrhizospheres, and reforestation: current knowledge and research needs. *Canadian Journal of Forest Research*, **17**, 929-940.

Perry DA, Amaranthus MP, Borchers JG, Borchers SL, Brainerd RE (1989) Bootstrapping in ecosystems. *Bioscience*, **39**, 230-237.

Perry DA, Margolis H, Choquette C, Molina R, Trappe JM (1992) Ectomycorrhizal mediation of competition between coniferous tree species. *New Phytologist,* **112**, 501-511.

Pilz D, Molina R (2002) Commercial harvests of edible mushrooms from the forests

of the Pacific Northwest United States: issues, management, and monitoring for sustainability. *Forest Ecology and Management,* **155**, 3-16.

Pilz D, Perry DA (1984) Impact of clearcutting and slash burning on ectomycorrhizal associations of Douglas-fir seedlings. *Canadian Journal of Forest Research,* **14**, 94-100.

Pugh GJF, Boddy L (1988) A view of disturbance and life strategies in fungi. *Proceedings of the Royal Society Edinburgh*, **94B**, 3-11.

Read DJ, Francis R, Finlay RD (1985) Mycorrhizal mycelia and nutrient cycling in plant communities. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds.). *Ecological interactions in soils*. Oxford, Blackwell Scientific Publications. pp. 193-218.

Redhead SA, Norvell LL, Danell E (1997) *Cantharellus formosus* and the Pacific Golden Chanterelle harvest in Western North America. *Mycotaxon*, **65**, 285-322.

Robbins WG (1988) Hard times in paradise, Coos Bay, Oregon 1850-1986. University of Washington Press, Seattle, WA.

Rydin H, Martin D, Hallingbäck T (1997) Biological characteristics, habitat associations, and distribution of macrofungi in Sweden. *Conservation Biology,* **11**, 628-640.

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R (1997) Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature*, **388**, 579-582.

Smith SE, Read DJ (1997) *Mycorrhizal symbiosis*, 2nd ed. Academic Press.

Spies TA, Franklin JF (1988) Old-growth and forest dynamics in the Douglas-fir region of western Oregon and Washington. *Natural Areas Journal,* **8**, 190-201.

Spies TA, Franklin JF (1989) Gap characteristics and vegetation response in coniferous forests of the Pacific Northwest. *Ecology*, **70**, 543-545.

Spies TA, Franklin JF (1991) The structure of natural young, mature, and old-growth Douglas-fir forests in Oregon and Washington. In *Wildlife and vegetation of unmanaged Douglas-fir forests*. Technical Coordinators, Ruggiero LF, Aubry KB, Carey AB, Huff MH USDA Forest Service General Technical Report PNW-GTR-285. pp 91-109.

Spies TA, Franklin JF, Thomas TB (1988) Coarse woody debris in Douglas-fir forests

of western Oregon and Washington. *Ecology*, **69**, 1689-1702.

Spies TA, Ripple WJ, Bradshaw GA (1994) Dynamics and pattern of a managed coniferous forest landscape. *Ecological Applications,* 4, 555-568.

Taylor DL, Bruns TD (1999) Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. *Molecular Ecology,* **8**, 1837-1850.

Thomas JW, Raphael MG, Anthony RG, Forsman ED, Gunderson AG, Holthausen RS, Marcot BG, Reeves GH, Sedell JR, Solis DM (1993) *Viability assessments and management considerations for species associated with late-successional and old-growth forests of the Pacific Northwest*. Portland, Oregon. USDA Forest Service. 523 p.

Trappe J (1977) Selection of fungi for ectomycorrhizal inoculation in nurseries. *Annual Review of Phytopathology,* **15**, 203-222.

U.S. Department of Agriculture, Forest Service; U.S. Department of the Interior, Bureau of Land Management. 1994. Record of decision for amendments to Forest Service and Bureau of Land Management planning documents within the range of the northern spotted owl. (place of publication unknown).

Visser S (1995) Ectomycorrhizal fungal succession in jack pine stands following wildfire. *New Phytologist,* **129**, 389-401.

Vogt KA, Bloomfield J, Ammirati JF, Ammirati SR (1992) Sporocarp production by Basidiomycetes, with emphasis on forest ecosystems. In Carroll GC, Wicklow DT (eds.) *The Fungal Community: Its Organization and Role in the Ecosystem*, 2nd Edition. Marcel Dekker, New York, NY, pp. 563-581.

Waddell KL, Oswald DD, Powell DS (1989) *Forest statistics of the United States, 1987.* Pacific Northwest Forest and Range Experiment Station, U.S. Department of Agriculture Forest Service, Resource Bulletin PNW-RB-168.

Wallin DO, Swanson FJ, Marks B, Cissel JH, Kertis J (1996) Comparison of managed and pre-settlement landscape dynamics in forests of the Pacific Northwest, USA. *Forest Ecology and Management,* **85**, 291-309.

Waters JR, McKelvey KS, Luoma DL, Zabel CJ (1997) Truffle production in old-growth and mature fir stands in northeastern California. *Forest Ecology and Management,* **96**, 155-166.

Waters JR, McKelvey KS, Zabel CJ, Oliver WW (1994) The effects of thinning and

broadcast burning on sporocarp production of hypogeous fungi. *Canadian Journal of Forest Research*, **24**, 1516-1522.

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: A Guide to methods and Applications* (ed. N Innis, D Gelfand, J Sninsky, T White). New York Academic Press, New York, NY, USA.

SPATIAL AUTOCORRELATION ANALYSIS OF WITHIN POPULATION GENETIC STRUCTURE IN GOLDEN CHANTERELLES (*CANTHARELLUS FORMOSUS*)

Susie M. Dunham

Oregon State University, Dept. of Forest Science, 3200 SW Jefferson Way, Corvallis, OR 97331, U. S. A.

Thomas E. O'Dell

Current Address: Grand Staircase-Escalante National Monument, 190 E. Center, Kanab, UT 84741.

Randolph J. Molina

USDA Forest Service, Pacific Northwest Research Station, 3200 Jefferson Way, Corvallis, OR 97331, U.S.A

Abstract

In order to balance resource extraction with protection of Pacific Northwest forest ecosystems we must understand the population biology of key species like ectomycorrhizal fungi. Cryptic growth habits make fungal individuals difficult to observe and life history information critical for management efforts is lacking. Knowledge of within-population genetic structure is a prerequisite for planning larger scale studies upon which management decisions are based. Using spatial autocorrelation analysis we examined the within-population genetic structure of the Pacific golden chanterelle (*Cantharellus formosus*) in 50 year old Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*). The main hypothesis tested is that basidiospore dispersal and subsequent genet establishment probabilities decline as a function of geographic distance in *C. formosus*. Fruit-bodies from 183 *C. formosus* genets were mapped in and collected from a 50 ha study plot. Sixty-five unique genotypes resulted from scoring these collections at four microsatellite loci. Statistically significant but weak spatial autocorrelation was detected in the two smallest distance classes estimating a 200-250 radius for genetically homogeneous patches for *C. formosus*. This result indicates that limited spore dispersal possibly coupled with inbreeding works to maintain fine scale genetic structure in this species. The implications of this research for future broad-scale population genetic studies of *C. formosus* are that collections should be separated by at least 250 m to be considered independent. Sampling designs that account for fine-scale genetic structure will better characterize the genetic heterogeneity distributed across

the landscape by avoiding pseudoreplication of genetic variability.

Introduction

The association between ectomycorrhizal (EM) fungi and most forest trees is a common form of mutualistic symbiosis known to play a fundamental role in the dynamic functioning of many Pacific Northwest ecosystems (Molina *et al.* 2001). The EM symbiosis improves host plant growth by increasing nutrient uptake efficiency and drought stress resistance (Smith & Read 1997). Because the mycelia of EM fungi are ubiquitous in forest soils, they also create below ground linkages between plants and strongly influence community development and resiliency by mediating inter- and intra-specific competition (Read *et al.* 1985; Molina *et al.* 1992; Simard *et al.* 1997). In addition to their ecological importance, EM fungi also are of great economic importance because many commercially harvested tree species are dependent on the benefits derived from this symbiotic relationship.

Concern for the population viability of many EM fungi has drawn attention to the need for integrating them into Pacific Northwest forest management plans (Molina *et al.* 2001). This task requires a basic understanding of population biology, including knowledge of how genetic variability within and among populations is structured across landscapes. The patterns revealed by landscape-scale population genetic studies yield important clues about the evolutionary significance of past and present ecological and demographic events (Avise 1994), particularly for species not easily observed during most of their life cycle. In addition, broad-scale patterns in genetic variability are widely used to prioritize intra-specific units for conservation efforts (Moritz 1994; Avise & Hamrick 1996; Newton *et al.* 1999; Crandall *et al.* 2000).

While much research has been conducted on gene flow and broad-scale population structure of fungal pathogens (McDonald 1997), studies characterizing population-level variation in EM fungi have primarily focused on characterizing genet size for various basidiomycete species. Collectively, these studies demonstrate significant differences in age structure, clonality, and genet recruitment patterns among the many EM species examined (Dahlberg 2001; Guidot *et al.* 2002). These studies have yielded important insights into the relative roles of basidiospore dispersal and vegetative growth in the life histories of EM fungi. Several have shown that genet establishment from basidiospores occurs frequently (De La Bastide *et al.* 1994; Gryta *et al.* 1997, 2000; Anderson *et al.* 1998; Selosse *et al.* 1998; Gherbi *et al.* 1999; Redecker *et al.* 2001; Guidot *et al.* 2002) indicating that this mode of genet establishment may be an important for the maintenance of genetic diversity in EM fungal populations. Building on this knowledge by expanding research efforts to estimate long-distance dispersal ability of EM fungi will greatly increase our ability to make informed management decisions.

Theoretically, long-distance dispersal of fungal spores is supported by the observation that fungi are ubiquitous and many species are cosmopolitan in distribution. Empirical evidence for the long-distance capabilities of fungi come from atmospheric sampling studies that have detected large numbers of spores in the air, even upper strata of the atmosphere (Ingold 1971). In contrast, evidence for short-distance dispersal (< 100 m) of fungal spores has come from direct measurements of spore settlement. These studies have characterized steep gradients of reduced spore

deposition with increasing distance, demonstrating that most spores are deposited in the vicinity of fruit-bodies (Wolfenbarger 1946; Gregory 1973; Lacey 1996; Morkkynen *et al.* 1997). Results from these fine-scale spore settlement studies are complemented by recent estimates of landscape-scale spore dispersal distances in the saprotrophic basidiomycete *Schizophyllum commune*. Using traps baited with homokaryon cultures James & Vilgalys (2001) determined that trapped spores most likely originated from local populations and that long-distance spore dispersal was rare despite the observation that spores of *S. commune* were common in the air.

In reality, spore dispersal rates and distances only represent the potential for dispersal. Actual genet establishment rates also depend on basidospore germination, growth of monokaryotic mycelia, fusion between compatible monokaryotic mycelia to form dikaryotic mycelia, and successful colonization of host plant roots. Germination rates of EM basidiospores are thought to be very low, usually <1% (Bonello *et al.* 1998, Chamber & Cairney 1999), thus the successful fusion between monokaryotic hyphae and colonization of host root systems may be rare (Dahlberg & Stenlid 1995) relative to the large number of spores typically generated by fruit-bodies of EM species. A few recent studies have examined the genetic structure of EM fungal populations at watershed and landscape scales in order to indirectly estimate gene flow, but have produced conflicting or inconclusive results. Zhou *et al.* (2001) described clustering of simple sequence repeat (SSR) genotypes at fine spatial scales and proposed that the patterns observed may have resulted from inbreeding caused by deposition of *Suillus grevillei* basidiospores within relatively short distances from

sporocarps. Given these non-random fine-scale genetic patterns one would expect genetic structure among *S. grevillei* populations sampled at larger spatial scales. While both study populations (~ 700 m apart) did contain unique alleles, Zhou *et al.* (2001) were not able to detect significant allele frequency differences between their study sites. Alternatively, recent population genetic research on the EM fungus *Cenococcum geophilum* demonstrated both high levels of genetic diversity within and genetic structure among populations sampled along a 250 km transect, but these results were confounded by ecological differences among study sites leaving limited gene flow as only one possible explanation for the patterns observed (Jany *et al.* 2002).

If genet establishment and allele spread via long-distance dispersal are relatively rare in EM fungal populations, the probability of random mating events will decrease as the distance between individuals increases (isolation-by-distance, Wright 1969) possibly resulting in increased mating among related individuals. Theoretical and empirical studies have shown that when such inbreeding is continued over generations the cumulative effect of genetic isolation-by-distance results in striking patterns of genetic structure within populations (Epperson *et al.* 1999; Ueno *et al.* 2000). Given the evidence for limited spore dispersal, it is reasonable to expect that fine-scale genetic structure may exist within EM fungal populations. At landscape scales, patterns of genetic isolation frequently are estimated using the variance in allele frequencies within and among sampling units (Wright 1951; Slatkin & Barton 1989). Precision of these statistics requires that sampling units do not encompass multiple random breeding units (genetic neighborhoods) (Magnussen 1993;

Ruckelshaus 1998). Wright (1969) showed analytically that as more neighborhoods are included within sampling units, the genetic variance among sampling units relative to the total variance declines, and the power to detect spatial genetic structure is lost. For conservation and management purposes, data from studies with this type of sampling error can lead to misguided decisions based on poor estimates of dispersal.

This logistical problem is important to take into consideration in the study of EM fungal populations because fruit-body production (and organism detection) only occurs during specific times of the year and can be variable from year-to-year. As a result, the distributions most species are not well documented (Dreisbach *et al.* 2002). Fortunately these sampling pitfalls can be avoided by using spatial autocorrelation analysis to characterize genetic structure because it makes no assumptions about the spatial scale of dispersal (Epperson 1993; Heywood 1991). These methods are powerful tools for detecting genetic differentiation over a range of spatial scales that allow definition of appropriate sampling networks and can help define optimum strategies for conserving genetic variability within species (Sokal & Oden 1978; Sokal & Wartenberg 1983; Epperson 1993; Smouse & Peakall 1999; Diniz-Filho & Telles 2002). An important advantage of spatial autocorrelation analysis over traditional techniques (e.g., hierarchical F-statistics) is that it provides a superior ability to detect the scale of spatial genetic patterns continuously repeated across landscapes (Epperson 1993; Sork *et al.* 1999). Spatial autocorrelation techniques directly analyze genotypes possessed by individuals with no information lost by pooling samples into arbitrary groups for subsequent comparisons of allele frequencies (Heywood 1991; Epperson

1993). Because of this, spatial autocorrelation analyses have increased power for detecting genetic spatial patterns (Epperson & Li 1996; Epperson 1997).

A practical approach to expanding our understanding of EM fungal population genetics to watershed and landscape spatial scales involves genetic research on species that fruit reliably and are broadly distributed. Research on such common species will provide initial studies with the statistical power sufficient to correctly identify evolutionarily important pattern-process relations (Dizon *et al.* 1995). Pacific golden chanterelles (*Cantharellus formosus*, Redhead *et al.* 1997) are commercially harvested basidiomycetes that form EM associations with a wide range of economically important host trees (Redhead *et al.* 1997; Pilz & Molina 2002). In the Pacific Northwest, this species fruits abundantly during the fall and winter months in several different habitats over a broad geographic range. Individual fruit-bodies of *C. formosus* can persist on the landscape, continuously producing basidiospores for over a month (Largent & Sime 1995). Assuming wind is the primary mechanism of spore dispersal for *C. formosus*, the potential for long-distance wind dispersal is high relative to other species of EM fungi with more ephemeral fruiting phenologies. Thus, *C. formosus* represents a distinct life history strategy for spore dispersal and provides a basis for comparison for spatial genetic analysis of other EM species. In this study we use recently developed microsatellite markers (Dunham *et al.* in press) to characterize the fine-scale genetic structure and spore dispersal distances in *C. formosus*. We employ quantitative criteria for assigning fruit-body collections to genets that allow us to assess the contribution of vegetative spread to genetic autocorrelation at short

distances. We also estimate the contribution of isolation-by-distance to fine-scale genetic structure by analyzing genotypes of individual genets separately. The main hypothesis tested is that basidiospore dispersal and subsequent genet establishment probabilities decline as a function of geographic distance in *C. formosus*. Thus, we predict that genetic similarity greater than expected by chance will be detected between genets that are close neighbors.

Methods

Study Area – Chanterelle fruit-body locations were mapped in and collections were taken from a 50 ha (1 Km X 0.5 Km) study plot located on the southeast boarder of the HJ Andrews Experimental forest (44.2° latitude by 122.2° longitude) in Oregon's central cascades. The plot represents a small portion of a larger forest of similar age and elevation (700-800 m) with interspersed old growth fragments at higher elevations. The overstory is dominated by Douglas-fir (*Pseudotsuga menziesii*), and western hemlock (*Tsuga heterophylla*) naturally regenerated from clear-cut harvesting that occurred ~50 years ago. Understory vascular plants include *Polystichum munitum* in wet sites, *Berberis nervosa* in mesic sites, and *Gautheria shallon* in drier sites. This area was chosen for several reasons. It is a replicate stand used in the Young Stand Thinning and Diversity Study (YSTDS), a joint research effort between the Cascade Center for Ecosystem Management, Oregon State University, USDA Forest Service Pacific Northwest Research Station, and the Willamette National Forest McKenzie, Blue River and Middle Fork Ranger Districts. The goal of the YSTDS is to investigate

several ecosystem variables including chanterelle productivity in young stands. During the course of the YSTDS the plot was identified as a high chanterelle productivity area (D. Pilz pers. comm.). Inclusion of the plot in YSTDS chanterelle productivity research required that it be closed to commercial and recreational harvest of fungi for several years prior to our research. As a result, local residents were aware of the closure and rarely entered the area to harvest chanterelles. In addition, the microsatellite markers employed here were characterized using an independent sample taken from the surrounding area and applied in a study of chanterelle genet size that included study sites within this plot (Dunham *et al.* in press).

Field Sampling - During the fall of 1998, a transect network was established (Fig. 5.1) and relative transect locations verified using a global positioning system. The network contained 12.2 km of transect length with a minimum of 20 m between any two transects. The spatial coordinates of chanterelle fruit-bodies within two meters of a transect were determined to the nearest 0.1 m by measuring from locator flags placed every 20 m along transects. To minimize re-sampling genets a minimum distance of 5 m was kept between any two fruit-bodies collected along the same transect. This distance is based on genet size estimates made for chanterelles in this stand (Dunham *et al.* in press). The entire transect network was sampled between Oct. 1 and Nov. 20, 1998. During this time 4.4 km of transect length (36%) dispersed evenly throughout the study area (Fig. 5.1) was re-sampled in order to include data from individuals with delayed fruiting phenology (Selosse *et al.* 2001). White chanterelles (*C. subalbidus*)

and a newly described yellow chanterelle species (*C. cascadensis* nom. prov.) also are present in the study area. We collected fruit-bodies of all three species in the manner described above to explore the possibility of conducting comparative spatial genetic analyses.

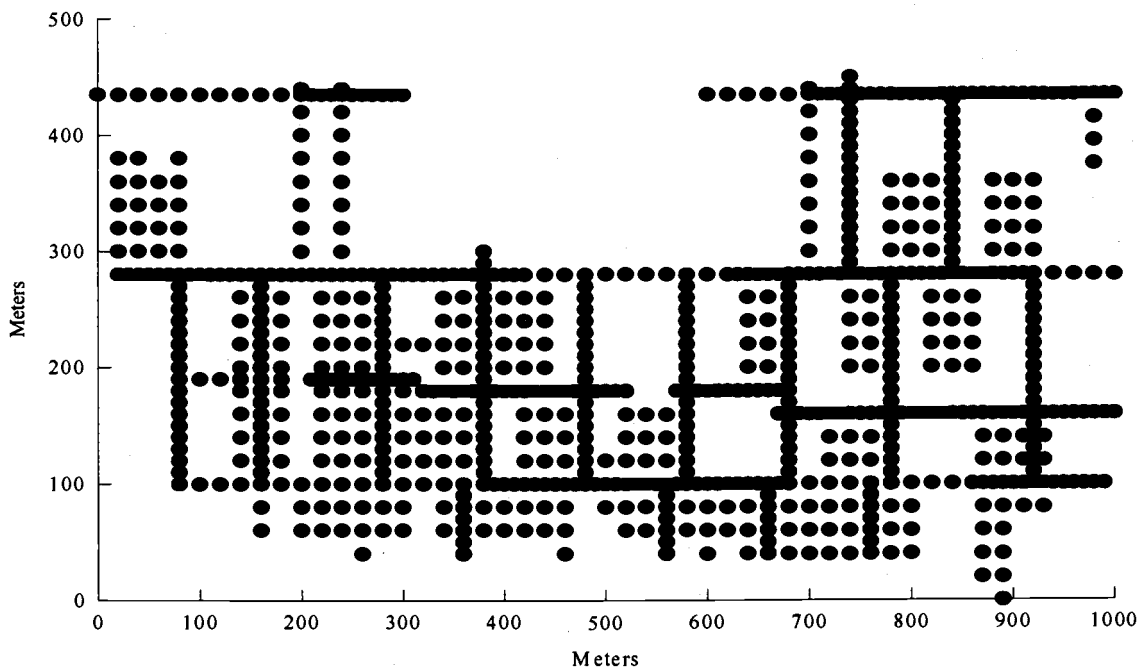


Figure 5.1. Locations of transects from which chanterelle fruit-bodies were sampled during the fall of 1998. Transects represented by densely dotted lines were sampled twice.

DNA Extraction and Amplification – C. cascadensis nom. prov.is difficult to differentiate from *C. formosus* in the field (Dunham *et al.* in press). To ensure that all spatial analyses were carried out on discrete taxa we analyzed all fruit-bodies using restriction fragment length polymorphism analysis of the internal transcribed spacer region of the nuclear ribosomal repeat (ITS-RFLP), a technique commonly used to characterize community level species diversity (Gardes & Bruns 1996; Horton & Bruns 2001). After grinding a small amount of fresh or dried tissue in 1 ml of lysis buffer (100 mM Tris, 10mM EDTA, 2% sodium dodecyl sulfate, 1mg/ml proteinase-K, pH 8.0), each sample was incubated for 1.5 hours at 55 °C and DNA was extracted using a standard phenol/chloroform emulsification and precipitation (Maniatis *et al.* 1982). Resulting pellets were vacuum-dried and re-suspended in 50 to 100 µl of sterile Tris-EDTA. One microliter of the unquantified DNA extracts were used in 40 µl polymerase chain reaction (PCR) amplifications. The ITS region, spanning the 3' end of the 18S, ITS-1, 5.8S, ITS-2, and 5' end of the 28S was amplified with the fungal specific primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). Final concentrations of PCR mix components were 0.2 mM of each dNTP, 0.2 µM of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, and 0.75 units of DNA polymerase. Reactions were subject to 35 PCR cycles with the following protocol: initial denaturation at 94 °C for 3 min, cycling denaturation at 94 °C for 50 s primer annealing at 55 °C for 50 s, and primer extension at 72 °C for 1.5 min. Samples were subjected to a final extension of 10 min following the 35th cycle.

Restriction Fragment Length Polymorphisms (RFLP's) –According to the manufacturer's recommendations (GIBCO BRL, Grand Island, NY), unpurified PCR products amplified with ITS1-F and ITS4 were digested separately with two restriction enzymes (*Hinf*I and *Hae*III) known to differentiate the three chanterelle species of interest. Restriction fragments were electrophoresed through 3% agarose gels (2% Nu-Sieve agarose, 1% SeaKem LE agarose; FMC BioProducts), stained with ethidium bromide, and scored against a 100 base-pair molecular weight standard using the Alpha Imager 2000 documentation and analysis system V 3.2 (Alpha Innotech Corp.). Each collection sampled in this study was assigned to an ITS-RFLP type that summarized information from the two restriction enzyme profiles. Collections with the same ITS type designation share identical restriction fragment patterns across both enzymes and, based on previous experience with ITS variability in *Cantharellus* (unpublished data) are considered to represent a single species.

Microsatellite screening - All samples were scored at two tri- and two tetra-nucleotide microsatellite loci developed for *C. formosus* (Dunham *et al.* in press). PCR reactions were carried out in 20 µl volumes and contained 2-3 µl genomic DNA (diluted as for ITS amplification), 1X Assay Buffer A (Fisher Scientific), 200 µM dNTPs each, 0.25 µM of each primer, 1 U Taq DNA polymerase (Fisher Scientific). The PCR profile consisted of initial denaturation at 95° C for 3 min followed by 35 cycles (95° C, 45s; 55° C, 60 s; 72° C, 60 s). Following the 35th cycle, samples were subjected to a final 72° C extension of 60 min to maximize the proportion of complete PCR products. PCR

products were analyzed on an ABI 377 automated sequencer using the GS500 Tamra internal size standard. Band sizes were determined using GENESCAN software (PE Applied Biosystems).

Data Analysis – The relative contributions of both vegetative growth (i.e., repetitive samples drawn from unique genets) and isolation-by-distance to fine-scale structure must be properly differentiated in order to develop an understanding of the true dispersal capabilities of EM fungi. Non-random spatial genetic patterns at fine spatial scales may result when the same genet (and thus an identical genotype) has been repeatedly sampled at nearby locations, or because of restricted gene flow (isolation-by-distance). Reliable assignment of chanterelle fruit-bodies to unique genets depends on the resolving power of the loci employed. During our study of *C. formosus* genet size (Dunham *et al.* in press) we found that the four microsatellite loci used here do not fully resolve all genets. This research also revealed that, even when overestimated, mean maximum genet width is 3.24 m ±3.6 m and 98% of genets are under 10 m maximum width. During field sampling we maintained a minimum distance of 5 m between any two fruit-bodies collected along each transect. The primary purpose of this was to decrease the number of samples that would need to be genotyped in order to examine spatial patterns while maximizing the number of discrete genets sampled. This field sampling strategy was not sufficient to avoid taking replicate fruit-bodies from many genets. To examine the effect of clonal propagation (vegetative growth) on spatial genetic structure across the study area we performed spatial autocorrelation

analyses on all fruit-bodies collected using this 5m-distance criterion. Significant correlation values in the smallest distance classes of this analysis are interpreted as resulting from vegetative growth of chanterelle genets.

To differentiate between clonal propagation and isolation-by-distance sources of spatial genetic structure, we developed criteria for assigning fruit-bodies to genets so any unique genet would be represented by only a single fruit-body. To accomplish this we first culled all collections if a single taxon using the criterion that fruit-bodies possessing identical multilocus genotypes must be separated by a minimum of 15 m to remain in the analysis. This distance exceeds the maximum width (13 m) of the largest chanterelle genet observed during our previous study of genet size in the study area (Dunham *et al.* in press). After reducing the data set by this distance criterion, we calculated the expected frequency of each multilocus genotype represented by more than one fruit-body using allele frequencies determined from an independent collection of chanterelles taken from the surrounding area (Dunham *et al.* in press). Ectomycorrhizal fungi do not produce mitotic (asexual) spores (Hutchinson 1989) that could allow long-distance dispersal of multilocus genotypes. If a multilocus genotype has many relatively low frequency alleles at several loci, the likelihood that it would occur more than once by chance (as opposed to vegetative growth) is extremely low. In contrast, if a multilocus genotype is composed of only a few high-frequency alleles at several loci the likelihood of multiple occurrences due to chance can be fairly high. Our previous chanterelle genet size research demonstrated that multilocus genotypes repeated over long distances (300 m – 11 km) had a probability ≥ 0.01 of being

encountered more than once due to chance. In contrast, genotypes found clustered only at fine spatial scales had expected frequencies < 0.1%. Based on these levels of expected multilocus genotype frequency, fruit-bodies with identical multilocus genotypes that had expected frequencies ≥ 0.01 were assigned to unique genets as long as they were separated by at least 15 m. Alternatively, multiple fruit-bodies possessing identical multilocus genotypes with expected frequencies < 0.01 were only included in spatial analyses if individual collections were separated by at least 50 m. Similar methodology and values for expected multilocus genotype frequencies have been used to assign collections to genets in several clonal plant species (Aspinwall & Christian 1992; Parks & Werth 1993; Reusch *et al.* 1999a, 1999b).

The spatial distribution of fruit-bodies determined to represent chanterelle genets (one fruit-body per genet) was analyzed using Spatial Genetic Software V1.0b (Degen 2000) analysis package. Ripley's K statistic (Bailey & Gatrell 1995) was used to test the null hypothesis that genets for each of the three chanterelle species collected were distributed randomly across the study area. To examine the spatial distribution of genotypes from individual microsatellite loci and for combined multilocus genotypes we used the program GenAlEx V5 (Peakall & Smouse 2001) This program employs multivariate analysis methods (Smouse & Peakall 1999) to calculate a correlation coefficient (r; range –1 to +1) between genetic and geographic distances for all pairs of individuals within an annulus of predefined radius (distance class). For example, r in the distance class 0 – 25 m would represent the correlation between genetic and geographic distances calculated between all pairs of individuals occurring within 0 –

25 m of one another. We used the genotypic distance option to calculate linear genetic distances between all possible pair-wise comparisons (pairs of individuals / genets) within each distance class (Peakall & Smouse 2001). Correlations between genetic distance and linear geographic distance were calculated for each of several distance classes selected to produce an even number of pair-wise comparisons within each class. The purpose of using this selection method is to reduce noise in confidence interval limits resulting from uneven numbers of pair-wise comparisons across distance classes. To increase pattern resolution the smallest distances classes that allowed sufficient sample size (at least 500 pair-wise comparisons) for calculating the *r* statistic were selected. To adjust for declining sample size (pair-wise comparisons) within distance classes and statistical power, intervals used to explore spatial autocorrelation resulting from isolation-by-distance were wider than those used to explore patterns due to vegetative growth.

For each distance class, statistical significance of *r* was determined by creating a randomized distribution of correlation values using 999 permutations that swapped genetic data across spatial locations. The resulting randomized distribution represents the expected behavior of the correlations statistic (given the data) under the null hypothesis that genotypes are distributed randomly across the study area. Tests of significance were computed for each distance class by comparing the correlation value calculated for the observed spatial data to those obtained from the 999 spatial permutations (Smouse & Peakall 1999). Patterns of spatial genetic autocorrelation were examined as a correlogram plotting r as a function of distance. For any

correlogram, the chance of committing a type-I error is increased because *r* is calculated as a separate test within each distance class. This requires a test of significance for the entire correlogram (Oden 1984). We predict (a priori) that spatial autocorrelation values will decrease as a function of distance due to either clonal propagation or isolation-by-distance. Therefore, the significance of the shortest distance class was considered a test of the entire correlogram (Heywood 1991; Reusch *et al.* 1999a). In situations were we extended interpretation of correlograms to additional distance classes a Bonferroni correction (alpha adjusted to 0.05/# distance classes, Oden 1984) was applied before interpreting the significance of the autocorrelation coefficient.

Results

Genet Assignment - Field sampling produced 465 chanterelle fruit-bodies from the 50 ha plot with 384 collections confirmed by ITS-RFLP analysis as *C. formosus*, 35 as *C. subalbidus*, and 28 as *C. cascadensis* nom. prov. *C. formosus* fruit-bodies possessed either two or three alleles for each microsatellite locus employed. *C. subalbidus* and *C. cascadensis* nom. prov. collections possessed multiple alleles (2-5 per locus) at only two loci each, greatly reducing the genetic information available for subsequent spatial genetic analyses. Scoring all collections with the four microsatellite loci produced 65 unique multilocus genotypes across the 384 *C. formosus* collections, 16 unique multilocus genotypes across the 35 *C. subalbidus* collections, and 10 unique multilocus genotypes for the 28 *C. cascadensis* nom. prov. collections. For *C.*

formosus, 20 multilocus genotypes were represented by single fruit-bodies and an additional 30 genotypes were represented by five or fewer fruit-bodies (Figure 5.2). Five or fewer fruit-bodies represented all multilocus genotypes found in *C. subalbidus* and *C. cascadensis* nom. prov. collections. Culling samples so that at least 15 m (expected frequency ≥ 0.01) or 50 m (expected frequency <0.01) separated fruit-bodies possessing identical multilocus genotypes reduced the *C. formosus* sample from 384 to 183 collections. Culling collections of *C. subalbidus* and *C. cascadensis* nom. prov. based the same distance criteria reduced sample sizes from 35 to 31 and 28 to 16 genets respectively. In the total *C. formosus* sample (384 collections) two multilocus genotypes were represented by 43 fruit-bodies each. These multilocus genotypes were composed of the most frequent alleles for each locus and had expected frequencies of 0.08 and 0.02. The number of fruit-bodies representing these multilocus genotypes was reduced to 19 and 23 (Figure 5.2) after distance criteria were applied (Figure 5.3). Figure 5.4 shows the actual spatial locations of chanterelle fruit-bodies representing unique chanterelle genets after each sample was reduced using distance criteria.

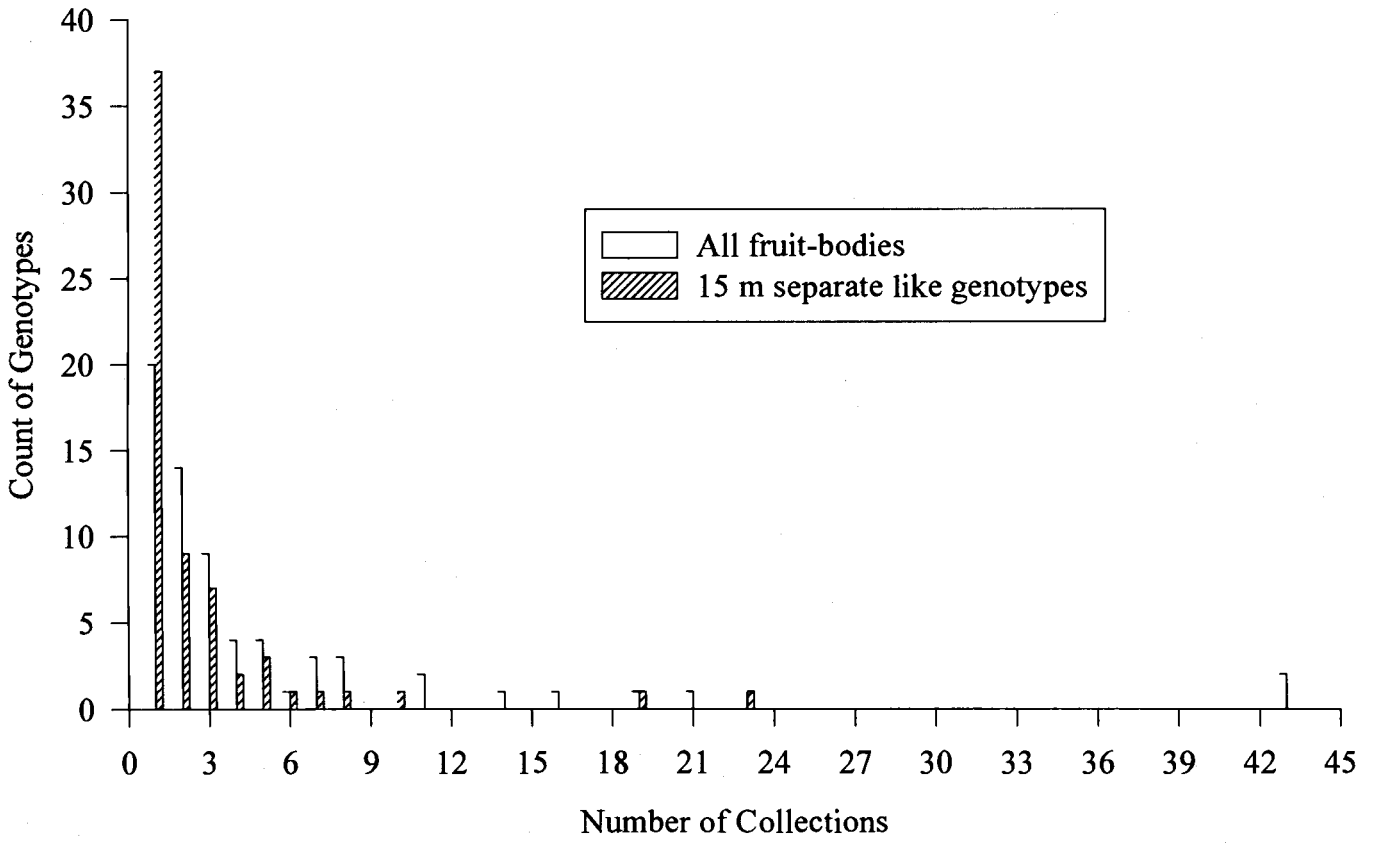


Figure 5.2. Plot showing the number of multilocus genotypes represented by different numbers of fruit-bodies in the total sample (open bars) and the sample reduced using distance criteria to assign fruit-bodies to unique genets (striped bars).

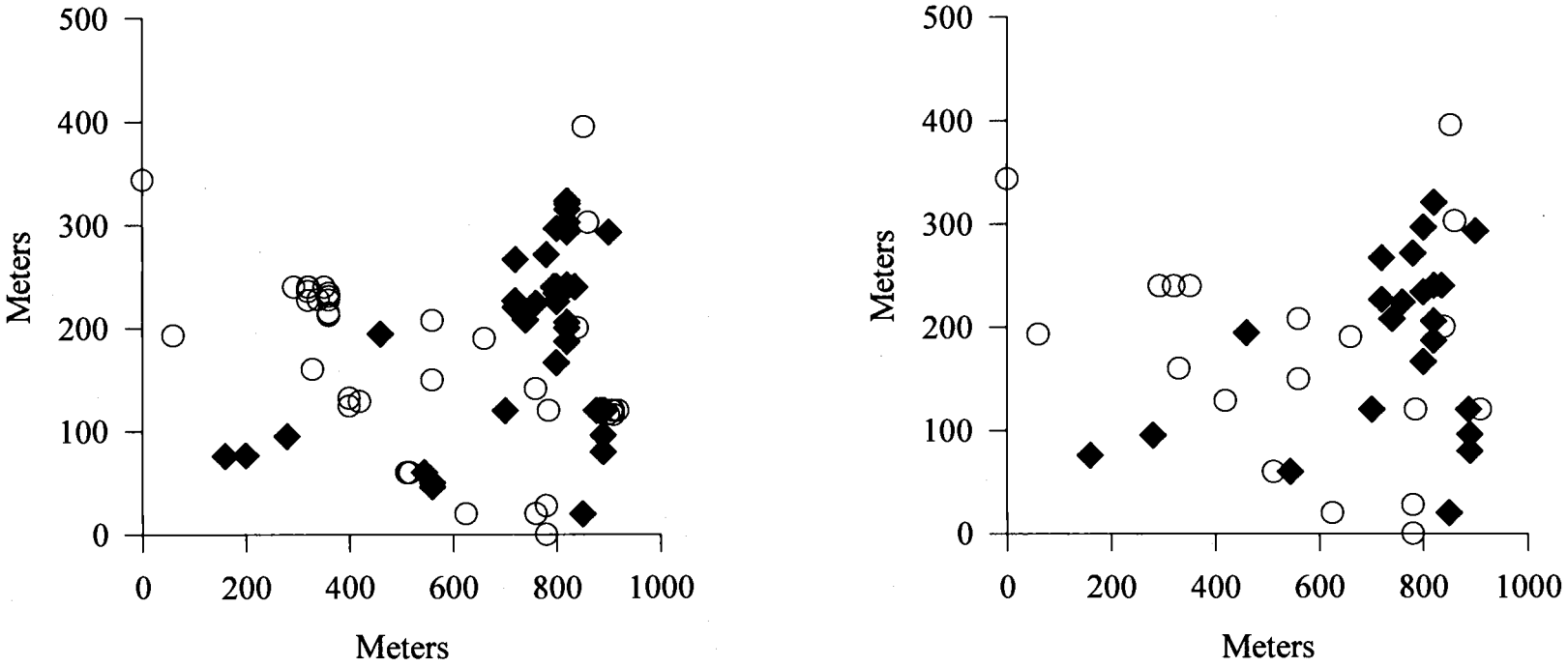


Figure 5.3. Side by side plots showing the effect of culling the total *C. formosus* sample with distance criteria used to assign fruit-bodies to unique genets. The plots show all collections of fruit-bodies possessing the two multilocus genotypes represented by 43 fruit-bodies each (left) and the same genotypes represented by 19 and 23 fruit-bodies in the reduced sample. Open circles and closed diamonds represent different genotypes.

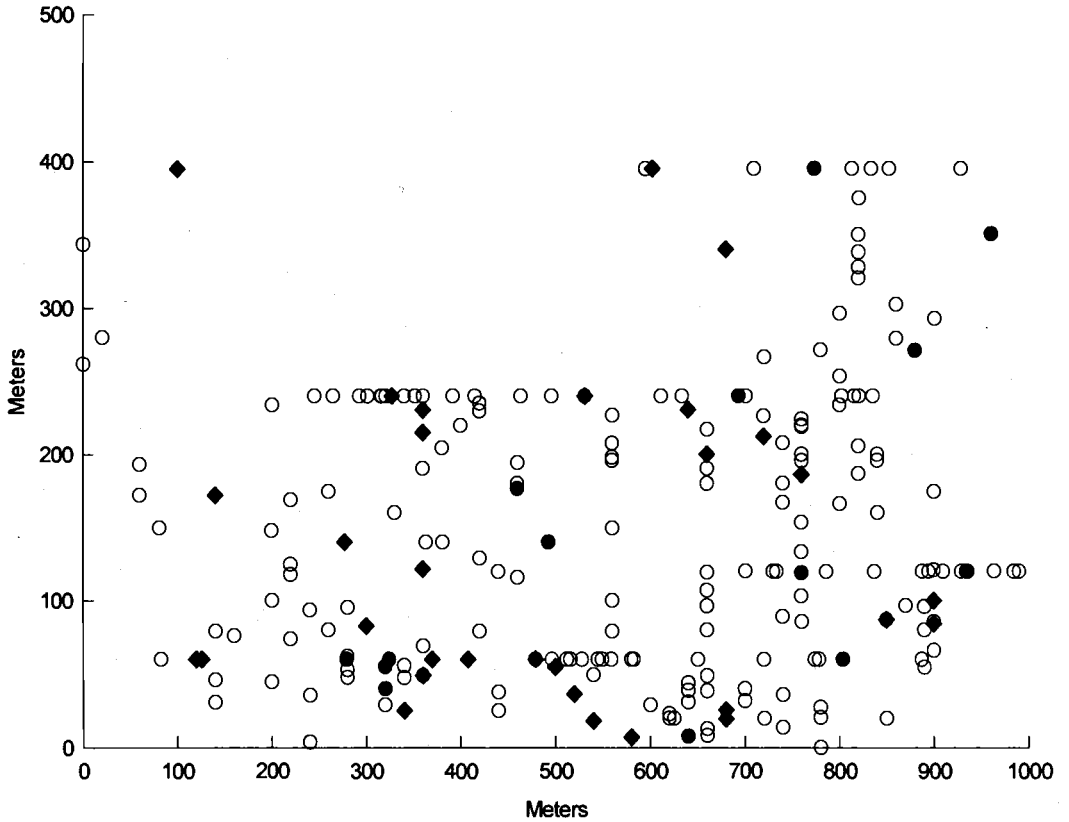


Figure 5.4. Locations of chanterelle genets identified using microsatellite loci. Fruit-bodies with identical multilocus genotypes were considered to represent separate genets when separated by 15 m (expected multilocus genotype frequency ≥ 0.01) or 50 m (expected multilocus genotypes frequency < 0.01). Open circles represent *C. formosus* genets, closed diamonds represent *C. subalbidus* genets, and closed circles represent *C. cascadensis* nom. prov. genets. Only the 183 *C. formosus* genets were included in final spatial autocorrelation analyses.

For each species, the total collection of fruit-bodies showed significant spatial aggregation across the study plot (Ripley's K: *C. formosus* = 0.53 $p<0.001$; *C. subalbidus* = 0.79 $p=0.05$; *C. cascadensis* nom. prov. = 0.24. $p<0.001$). After fruit-bodies were assigned to discrete genets and repetitive samples were dropped from the analysis both *C. subalbidus* and *C. cascadensis* nom. prov. genets showed a random spatial distribution (Ripley's K = 0.90; 0.85 respectively, $p>0.05$ for each) while *C. formosus* genets continued to show weak but statistically significant spatial aggregations (Ripley's K = 0.77 $p<0.001$). We cannot exclude the possibility that *C. subalbidus* and *C. cascadensis* nom. prov. genets were spatially aggregated but the low number of genets (resulting in low statistical power) for each species precluded detection of spatial patterns. Some researchers have pointed out that spatial autocorrelation analyses should not be carried out on fewer than 30 samples (Fortin *et al.* 1989; Sokal & Jackquez 1991). Sample sizes and levels of genetic variability were insufficient to warrant spatial analysis of *C. subalbidus* and *C. cascadensis* nom. prov. genotypes. Spatial autocorrelation analyses of microsatellite genotype distributions were performed only for *C. formosus*.

Spatial Genetic Structure Due to Vegetative Growth – The mean distance between fruit-bodies in the total *C. formosus* sample (n=384) was 9.4 m. Spatial autocorrelation analysis of genetic distances shows that multilocus genotypes are not distributed at random in the total *C. formosus* sample (Figure 5.5). Instead like genotypes cluster

tightly in groups at fine spatial scales with significant positive autocorrelation in the 0-24 m distance class ($r = 0.25$, $p < 0.001$). Statistically significant (after Bonferroni correction) but weak autocorrelation was also found in the 25 – 42 m distance class ($r = 0.06$, $p<0.001$) indicating that either we have underestimated the extent of vegetative growth in *C. formosus*, or isolation-by-distance is also contributing to this fine-scale spatial structure. To better evaluate the behavior of the autocorrelation coefficient as a function of short distances we repeated this analysis using smaller distance classes and included only pair-wise comparisons up to 100 m apart. This analysis shows that the strongest positive associations among multilocus genotypes occur between fruit-bodies within 17 m of one another (Figure 5.5). As distance increases, both correlograms show a fluctuating pattern expected for populations where genotypes are patchily distributed. The first x-axis intercept occurs at approximately 48 m. This represents the point at which two paired genets are equally likely to be in the same patch or different patches and therefore estimates the radius of genetically homogenous patches in the population (Sokal & Oden 1978; Sokal & Wartenberg 1983; Epperson 1995). After dropping below the upper confidence interval limit the autocorrelation coefficient oscillates between positive and negative values. This pattern is indicative of strong fine-scale spatial structure created by discrete patches of similar multilocus genotypes repeated across the study area (Smouse & Peakall 1999; Diniz-Filho & Telles 2002). Separate analysis of each microsatellite locus (correlograms not shown) revealed that single locus genotypes are similarly structured across the study area. All four showed significant ($p<0.001$) positive autocorrelation in

the 0 – 24 m distance class with r values ranging from 0.17 – 0.46. All loci showed positive autocorrelation in the 25 – 48 m distance class but only two correlations were statistically significant after Bonferroni correction.

Spatial Genetic Structure Due to Isolation-by-Distance – Fruit-body collections were assigned to unique genets and all collections determined to represent replicate samples from genets were excluded in order to subtract the contribution of vegetative growth from the spatial autocorrelation analysis. The mean distance between *C. formosus* genets included in the analysis of isolation-by-distance was 18.6 m. The correlation between geographic distance and multilocus genetic distance was weakly positive and statistically significant in the shortest distance class (0 – 55 m, $r = 0.02$, $p = 0.04$) but stronger and, after Bonferroni correction, highly significant in the 55 – 83 m distance class ($r = 0.034$, $p = 0.001$, Figure 5.6). The radius of genetically homogenous patches resulting from isolation-by-distance as determined from the x-axis intercept is ~ 200 m (Figure 5.6). Analysis of individual microsatellite loci (Figure 5.7) showed that two of the four loci mirrored the combined analysis in the 0-55 m distance class with a maximum r of 0.07 while genetic variation was distributed randomly across the plot for the other two loci. In the 55 – 83 m distance class all four loci show positive autocorrelation with r ranging from 0.04 – 0.06. The locus showing the strongest spatial pattern also had the highest observed heterozygosity (0.69 vs. 0.44, 0.31 & 0.09 for the other three loci). Variation in the direction and strength of signal in spatial autocorrelation analyses across loci (Figure 5.7) is commonly viewed as stochastic

variability due to random sampling of alleles during sexual reproduction (Smouse & Peakall 1999). Multilocus analyses better demonstrate the general pattern of spatial affinity because they smooth out the variance created by random genetic processes.Comparable to the correlogram analyzing the contribution of vegetative spread to spatial genetic patterns, the correlogram characterizing the contribution of isolation-by-distance shows a fluctuating pattern indicative of patchily distributed genotypes.

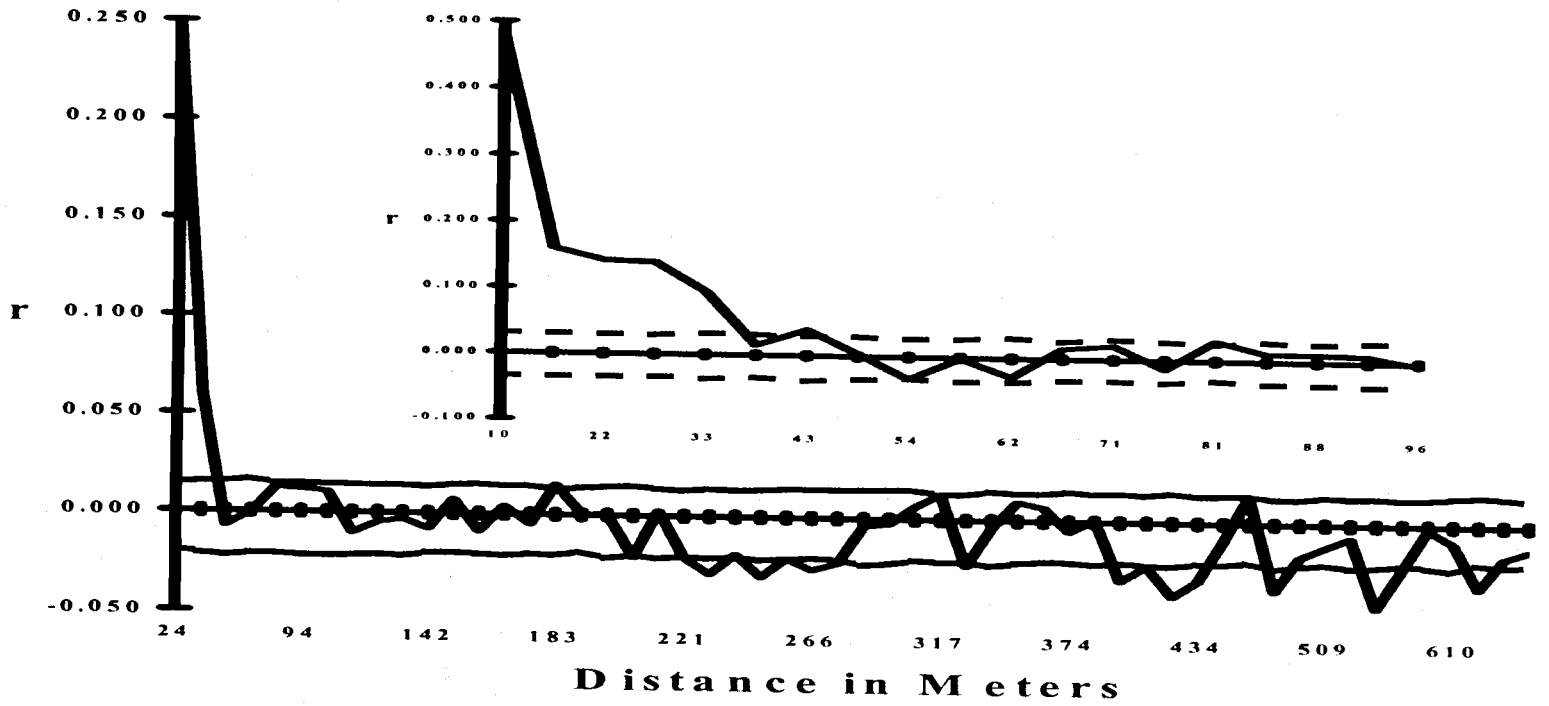


Figure 5.5. Correlograms plotting the mean spatial autocorrelation determined from all *C. formosus* fruit-bodies collected (n=384). The solid line represents the mean correlation value per distance class calculated across the four microsatellite loci scored. Dashed lines represent 95% confidence intervals determined using permutation tests. Correlation values outside the confidence intervals are statistically significant. The first x-axis intercept represents the extent of non-random genetic structure due to both clonal propagation and isolation-by-distance.

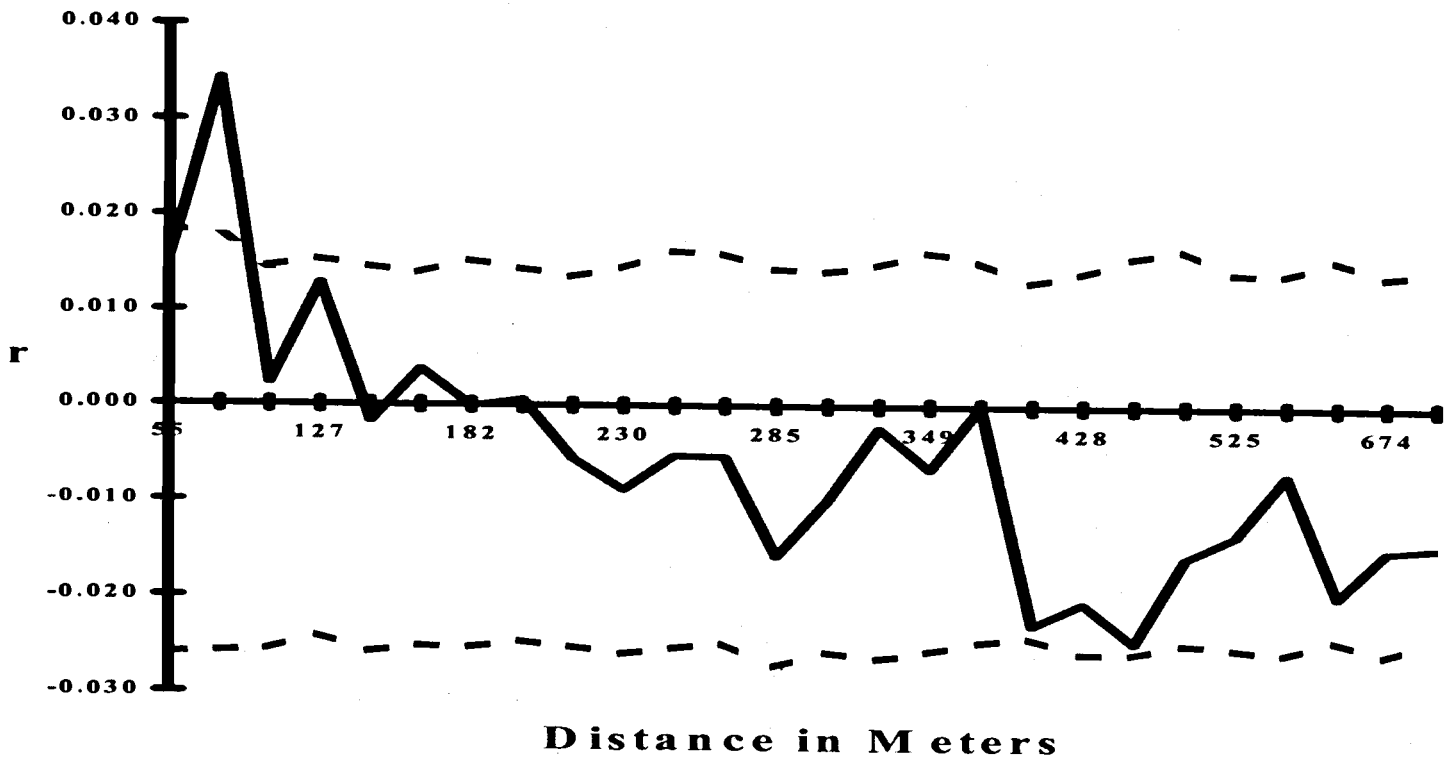


Figure 5.6. Correlogram plotting the mean spatial autocorrelation determined from *C. formosus* fruit-bodies that represent distinct genets (n=183). The solid line represents the mean correlation value per distance class calculated across the four microsatellite loci scored and dashed lines represent 95% confidence intervals. Correlation values outside the confidence intervals are statistically significant. The first x-axis intercept represents the extent of non-random genetic structure due isolation-by-distance.

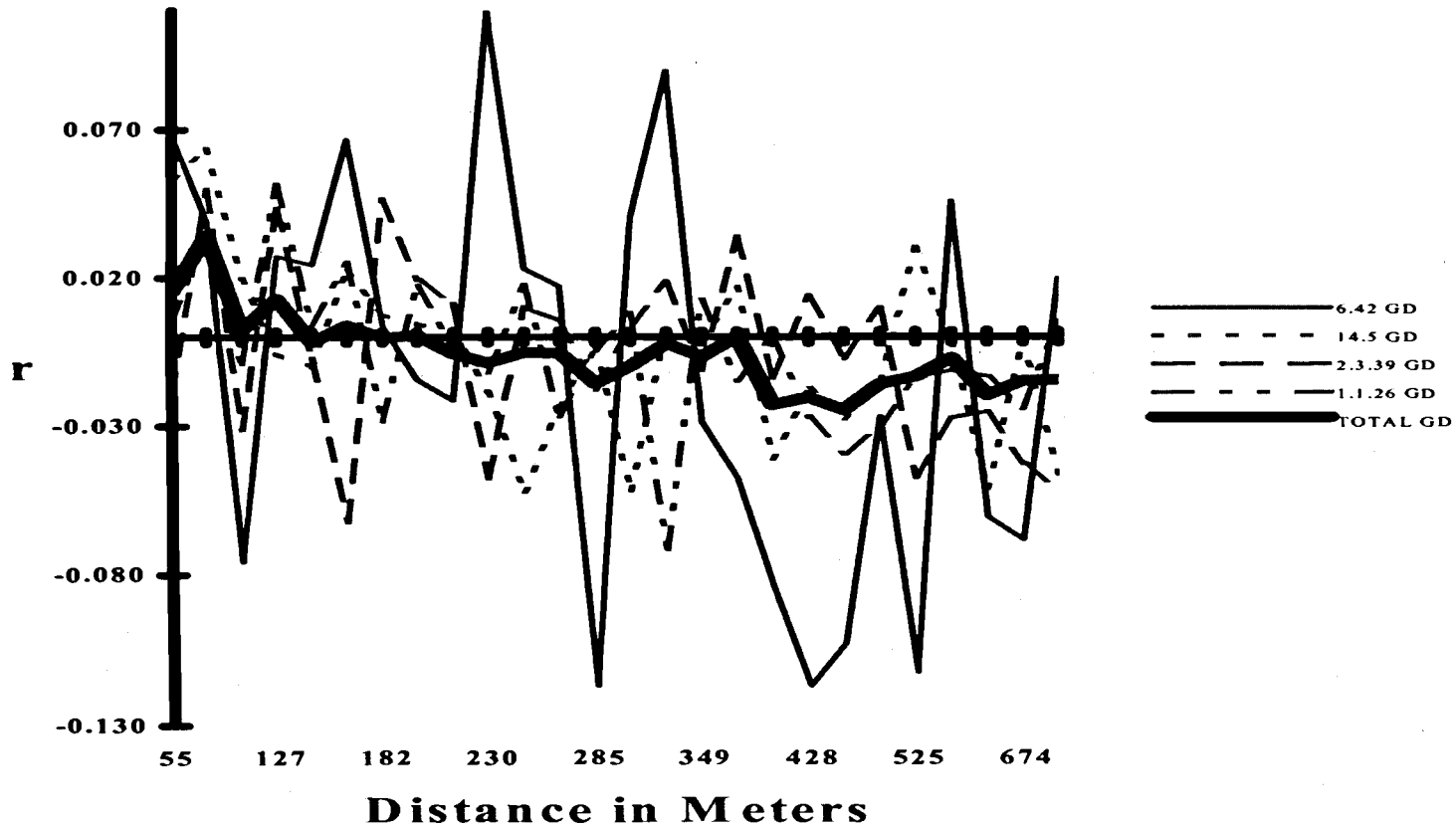


Figure 5.7. Variation in spatial autocorrelation direction and strength for each microsatellite locus scored compared to the mean autocorrelation averaged across loci for the analysis of isolation-by-distance.

Discussion and Conclusions

Over the last decade research into the relative roles of basidiospore dispersal and vegetative growth in the life histories of EM fungi have shown that frequent and repetitive genet establishment from basidiospores occurs in some EM species (Guidot *et al.* 2002). These results indicate that basidospore dispersal is important for the maintenance of genetic diversity in EM fungal populations. In order to improve the design of landscape scale studies of EM fungi and make better-informed management decisions we must also understand the spatial scale of basidiospore dispersal. We have demonstrated spatial clustering of multilocus genotypes at short distances (<250 m) after removing the effect of genetic similarity at short distances due to repetitively sampled genets. This fine-scale genetic structure indicates that gene flow is insufficient to counteract the effects of localized inbreeding and genetic drift despite the fact that *C. formosus* fruit-bodies can remain on the landscape continuously producing basidiospores for up to 90 days (Largent & Sime 1995). To our knowledge, this is the first demonstration of isolation-by-distance in a population of EM fungi. The spatial genetic patterns characterized here can be used to design sampling networks that will maximize sampling efficiency at larger spatial scales and accurately characterize genetic patterns and evolutionary processes at spatial scales relevant to management.

In a spatial autocorrelation analysis the sign and direction of change in the autocorrelation coefficient allows inference about evolutionary processes affecting individuals in the study area. Three common patterns detected include 1) random

fluctuation of the autocorrelation coefficient, 2) a stabilizing profile with strong positive autocorrelation at short distances then random fluctuation of the autocorrelation coefficient at longer distances, and 3) a long-distance cline with positive autocorrelation at short distances that decreases continuously with increasing geographic distance (Diniz-Filho & Telles 2002). Correlograms showing only random fluctuations about the x-axis are indicative of panmixia. The stabilizing profile represents genetic similarity within a well-defined spatial range, indicating a steep decline in dispersal distances with increasing geographic distance (Sokal & Wartenberg 1983). The long-distance cline is indicative of isolation-by-distance over long distances (Sokal *et al.* 1997). In contrast to the stabilizing profile, clinal patterns do not indicate that the continuum of genetic variation is partitioned into discrete groups of local populations, but both patterns allow estimation of the minimum distance at which samples become genetically independent (Diniz-Filho & Telles 2002). While interpreting the general meaning of a correlogram pattern is fairly straight forward, drawing inferences about patch size, dispersal, and isolation-by-distance requires the ability to differentiate between all potential causes leading to spatial clustering of genotypes. In fungi, clonal (vegetative) growth will tend to confound patterns of genetic structure and limit inference related to spore dispersal. The correlation between multilocus genetic distance and geographic distance in the *C. formosus* sample known to contain multiple collections from unique genets indicates that clonal propagation is very localized (Figure 5.5). The correlogram for this analysis shows a stabilizing profile indicating the presence of many discrete

homogeneous patches distributed throughout the study area. The point at which the autocorrelation coefficient first crosses the x-axis provides an estimate of patch radius (Sokal & Wartenberg 1983), which is approximately 48 m. Independent estimates of genet size for *C. formosus* (Dunham *et al.* in press) indicate that mean genet width for this species is much smaller than the patch size estimated here with 98% of genets less than 10 m in width and the largest genet observed 13 m wide. These genet width estimates agree with the strong pattern of genetic affinity found in 0-10 m and 10-17 m distance classes delineated in the fine-scale autocorrelation analysis within 100 m (Figure 5.5 inset). There are two possible explanations for the positive autocorrelation found distance classes beyond 17 m and the relatively large patch size. Genetic similarity at fine spatial scales due to isolation-by-distance was not excluded from this analysis and may inflate the patch size resulting from vegetative growth alone. Alternatively, short distance dispersal of genets could occur by vegetative growth of mycelia that subsequently become disjointed. The presence of disjointed genet fragments in close proximity to one another would increase patch size estimates and increase the influence of vegetative growth on fine-scale population genetic structure. Given that our markers to not fully resolve all genets we cannot exclude this as a possible mechanism driving fine-scale genetic structure in *C. formosus*.

In this study, weak but significantly positive autocorrelation of multilocus genotypes at short distances (<250 m) and stabilizing profile at longer distances was detectable after accounting for the effect of genetic similarity due to vegetative growth (Figure 5.6). The spatial analysis of collections from sexually reproduced individual

genotypes (genets) indicates that they are distributed in a structured, isolation-by-distance manner with a patch size of 200 m. Significant genetic structure in the 0-55 m distance class may be due to representation of some genets by repeated collections that occurred at distances longer than the criteria used to assign fruit-bodies to genets. This is unlikely given the highly significant genetic structure detected in the 55-83 m distance class. If the genetic structure in the first distance class were due to both clonal propagation and isolation-by-distance one would expect that relationship between genetic similarity and geographic distance would be stronger than that of the second distance class. Instead, lower genetic similarity in the first distance class indicates that the distance criteria we used to subtract the contribution of clonal growth were overly conservative and reduced the relative number of unique genets sampled at fine spatial scales.

Although statistically significant, the strength of spatial genetic structure observed was low compared to other studies using the same analysis techniques (e.g., Smouse & Peakall 1999; Chung & Epperson 1999). It may be the case that isolation-by-distance operates only weakly within *C. formosus* populations. Before drawing this conclusion we must consider the likelihood that our ability to detect spatial genetic patterns was compromised by low statistical power. Epperson *et al.* (1999) demonstrated that maximum statistical power results from sampling sufficient numbers of individuals in at least 4 to 9 patches. Based on the estimated patch size and the total size of our plot, only two to four patches were sufficiently sampled. In addition Smouse & Peakall (1999) showed that the strength of spatial genetic signal

increases with the level of polymorphism at each locus rather than with the total number of alleles sampled. Three of our four loci exhibited low heterozygosities (0.09, 0.3 & 0.4) compounding the reduced power from inadequate sampling of patches.

Weak (although significant) spatial autocorrelation in the smallest distance classes indicates that close genets were not very similar. In other words, the spatial pattern observed in overall genetic distances is better explained by long-distance differentiation than by short-distance similarity. Low positive autocorrelation at short distances coupled with slightly negative autocorrelation at longer distances can be interpreted two ways. The first explanation to consider is that despite the fact that *C. formosus* fruit-bodies are known to remain on the landscape for up to 90 days (Largent & Sime 1995) gene flow is sufficient to only partially counteract fine-scale structure resulting from local inbreeding and genetic drift. Although EM fungi possess mating-type genes that function to reduce inbreeding, basidiospores from the same genet can germinate and fuse to form a dikaryotic mycelium capable of fruit-body formation. Resulting fine-scale structure may be driven by variance in genet size with larger individuals disproportionately contributing to the establishment of new genets in local areas (e.g., Gryta *et al.* 2000). Patterns of fine-scale genetic structure may also be influence by the spatial aggregation of genets used in the isolation-by-distance analysis. The effect of spatially aggregated samples on genetic autocorrelation analyses have been investigated (Doligez *et al.* 1998) but were found to have only minor effects on the presence of spatial structure relative to dispersal.

Alternatively, we must consider that spore dispersal only represents potential gene flow and must be followed by successful germination, dikaryon formation, and EM colonization for actual genet establishment to occur. Spores may be able to travel long distances but migrants may be selected against if they are not genetically compatible with EM host genotypes in the local area. Selection against spores from distant populations could create the patterns we observed if the monokaryons produced long-distance dispersers are dependent on forming dikaryons with monokaryons produced locally. Spores remaining locally would exhibit strong isolation-by-distance diluted by the introgression of alleles from outside the area.

Implications for future research on C. formosus – Taken together, the data presented above strongly support the hypothesis that spore dispersal and or genet establishment is restricted in *C. formosus*. Regardless of the evolutionary mechanism driving the observed spatial genetic structure, our results have important implications for the design of population genetic studies at larger spatial scales and can be used to design sampling networks that will capture informative variability in genetic patterns and processes at spatial scales relevant to management. The x-axis intercept defines geographic distance between genetically independent samples (Diniz-Filho & Telles 2002). For sampling purposes, collecting samples separated by this minimum distance will better characterize the genetic heterogeneity distributed across the landscape by avoiding pseudoreplication of genetic variability distributed within patches. For *C. formosus* these inferences currently are limited to the single site that we sampled in 50

year-old second-growth Douglas-fir. Our research needs to be replicated under an array of environmental conditions before these conclusions could be considered widely applicable.

Implications for other EM species - We know of only one other study on EM fungi where the spatial patterns of genotypes were examined after genet structure was determined. In this study Zhou *et al.* (2001) visually detected clustered genotypes on their study plots but were unable to detect genetic differentiation at larger spatial scales using F-statistics. Given the fine scale patterns they observed Zhou *et al.* (2001) may have pooled information from several genetically homogenous patches. The conclusion that their study populations were not genetically differentiated may have resulted from low statistical power (Wright 1951, 1969; Magnussen 1993, Ruckelshaus 1998) rather than the presence of gene flow.

Population structure results from complex interactions between genetic, demographic, and environmental factors. Characterizing fine-scale spatial genetic structure at multiple spatial scales provides a way to examine the processes that could affect the evolutionary potential and viability of a population (Dunham *et al.* 1999). Studies on terrestrial plants have shown that dispersal capability and reproductive biology are correlated with genetic diversity, population structure, and gene flow (Loveless & Hamrick 1984). Several studies of spore settlement have shown that most spores do not disperse very far from the source (Wolfenbarger 1946, Gregory 1973, Lacey 1996, Morkkynen *et al.* 1997). James and Vilgalys (2001) demonstrated that

even though spores of *S. commune* were abundant in the air and sedimentation rates were high, population genetic characterization of trapped spores showed population subdivision indicating lack of long-distance dispersal. In this study we have demonstrated that multilocus genotypes of *C. formosus* are structured at fine spatial scales in patterns consistent with isolation-by-distance despite the fact that fruit-bodies persist on the landscape for long periods continually producing basidiospores. Ectomycorrhizal fungi show substantial life history variability controlled both by genetic and environmental factors and offer numerous opportunities to investigate the relationship between life history strategy and dispersal capacity (Dahlberg 2001, Guidot *et al.* 2002). General characterizations of dispersal capabilities for EM fungi will benefit a number of current research efforts aimed at understanding EM population biology and ecology by improving both predictions about establishment probability of for various species and knowledge of the scale at which sample genetic independence occurs across landscapes (Wiens 1989).

References

Anderson IC, Chambers SM, Cairney JWG (1998) Use of molecular methods to estimate the size and distribution of mycelial individuals of the ectomycorrhizal basidiomycete, *Pisolithus tinctorius*. *Mycological Research*, **102**, 295-300.

Aspinwall N, Christian T (1992) Clonal structure, genotypic diversity, and seed production in populations of *Filipendula rubra* (Rosaceae) from the north central United States. *American Journal of Botany*, **79**, 294-299.

Avise JC (1994) *Molecular Markers, Natural History, and Evolution*. Chapman and Hall New York.

Avise JC, Hamrick JL (1996) *Conservation genetics: case histories from nature*.

Chapman & Hall, London.

Bailey TC, Gatrell AT (1995) *Interactive spatial data analysis*. Longman Scientific & Technical. Essex, England; pp. 90-95, 103-5.

Bonello P, Bruns T, Gardes M (1998) Genetic structure of a natural population of the ectomycorrhizal fungus *Suillus pungens*. *New Phytologist,* **138**, 533-542.

Chamber SM, Cairney JWG (1999). *Pisolithus*. In: Cairney JWG, Chamber SM, eds. *Ectomycorrhizal fungi-key genera in profile*. Berlin, Germany: Springer-Verlag, 1-31.

Chung MG, Epperson BK (1999) Spatial genetic structure of clonal and sexual reproduction in populations of *Adenophora grandiflora* (Campanulaceae). *Evolution*, **53**, 1068-1078.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000). Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution*, **15**, 290-295.

Dahlberg A (2001) Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. *New Phytologist,* **150**, 555-562.

Dahlberg A, Stenlid J (1995) Spatiotemporal patterns in ectomycorrhizal populations. *Canadian Journal of Botany,* **73**, 1222-1230.

Degen B (2000) SGS: Spatial Genetic Software. Computer program and users manual. Http://kourou.cirad.fr/genetique/software.html

De La Bastide PY, Kropp BR, Piché Y (1994) Spatial distribution and temporal persistence of discrete genotypes of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton. *New Phytologist,* **127**, 547-556.

Diniz-Filho JAF, Telles MPD (2002) Spatial autocorrelation analysis and the identification of operational units for conservation in continuous populations. *Conservation Biology*, **16**, 924-935.

Dizon AE, Taylor BL, O'Corry-Crowe GM (1995) Why statistical power is necessary to link analyses of molecular variation to decisions about population structure. In *Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation* (ed. Nielsen JL, Powers DA). American Fisheries Society: Bethesda, Maryland.

Doligez A, Baril C, Joly HI (1998) Fine-scale spatial genetic structure with

nonuniform distribution of individuals. *Genetics*, **148**, 905-919.

Dreisbach TA, Smith JE, Molina R (2002) Challenges of modeling fungal habitat: when and where do you find chanterelles? In *Predicting Species Occurrence: Issues of Scale and Accuracy* (eds. Scott JM, Heglund PJ, Morrision M, Raphael M, Haufler J, Wall B). Island Press, Covello, CA.

Dunham J, Peacock M, Tracy CR, Nielsen J, Vinyard G (1999) Assessing extinction risk: integrating genetic information. *Conservation Ecology* [online], **3**, 2.

Dunham SM, Kretzer A, Pfrender ME (In Press) Characterization of Pacific golden chanterelle (*Cantharellus formosus*) genet size using co-dominant microsatellite loci. *Molecular Ecology.*

Epperson BK (1993) Recent advances in correlation studies of spatial patterns of genetic variation. *Evolutionary Biology,* **2,**: 95-155.

Epperson BK (1995) Spatial distributions of genotypes under isolation by distance. *Genetics,* **140**, 1431-1440.

Epperson BK (1997) Gene dispersal and spatial genetic structure. *Evolution,* **51**, 672-681.

Epperson BK, Huang Z, Li TQ (1999) Measures of spatial structure in samples of genotypes for multiallelic loci. *Genetical Research,* **73**, 251-261.

Epperson BK, Li TQ (1996) Measurement of genetic structure within populations using Moran's spatial autocorreation coefficient. *Proceedings of the National Academy of Science USA*, **93**, 10528-11032.

Fortin M-J, Drapeau P, Legendre P (1989) Spatial autocorrelation and sampling design in plant ecology. *Vegetatio*, **83**, 209-222.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for Basidiomycetes: Applications to the identification of mycorrhizae and rusts. *Molecular Ecology,* **2**, 113-118.

Gardes M, Bruns TD (1996) ITS-RFLP matching for identification of fungi. *Methods in Molecular Biology,* **50**, 177-186.

Gherbi H, Delaruelle C, Selosse M-A, Martin F (1999) High genetic diversity in a population of the ectomycorrhizal basidiomycete *Laccaria amethystina* in a 150 year-old beech forest. *Molecular Ecology,* **8**, 2003-2013.

Gregory PH (1973) *Microbiology of the atmosphere*, 2nd edn. Leonard Hill Books, Aylesbury, UK.

Gryta H, Debaud J-C, Effosse A, Gay G, Marmeisse R (1997) Fine-scale structure of populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum* in coastal sand dune forest ecosystems. *Molecular Ecology,* **6**, 353-364.

Gryta H, Debaud J-C, Marmeisse R (2000) Population dynamics of the symbiotic mushroom *Hebeloma cylindrosporum*: mycelial persistence and inbreeding. *Heredity,* **84**, 294-302.

Guidot A, Gryta H, Gourbière F, Debaud J, Marmeisse R. (2002) Forest habitat characteristics affect balance between sexual reproduction and clonal propagation of the ectomycorrhizal mushroom *Hebeloma cylindrosporum*. *Oikos,* **99**, 25-36.

Heywood JS (1991) Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics,* **22**, 335-355.

Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black box. *Molecular Ecology,* **10**, 1855-1871.

Hutchinson A (1989) Absence of conidia as a morphological character in ectomycorrhizal fungi. *Mycologia,* **81**, 587-594.

Ingold CT (1971) *Fungal spores: Their liberation and dispersal*. Clarendon Press, Oxford.

James TY, Vilgalys R (2001) Abundance and diversity of *Schizophyllum commune* spore clouds in the Caribbean detected by selective sampling. *Molecular Ecology*, **10**, 471-479.

Jany J-L, Garbaye J, Martin F (2002) *Cenococcum geophilum* populations show a high degree of genetic diversity in beech forests. *New Phytologist*, **154**, 651-659.

Lacey J (1996) Spore dispersal – its role in ecology and disease: the British contribution to fungal aerobiology. *Mycological Research*, **100**, 641-660.

Largent DL, Sime AD (1995) A preliminary report on the phenology, sporulation, and lifespan of *Cantharellus cibarius* and *Boletus edulis* basidiomes in Patrick Point State Park. In, *Symposium Proceedings, 43rd Annual Meeting of the California Forest Pest Council*, Nov. 16-17, 1994, (ed. Adams DH, Rios JE, Stoere AJ). Rancho Cordova, California.

Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics*, **15**, 65-95.

Magnussen S (1993). Bias in genetic variance estimates due to spatial autocorrelation. *Theoretical Applied Genetics,* **86**, 349-355.

Maniatis T, Fritsch EF, Sambrook J (1982) *Molecular cloning. A laboratory manual.* Cold Spring Harbor Laboratory, Cold Springs: Harbor, New York.

Manly BFJ (1997) *Randomization, Bootstrap and Monte Carlo Methods in Biology.* Chapman & Hall, London.

McDonald BA (1997) The population genetics of fungi: tools and techniques. *Phytopathology,* **87**, 448-453.

Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In. Allen, M. (ed.) *Mycorrhizal functioning, and integrative plant-fungal process.* New York, Chapman and Hall. Pp. 357-423.

Molina R, Pilz D, Smith J, Dunham S, Dreisbach T, O'Dell T, Castellano M (2001) Conservation and management of forest fungi in the Pacific Northwestern United States: An integrated ecosystem approach. In: *Fungal Conservation.* (eds. Moore D, Nauta M, Rotheroe M) Cambridge University Press.

Morkkynen T, Weissenbergh KV, Pappine A (1997) Estimation of dispersal gradients of S- and P-type basidiospores of *Heterobasidion annosum. European Journal of Forest Pathology,* **27**, 291-300.

Moritz C (1994) Defining "evolutionarily significant units" for conservation. *Trends in Ecology & Evolution*, **9**, 373-375.

Newton AC, Allnutt TR, Gillies ACM, Lowe AJ, Ennos RA (1999) Molecular phylogeography, intraspecific variation and the conservation of tree species. *Trends in Ecology and Evolution,* **14**, 140-145.

Oden NL (1984) Assessing the statistical significance of a spatial correlogram. *Geographical Analysis,* **16**, 1-16.

Parks JC, Werth CR (1993) A study of spatial features of clones in a population of bracken fern, *Peteridium aquilinum* (Dennstaedtiaceae). *American Journal of Botany,* **80**, 537-544.

Peakall R, Smouse PE (2001) GenAlEx V5: Genetic Analysis in Excel. Population

genetic software for teaching and research. Australian National University, Canberra, Australia. http://www.anu.edu.au/BoZo/GenAlEx/

Pilz D, Molina R (2002) Commercial harvests of edible mushrooms from the forests of the Pacific Northwest United States: issues, management, and monitoring for sustainability. *Forest Ecology and Management,* 155, 3-16.

Read DJ, Francis R, Finlay RD (1985) Mycorrhizal mycelia and nutrient cycling in plant communities. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds.). *Ecological interactions in soils.* Oxford, Blackwell Scientific Publications. pp. 193-218.

Redecker D, Szaro TM, Bowman RJ, Bruns TD (2001) Small genets of *Lactarius xanthogalactus, Russula cremoricolor* and *Amanita francheti* in late-stage ectomycorrhizal successions. *Molecular Ecology,* **10**, 1025-1034.

Redhead SA, Norvell LL, Danell E (1997) *Cantharellus formosus* and the Pacific Golden Chanterelle harvest in Western North America. *Mycotaxon* **65**: 285-322.

Reusch TBH, Hukriede W, Stam WT, Olsen JL (1999a) Differentiating between clonal growth and limited gene flow using spatial autocorrelation of microsatellites. *Heredity,* **83**, 120-126.

Reusch TBH, Stam WT, Olsen JL (1999b) Size and estimated age of genets in eelgrass *Zostera marina* L. assessed with microsatellite markers. *Marine Biology,* **133**, 519-525.

Ruckelshaus MH (1998) Spatial scale of genetic structure and an indirect estimate of gene flow in eelgrass, *Zostera marina. Evolution,* **52**, 330-343.

Selosse M-A, Jacquot D, Bouchard D, Martin F, Le Tacon F (1998) Temporal persistence and spatial distribution of an American inoculant strain of the ectomycorrhizal basidiomycete *Laccaria bicolor* in European forest plantations. *Molecular Ecology,* **7**, 561-573.

Selosse M-A, Martin F, Le Tacon F (2001) Intraspecific variation in fruiting phenology in an ectomycorrhizal *Laccaria* population under Douglas fir. *Mycological Research,* **105**, 524-531.

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina, R (1997) Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature,* **388**, 579-582.

Slatkin M, Barton NH (1989). A comparison of three indirect methods for estimating

average levels of gene flow. *Evolution,* **4**, 1349-1368.

Smith SE, Read DJ (1997) *Mycorrhizal symbiosis*, 2nd ed. Academic Press.

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity,* **82**, 561-573.

Sokal RR, Jacquez GM (1991) Testing inferences about micro-evolutionary processes by means of spatial autocorrelation analysis. *Evolution*, **45**, 152-168.

Sokal RR, Oden NL (1978) Spatial autocorrelation in biology 1. *Methodology Biological Journal of the Linnean Society,* **10**, 199-228.

Sokal RR, Oden NL, Thomson BA (1997) A simulation study of microevolutionary inferences by spatial autocorrelation analysis. *Biological Journal of the Linnean Society*, **65**, 41-62.

Sokal RR, Wartenberg DE (1983) A test of spatial autocorrelation analysis using an isolation-by-distance model. *Genetics,* **105**, 219-237.

Sork VL, Nason J, Campbell DR, Fernandez JF (1999) Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology & Evolution*, **14**, 219-224.

Ueno S, Tomaru N, Yoshimaru H, Manabe T, Yamamoto S (2000) Genetic structure of *Camellia japonica* L. in an old-growth evergreen forest, Tsushima, Japan. *Molecular Ecology,* **9**, 647-656.

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: A Guide to methods and Applications* (ed. N Innis, D Gelfand, J Sninsky, and T White). New York Academic Press, New York, NY, USA.

Wiens JA (1989) Spatial scaling in ecology. *Functional Ecology,* **3**, 385-397.

Wolfenbarger DO (1946) Dispersion of small organisms. *American Midland Naturalist*, **35**, 1-152.

Wright S (1951). The genetical structure of populations. *Annual Eugenetics,* **15**, 323-354.

Wright S (1969) *Evolution and the Genetics of Populations*. Vol. 2. The Theory of Gene Frequencies. University of Chicago Press, Chicago.

Zhou Z, Miwa M, Hogetsu T (2001) Polymorphism of simple sequence repeats reveals gene flow within and between ectomycorrhizal *Suillus grevillei* populations. *New Phytologist,* **149**, 339-348.

CONCLUSIONS

Contributions to Knowledge of Chanterelle Ecology

Genet size - Five novel co-dominant microsatellite markers were used to study Pacific golden chanterelle genet size and its relation to forest age and disturbance in stands dominated by Douglas-fir. Putative '*C. formosus*' fruit-body collections included a cryptic chanterelle species and all genetic evidence indicated that the two are well differentiated from each other. Small genets were characterized for both species with mean maximum widths of 3.2 m ± 3.6 m for *C. formosus* and 1.5 m ± 1.7 m for the alternate genetic group (named *C. cascadensis* nom. prov. in Ch. 2). Variance in genet size was high but it is not clear if this simply is due to lack of resolving power at the loci used. Despite the error associated with genet size estimates there was no evidence that genet size differed across the three disturbance treatments.

Taxonomy - Researchers recently have used morphological and genetic data to identify the yellow chanterelle most frequently harvested from the Pacific Northwest forests as *C. formosus*, a species once thought to be rare in the region. Chapter 2 contains three genetic data sets and one morphological data set that characterize a previously undescribed, species of yellow chanterelle (*C. cascadensis* nom. prov.) with morphology very similar to *C. formosus*. Phylogenetic analyses of the nrDNA large subunit and ITS regions show that *C. cascadensis* nom. prov., along with two other yellow chanterelle taxa (*C. cibarius* var. *roseocanus* and European *C. cibarius*), are more closely related to white chanterelles (*C. subalbidus*) than they are to *C.*

formosus. Data from five microsatellite loci provide evidence that *C. formosus*, *C. subalbidus*, and *C. cascadensis* nom. prov. do not interbreed when they co-occur spatially and temporally in Douglas fir forests. This demonstrates that these three sympatric chanterelles are biological species with boundaries congruent with those delineated by nrDNA phylogenetic clades. Morphological data indicate that careful examination of pileus color and stipe shape can be used to separate fresh collections of the two yellow species.

Chanterelle Habitat Associations within Douglas-fir – Watershed-scale habitat associations were determined for three chanterelle species with respect to stand age and harvesting disturbance across the HJ Andrews experimental forest (HJA) in 1998 and 2000. Changes in precipitation reduced chanterelle productivity by 52 % in 2000 compared to 1998, with larger reductions occurring in old growth stands. At the watershed scale where stands of 10-20 ha are the sampling units stand age is a good predictor of the distribution of *C. subalbidus* and *C. formosus*, but is only marginally important for *C. cascadensis* nom. prov. The habitat associations of *C. cascadensis* nom. prov.were difficult to model using this study design because the species is rare throughout the study area. Abundance of *C. cascadensis* nom. prov. individuals increased substantially with decreasing elevation in both study years indicating that landscape features other than stand age may be more important in modeling its distribution.

Fine Scale Genetic Structure - Little is currently known about the origin and maintenance of genetic diversity in EM fungal populations beyond the scale of plots used to study genet size. In broadly distributed species like *C. formosus*, knowledge of within-population genetic structure is a prerequisite for planning larger scale studies upon which management decisions are based and contributes to our understanding of life history parameters difficult to measure without genetic data. Fruit bodies from 183 *C. formosus* genets were mapped and collected from a 50 year old, 50 ha stand of Douglas-fir naturally regenerated from clear-cut harvest. The spatial distribution of multilocus genotypes was studied using spatial autocorrelation analysis. Statistically significant but weak spatial autocorrelation was detected in the two smallest distance classes estimating a 200-250 meter patch size for *C. formosus*. This result indicates that either limited spore dispersal possibly coupled with inbreeding, long-lived individuals fragmented over large areas, or some interaction between these processes works to maintain fine scale genetic structure in this species.

Considerations for Co-dominant Marker Development in EM Fungi

Development of population genetic markers - The overall efficiency of attempts isolate microsatellites from the *C. formosus* genome was low relative to that observed for non-fungal taxa. Prior to performing enrichment attempts several *C. formosus* collections were screened with ISSR primers based on various microsatellite motifs. ISSR fingerprint profiles indicated that the repeat types used in enrichments were fairly common in *C. formosus*. Use of these markers to guide motif selection may have

resulted in poor choices if the primers were able to anneal to imperfect repeats or if ISSR profiles exhibiting discrete bands (rather than smears) are actually indicative of low repeat abundance.

The low efficiency of enrichments may also reflect the true abundance of microsatellite motifs in the *C. formosus* genome. Several studies have reported a positive correlation between the frequency of microsatellite regions and total genome size (e.g., Field & Wills 1998; Kubis *et al.* 1998; Primmer *et al.* 1997; Hancock 1999). For example the avian genome is roughly one-third the size of the human genome (~1,200 Mb for the domestic chicken *Gallus gallus*; Bloom *et al.* 1993; Wachtel & Tiersch 1993) and recent research has demonstrated that microsatellite repeats occur less frequently in birds relative to other vertebrates (Primmer *et al.* 1997). The few fungal genomes that have been well characterized are modest in size with high gene density (Goffeau *et al.* 1996) with average basidiomycete genome size ranging around 35-40 Mb (e.g., Ramírez *et al.* 2000), roughly 30 times smaller than the typical avian genome. Microsatellites predominantly occur in noncoding regions, and if the proportion of noncoding DNA in the genome acts as the main constraint for the evolution of simple repeats, both the relative and absolute numbers of repeats in fungal genomes may naturally be low.

There are several recent examples of attempts to isolate microsatellite loci from fungal genomes (Kretzer *et al.* 2000; Langrell *et al.* 2001; Sirjusingh & Kohn 2001; Zhou *et al.* 2001) but the efficiency of each is either not well described or not comparable to those described here (e.g., no enrichment protocols were used), making

it difficult to discern from these studies if microsatellites truly are rare in fungi. Total repeat length in the loci described here, and for other fungal species generally are shorter than those published for other (non-fungi) taxa, which limits their variability. Further research on the abundance of microsatellites in fungi is needed to clarify if the low number of loci characterized in this study is the result of factors involved in the generation of simple repeats, selection against "superfluous" repetitive DNA, or low abundance of the repeat motifs we enriched for in the *C. formosus* genome.

Management implications

Sampling populations of C. formosus and other EM fungi - Wright (1969) showed analytically that when sampling units contain multiple genetic neighborhoods the genetic variance among sampling units relative to the total variance is reduced and the power to detect spatial genetic structure is lost. This logistical problem is important to take into consideration in the study of EM fungal populations because fruit-body production (and organism detection) only occurs during specific times of the year and can be variable from year-to-year. As a result, the distributions of most EM species are not well documented (Dreisbach *et al.* 2002). Spatial autocorrelation methods are powerful tools for detecting genetic differentiation over a range of spatial scales that allow definition of appropriate sampling networks. Spatial analysis of multilocus genotypes from sexually reproduced *C. formosus* genets indicates that the radius of genetically homogenous patches resulting from isolation-by-distance is 200 m. This strongly supports the hypothesis that spore dispersal and genet establishment is

spatially restricted in *C. formosus*. Regardless of the evolutionary mechanism driving the observed spatial genetic structure, these results have important implications for the design of population genetic studies at larger spatial scales and can be used to design sampling networks that will capture informative variability in genetic patterns and processes at spatial scales relevant to management. The x-axis intercept of a correlogram defines geographic distance between genetically independent samples (Diniz-Filho & Telles 2002). Collecting samples separated by this minimum distance will better characterize the genetic heterogeneity distributed across the landscape by avoiding pseudoreplication of genetic variability distributed within patches. For the purpose of conservation and management, all genets situated at geographic distances shorter than 250 m could be considered unique genetic units.

The genetic structure of EM fungal populations is likely intimately connected to many aspects of EM ecology. Because of this, the scale of genetic population structure may be important to consider in studies designed to determine habitat associations or community structure. For example, stand level data collected to characterize chanterelle habitat associations (Chapter 3) may suffer from pseudoreplication resulting in low statistical power and broad confidence intervals around the logistic regression parameter estimates. For conservation and management purposes, data from studies with this type of sampling error can lead to misguided decisions because the chance of committing type II statistical errors (accepting the null hypothesis of no difference when this conclusion is false) is increased. For *C. formosus* these conclusions are tentative because the scope of inference for this study

is limited to the single 50 year-old Douglas-fir stand sampled. Replication of this research across environmental conditions and EM species will increase the rigor of these conclusions and the strength of recommendations for sampling EM fungal populations.

Conservation issues

Rarity of C. cascadensis nom. prov. – To date, *C. cascadensis* nom. prov. has been collected only in forests dominated by Douglas-fir despite efforts to locate this species in other forest types. Relative to *C. formosus* and *C. subalbidus* the total number of *C. cascadensis* nom. prov. collections observed during the course of my research was much lower and detection of this species across the HJA varied greatly between 1998 and 2000. The patchy distribution of *C. cascadensis* nom. prov. across the study area made delineation of its habitat associations difficult given the study design employed. While, the association of *C. cascadensis* nom. prov. with OG habitats over 600 m in elevation was statistically significant in 1998, the over all implications of this result for the species are likely of marginal importance. It is clear that environmental variables correlated with stand age have some control over the distribution of *C. cascadensis* nom. prov. but this relationship is not constant over the elevation range sampled. In addition, factors that control the abundance of *C. cascadensis* nom. prov. genets present in a stand are unclear. This study clearly did an inadequate job of sampling the range of habitats in which this species occurs. It was clear that *C. cascadensis* nom. prov. is rare in the study area relative to other chanterelles and this

taxon currently is known only from a restricted range in and adjacent to the HJA with single collections from two sites in southern Oregon. Much survey work still is required to fully understand its distribution in Oregon and future survey work should focus on lower elevation habitats. Given the potential rarity of *C. cascadensis* nom. prov.it might qualify for protection under survey and manage guidelines outlined in the Northwest Forest Plan.

Old growth association of C. subalbidus – Results from the study of chanterelle habitat associations demonstrate that the probability of locating *C. subalbidus* fruit bodies increases significantly in OG habitats. The Pacific Northwest (PNW) region provides a large share of the world timber supply (Waddell *et al.* 1989). Clear cut harvesting followed by subsequent even-aged forest management has been ongoing on private lands for well over a century (Robbins 1988) and on federal lands since the 1940's (Harris 1984). During the past few decades, rotation lengths on public lands have generally been in the range of 80-100 years on 10-20 ha cutting units while private landowners generally cut larger areas on rotation lengths of 50 years (Spies *et al.* 1994). This intense forest harvest activity has resulted in profound alterations in the age structure of the forest environment since European settlement (Wallin *et al.* 1996). Recent estimates of the percentage of old growth forests > 200 years old remaining from pre-logging time periods range from 13.1% (Spies & Franklin 1988; Booth 1991) to 17.5% (Haynes 1986). Conversion of PNW forests from OG to SG may have significantly reduced the distribution of *C. subalbidus* in the western Cascades.

If these results from the HJA can be extended to other areas, *C. subalbidus* has likely been largely excluded from private lands with short cutting rotations and possibly also from public lands if 80-100 year rotation cycles are not long enough for it to establish and maintain viable populations. The impact of habitat loss and fragmentation may be exacerbated by year-to-year variation in *C. subalbidus* productivity. The total number of chanterelle fruit-bodies detected during the second year of the habitat association study (2000) was 52% of the number collected in 1998 but the reduction in fruiting was not uniform across the three species or the two habitats sampled. The reduction in fruiting abundance in 2000 was most evident for *C. subalbidus* with the number of fruit bodies collected declining by 69% compared to 1998. For this species, reductions in abundance were most prevalent in OG with all nine stands showing at least a 2-fold reduction in abundance in 2000 compared to only four SG stands showing reductions of similar magnitude. In contrast, *C. formosus* numbers in 2000 were only 31% lower that those in 1998 with five OG and four SG stands showing 2-fold or greater abundance reductions. The ability of *C. subalbidus* populations isolated in small habitat fragments to persist through periods of climatic fluctuations will depend on genet longevity and how long spores remain viable in the soil prior to germination. We do not understand these life history variables very well for any species of EM fungi but they are important to consider in predicting the long-term impacts of habitat fragmentation on *C. subalbidus*. Future research on this species should be directed towards identifying the suite of factors that limit its distribution and determining how rapidly optimal conditions for its growth develop as

SG stands continue to mature beyond 50 years.

Cryptic species – Morphological analysis of a suite of phenotypic characters that exhibit variability resulting from both evolutionary divergence and environmental plasticity has led to a taxonomy that inadequately characterizes the species diversity within *Cantharellus*. Achieving a taxonomy based on biological species boundaries requires characters that can demonstrate the genetically based discontinuities between evolutionarily independent groups. The molecular analyses presented in Chapter 2 represent the most rigorous estimation of species boundaries in *Cantharellus* to date. Both phylogenetic and biological species recognition criteria (Taylor *et al.* 2000) were used to diagnose a cryptic species within *Cantharellus* in the PNW.

Molecular markers have been used in other genera to distinguish fungal taxa difficult to characterize by traditional morphological means (Anderson *et al.* 1987; Bruns *et al.* 1991; Fukuda *et al.* 1994). Global F_{st} values indicate that *C. cascadensis* nom. prov. is differentiated from *C. subalbidus* and *C. formosus* at similar levels even though nrDNA sequences divergence values differ drastically in these pairwise comparisons. *C. cascadensis* nom. prov. differs from *C. subalbidus* at only 1.5-2.2% of the 989 ITS nucleotide sites examined while it differs from *C. formosus* at 7.5-8.0% of nucleotide sites. The 1.5-2.2% divergence observed between *C. cascadensis* nom. prov. and *C. subalbidus* is within the range of intraspecific ITS sequence variability reported for *Cenococcum geophilum* (0.0-4.0%; Shinohara *et al.* 1999), *Marasmius quercophilus* (1.11-1.25%; Farnet *et al.* 1999), *Galerina marginata* (0.3-2.7%; Gulden

et al. 2001), and *Phialophora americana* (up to 2%; Yan *et al.* 1995), and similar to that observed between closely related species in *Hebeloma* (Aanen *et al.* 2000), *Dermocybe* (Liu *et al.* 1997), *Sarcodon* (Johannesson *et al.* 1999), and *Cortinarius* (Høiland & Holst-Jensen 2000). Other studies have reported identical ITS sequences for morphologically distinguishable ascomycete species (Harrington & Potter 1997; Seifert *et al.* 1995) including those demonstrated to differ in ecology and isozyme variability (Harrington & McNew 1998; Harrington *et al.* 1996; Witthuhn *et al.* 1998). Collectively, these results demonstrate that, when working at the interface between population level variation and species boundaries, the meaning of sequence variability in these regions requires case specific interpretation. For example, the substantial sorting of microsatellite alleles within *C. cascadensis* nom. prov. and *C. subalbidus* indicates that the 0.7-0.8% divergence observed between *C. cibarius* var. *roseocanus* and *C. cibarius* var. *cibarius* in the ITS region may be large enough to indicate species level differentiation.

A solid taxonomic underpinning is critical for conservation, because we must first know the abundance and distribution of species before we can ascertain that a species or community is in need of protection. Since many conservation efforts focus on maintaining species level biodiversity, it is important for taxonomy to accurately reflect species diversity (Avise 1989). Accurate delineation of fungal species is required for the preservation of biodiversity and ecosystem function because many regions are rich in ectomycorrhizal diversity. PNW forest ecosystems are dependent on ectomycorrhizal symbioses, and we do not understand which taxa are most

important to the maintenance of ecosystem health. If our knowledge of *Cantharellus* species concepts is similar to those for other major genera of ectomycorrhizal fungi in the PNW, these species-based conservation efforts will likely miss many rare, cryptic species of unknown ecological importance. There currently is no way to determine if the conservation of other well described but rare taxa will protect this unknown diversity in PNW forests.

References

Aanen DK, Kuyper TW, Boekhout T, Hoekstra RF (2000). Phylogenetic relationships in the genus *Hebeloma* based on ITS 1 and 2 sequences, with special emphasis on the *Hebeloma crustuliniforme* complex. *Mycologia,* **92**, 269-281.

Anderson JB, Petsche DM, Smith ML (1987) Restriction fragment polymorphisms in biological species of *Armillaria mellea. Mycologia,* **79**, 69-76

Avise JC (1989) A role for molecular genetics in the recognition and conservation of endangered species. *Trends in Ecology and Evolution,* **4**, 279-281.

Bloom SE, Delany ME, Muscarella DE (1993) Constant and variable features of avian chromosomes. In *Manipulation of the avian genome* (ed. Etches RJ, Verrinder Gibbins AM), pp. 39–60. CRC Press, Boca Raton, Florida.

Booth DE (1991) Estimating prelogging old-growth in the Pacific Northwest. *Journal of Forestry*, **89**, 25-29

Bruns TD, White TJ, Taylor JW (1991) Fungal molecular systematics. *Annual Review of Ecology and Systematics,* **22**, 525-564.

Diniz-Filho JAF, Telles MPD (2002) Spatial autocorrelation analysis and the identification of operational units for conservation in continuous populations. *Conservation Biology*, **16**, 924-935.

Dreisbach TA, Smith JE, Molina R (2002) Challenges of modeling fungal habitat: when and where do you find chanterelles? In *Predicting Species Occurrence: Issues of Scale and Accuracy* (eds. Scott JM, Heglund PJ, Morrision M, Raphael M, Haufler J, Wall B). Island Press, Covello, CA.

Farnet AM, Roux M, Le Petit J (1999) Genotypic variations among isolates of *Marasmius quercophilus*, a white-rot fungus isolated from evergreen oak litter. *Canadian Journal of Botany,* **77**, 884-890.

Field D, Wills C (1998) Abundant microsatellite polymorphism in *Saccharomyces cerevisiae*, and the different distributions of microsatellites in eight prokaryotes and *S. cerevisiae*, result from strong mutation pressures and a variety of selective forces. *Proceedings of the National Academy of Science* U.S.A., **95**, 1647–1652.

Fukuda M, Nakai YF, Hibbett DS, Matsumoto T, Hayashi Y (1994) Mitochondrial DNA restriction fragment length polymorphisms in natural populations of *Lentinula edodes. Mycological Research,* **98**, 169-175.

Goffeau A, Barrell BG, Bussey H, *et al.* (1996) Life with 6000 genes. *Science,* **274**, 563-567.

Gulden G, Dunham S, Stockman J (2001) DNA studies in the *Galerian marginata* complex. *Mycological Research,* **105**, 432-440.

Hancock JM (1999) Microsatellites and other simple sequences: genomic context and mutational mechanisms. In *Microsatellites: evolution and applications*. (ed. Goldstein DB, Schlötterer C. Oxford University Press, Oxford. pp. 1–9.

Harrington FA, McNew DL (1998) Partial interfertility among the *Ceratocystis* species on conifers. *Fungal Genetics and Biology,* **25**, 44-53.

Harrington FA, Potter D (1997) Phylogenetic relationships within *Sarcoscypha* based on nucleotide sequences of the internal transcribed spacer of nuclear ribosomal DNA *Mycologia,* **89**, 258-267.

Harrington TC, Steimel J, Wingfield MJ, Kile GA (1996) Isozyme variation and species delimitation in the *Ceratocystis coerulescens* complex. *Mycologia,* **88**, 104-113.

Harris LD (1984) *The Fragmented Forest*. University of Chicago Press, Chicago, IL.

Haynes RW (1986) *Inventory and value of old-growth in the Douglas-fir region.* USDA Forest Service Research Note PNW-437, 18p.

Høiland K, Holst-Jensen A (2000) *Cortinarius* phylogeny and possible taxonomic implications of ITS rDNA sequences. *Mycologia,* **92**, 694-710.

Johannesson H, Ryman S, Lundmark H, Danell E (1999) *Sarcodon imbricatus* and *S. squamosus* – two confused species. *Mycological Research,* **103**, 1447-1452.

Kubis S, Schmidt T, Heslop-Harrison JS (1998) Repetitive DNA elements as a major component of plant genomes. *Annals of Botany,* **82**(Suppl. A), 45–55.

Kretzer AM, Molina R, Spatafora JW (2000) Microsatellite markers for the ectomycorrhizal basidiomycete *Rhizopogon vinicolor. Molecular Ecology* **9**, 1171-1193.

Langrell SRH, Lung-Escarmant B, Decroocq S (2001) Isolation and characterization of polymorphic simple sequence repeat loci in *Armillaria ostoyae. Molecular Ecology Notes* **1**, 305-307.

Liu Y, Ammirati JF, Rogers SO (1997) Phylogenetic relationships in *Dermocybe* and related *Cortinarius* taxa based on nuclear ribosomal RNA internal transcribed spacers. *Canadian Journal of Botany,* **75**, 519-532.

Peakall R, Smouse PE (2001) GenAlEx V5: Genetic Analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia. http://www.anu.edu.au/BoZo/GenAlEx/

Primmer CR, Raudsepp T, Chowdhary BP, Møller AP, and Ellegren H (1997) Low frequency of microsatellites in the avian genome. *Genome Research,* **7**, 471–482.

Ramírez L, Larraya LM, Pisabarro AG (2000) Molecular tools for breeding basidiomycetes. *International Microbiology,* **3**, 147-152.

Robbins WG (1988) Hard times in paradise, Coos Bay, Oregon 1850-1986. University of Washington Press, Seattle, WA.

Seifert KA, Wingfield BD, Wingfield MJ (1995) A critique of DNA sequence analysis in the taxonomy of filamentous Ascomycetes and ascomycetous anamorphs. *Canadian Journal of Botany,* **73** (Suppl. 1), 760-767.

Shinohara ML, Lobuglio KF, Rogers SO (1999) Comparison of ribosomal DNA ITS regions among geographic isolates of *Cenococcum geophilum. Current Genetics,* **35**, 527-535.

Sirjusingh C, Kohn LM (2001) Characterization of microsatellites in the fungal plant pathogen, *Sclerotinia sclerotiorum. Molecular Ecology Notes* **1**, 267-269.

Sokal RR, Wartenberg DE (1983) A test of spatial autocorrelation analysis using an

isolation-by-distance model. *Genetics,* **105**, 219-237.

Spies TA, Franklin JF (1988) Old-growth and forest dynamics in the Douglas-fir region of western Oregon and Washington. *Natural Areas Journal,* **8**, 190-201.

Spies TA, Ripple WJ, Bradshaw GA (1994) Dynamics and pattern of a managed coniferous forest landscape. *Ecological Applications,* 4, 555-568.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology,* **31**, 21-32.

Wachtel SS, Tiersch TR (1993) Variations in genome mass. *Comparative Biochemistry and Physiology,* **104B**, 207–213.

Waddell KL, Oswald DD, Powell DS (1989) *Forest statistics of the United States, 1987.* Pacific Northwest Forest and Range Experiment Station, U.S. Department of Agriculture Forest Service, Resource Bulletin PNW-RB-168.

Wallin DO, Swanson FJ, Marks B, Cissel JH, Kertis J (1996) Comparison of managed and pre-settlement landscape dynamics in forests of the Pacific Northwest, USA. *Forest Ecology and Management,* **85**, 291-309.

Witthuhn RC, Wingfield BD, Wingfield MJ, Wolfaard M, Harrington TC (1998) Monophyly of the conifer species in the *Ceratocystis coerulescens* complex using DNA sequence data. *Mycologia,* **90**, 96-101.

Wright S (1969) *Evolution and the Genetics of Populations*. Vol. 2. The Theory of Gene Frequencies. University of Chicago Press, Chicago.

Yan ZH, Rogers SO, Wang CJK (1995) Assessment of *Phialophora* species based on ribosomal DNA internal transcribed spacers and morphology. *Mycologia,* **87**, 72-83.

Zhou Z, Miwa M, Hogetsu T (2001) Polymorphism of simple sequence repeats reveals gene flow within and between ectomycorrhizal *Suillus grevillei* populations. *New Phytologist* **149**, 339-348.

BIBLIOGRAPHY

Aanen DK, Kuyper TW, Boekhout T, Hoekstra RF (2000). Phylogenetic relationships in the genus *Hebeloma* based on ITS 1 and 2 sequences, with special emphasis on the *Hebeloma crustuliniforme* complex. *Mycologia,* **92**, 269-281.

Agee JK (1993) Fire ecology of Pacific Northwest forests. Island Press, Washington, D.C.

Alig RJ, Zheng D, Spies TA, Butler BJ (2000) *Forest cover dynamics in the Pacific Northwest west side: Regional trends and projections*. USDA Forest Service Research Paper PNW-RP-522.

Allison PD (1999*) Logistic Regression using the SAS System: Theory and Application.* SAS Institute Inc., Cary, NC, USA.

Amaranthus MP, Parrish DS, Perry DA (1989) Decaying logs as moisture reservoirs after drought and wildfire. In *Stewardship of Soil, Air and Water Resources, Proceedings of Watershed 89* (ed. E Alexander), pp. 191-194. USDA Forest Service. R10-MB-77. USDA Forest Service, Alaska Region: Juneau, AK.

Amaranthus MP, Perry DA (1987) Effect of soil transfer on ectomycorrhiza formation and survival and growth of conifer seedlings in disturbed forest sites. *Canadian Journal of Forest Research,* **17**, 944-950.

Anderson IC, Chambers SM, Cairney JWG (1998) Use of molecular methods to estimate the size and distribution of mycelial individuals of the ectomycorrhizal basidomycete *Pisolithus tinctorius. Mycological Research,* **102**, 295-300.

Anderson JB, Petsche DM, Smith ML (1987) Restriction fragment polymorphisms in biological species of *Armillaria mellea. Mycologia,* **79**, 69-76.

Anderson JB, Stasovski E (1992) Molecular phylogeny of northern hemisphere species of *Armillaria* species. *Mycologia,* **84**, 505-516.

Arnolds E (2001) The future of fungi in Europe: threats, conservation and management. In Moore D, Nauta MM, Evans SE, Rotheroe M. *Fungal Conservation: Issues and Solutions*. Cambridge University Press.

Aspinwall N, Christian T (1992) Clonal structure, genotypic diversity, and seed production in populations of *Filipendula rubra* (Rosaceae) from the north central United States. *American Journal of Botany,* **79**, 294-299.

Avise JC (1989) A role for molecular genetics in the recognition and conservation of endangered species. *Trends in Ecology and Evolution,* **4**, 279-281.

Avise JC (1994) *Molecular Markers, Natural History, and Evolution*. Chapman and Hall New York.

Avise JC, Ball Jr. RM (1990) Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Survey of Evolutionary Biology,* **7**, 45-67.

Avise JC, Hamrick JL (1996) *Conservation genetics: case histories from nature.* Chapman & Hall, London.

Baar J, Horton TR, Kretzer AM, Bruns TD (1999) Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. *New Phytologist,* **143**, 409-418.

Baar J, Ozinga WA, Kuyper TW (1994) Spatial distribution of *Laccaria bicolor* genets reflected by sporocarps after removal of litter and humus layers in a *Pinus sylvestris* forest. *Mycological Research,* **98**, 726-728.

Bailey TC, Gatrell AT (1995) *Interactive spatial data analysis*. Longman Scientific & Technical. Essex, England; pp. 90-95, 103-5.

Berg Å, Ehnström B, Gustafsson L, Hallingbäck T, Jonsell M, Weslien J (1994) Threatened plant, animal, and fungus species in Swedish forests: Distributions and habitat associations. *Conservation Biology*, **8**, 718-731.

Bergemann SE, Largent DL (2000) The site specific variables that correlate with the distribution of the Pacific Golden Chanterelle, *Cantharellus formosus. Forest Ecology and Managemen,t* 130, 99-107.

Bloom SE, Delany ME, Muscarella DE (1993) Constant and variable features of avian chromosomes. In *Manipulation of the avian genome* (ed. Etches RJ, Verrinder Gibbins AM), pp. 39–60. CRC Press, Boca Raton, Florida.

Bonello P, Bruns TD, Gardes M (1998) Genetic structure of a natural population of the ectomycorrhizal fungus *Suillus pungens. New Phytologist,* **138**, 533-542.

Booth DE (1991) Estimating prelogging old-growth in the Pacific Northwest. *Journal of Forestry*, **89**, 25-29.

Bramley PM, Mackenzie A (1992) Carotenoid biosynthesis and its regulation in fungi.

Pgs. 401-444 *in* Aroroa D, Elander R, Mukerji K (eds.) *Hanbook of applied mycology*. Vol. 4. Fungal biotechnology. Marcel Dekker, New York.

Bruns TD, White TJ, Taylor JW (1991) Fungal molecular systematics. *Annual Review of Ecology and Systematics,* **22**, 525-564.

Bruns TD, Vilgalys R, Barns SM, Gonzales D, Hibbett DS, Lane DJ, Simon L, Stickel S, Szaro TM, Weisberg WG, Sogin ML (1992) Evolutionary relationships within the fungi: Analyses of nuclear small subunit rRNA sequences. *Molecular Phylogenetics and Evolution,* **1**, 231-241.

Burt A, Carter DA, Koenig GL, White TJ, Taylor JW (1996) Molecular markers reveal cryptic sex in the human pathogen *Coccidioides immitis*. *Proceedings of the National Academy of Science, USA,* **93**, 770-773.

Burt A, Dechairo BM, Koenig GL, Carter DA, White TJ, Taylor JW (1997) Molecular markers reveal differentiation among isolates of *Coccidioides immitis* from California, Arizona, and Texas. *Molecular Ecology,* **6**, 781-786.

Chakraborty R, Zhong Y (1994) Statistical power of an exact test of Hardy-Weinberg proportions of genotypic data at a multiallelic locus. *Human Heredity*, **44**, 1-9.

Chamber SM, Cairney JWG (1999). *Pisolithus*. In: Cairney JWG, Chamber SM, eds. *Ectomycorrhizal fungi-key genera in profile*. Berlin, Germany: Springer-Verlag, 1-31.

Chung MG, Epperson BK (1999) Spatial genetic structure of clonal and sexual reproduction in populations of *Adenophora grandiflora* (Campanulaceae). *Evolution*, **53**, 1068-1078.

Cissel JH, Swanson FJ, Weisberg PJ (1999) Landscape management using historical fire regimes: Blue River, Oregon. *Ecological Applications,* 9, 1217-1231.

Colgan W, Carey AB, Trappe JM, Molina R, Thysell D (1999) Diversity and productivity of hypogeous fungal sporocarps in a variably thinned Douglar-fir forest. *Canadian Journal of Forest Research*, **29**, 1259-1268.

Corner EJH (1966) *A monograph of Cantharelloid fungi*. Oxford University Press, London 255 pgs.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000). Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution*, **15**, 290-295.

Dahlberg A (1997) Population ecology of *Suillus variegatus* in old Swedish Scots pine forests. *Mycological Research,* **101**, 47-54.

Dahlberg A (2001) Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. *New Phytologist,* **150**, 555-562.

Dahlman M, Danell E, Spatafora JW (2000) Molecular systematics of *Craterellus*: cladistic analysis of nuclear LSU rDNA sequence data. *Mycological Research,* **104**, 388-394.

Dahlberg A, Stenlid J (1990) Population structure and dynamics in *Suillus bovinus* as indicated by spatial distribution of fungal clones. *New Phytologist,* **115**, 487-493.

Dahlberg A, Stenlid J (1994) Population ecology of *Suillus variegatus* in old Swedish Scots pine forests. *Mycological Research,* **101**, 47-54.

Dahlberg A, Stenlid J (1995) Spatiotemporal patterns in ectomycorrhizal populations. *Canadian Journal of Botany,* **73**, S1222-S1230.

Danell E (1995) Comparisons between Swedish *Cantharellus cibarius* and *Cantharellus* spp. in the Pacific Northwest, based on differences in RFLP patterns of the ITS region. Abstract in *Inoculum,* **46**, 10.

Danell E (1999) *Cantharellus*. In Cairney JWG, Chambers SM (eds), *Ectomycorrhizal Fungi: Key Genera in Profile*. Springer-Verlag, pp 253-267.

Dataxiom Software Inc. (2001) *StatMost: Statistical analysis and graphics, 4th edition*. Los Angeles, CA.

Degen B (2000) SGS: Spatial Genetic Software. Computer program and users manual. Http://kourou.cirad.fr/genetique/software.html

De La Bastide PY, Kropp BR, Piché Y (1994) Spatial distribution and temporal persistence of discrete genotypes of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton. *New Phytologist,* **127**, 547-556.

Dickie IA, Xu B, Koide RT (2002) Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytologist,* **156**, 527-535.

Diniz-Filho JAF, Telles MPD (2002) Spatial autocorrelation analysis and the identification of operational units for conservation in continuous populations. *Conservation Biology*, **16**, 924-935.

Dizon AE, Taylor BL, O'Corry-Crowe GM (1995) Why statistical power is necessary to link analyses of molecular variation to decisions about population structure. In *Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation* (ed. Nielsen JL, Powers DA). American Fisheries Society: Bethesda, Maryland.

Doligez A, Baril C, Joly HI (1998) Fine-scale spatial genetic structure with nonuniform distribution of individuals. *Genetics*, **148**, 905-919.

Dreisbach TA, Smith JE, Molina R (2002) Challenges of modeling fungal habitat: when and where do you find chanterelles? In *Predicting Species Occurrence: Issues of Scale and Accuracy* (eds. Scott JM, Heglund PJ, Morrision M, Raphael M, Haufler J, Wall B). Island Press, Covello, CA.

Dunham J, Peacock M, Tracy CR, Nielsen J, Vinyard G (1999) Assessing extinction risk: integrating genetic information. *Conservation Ecology* [online], **3**, 2.

Dunham SM, Kretzer A, Pfrender ME (In Press) Characterization of the extent and distribution of Pacific golden chanterelle (*Cantharellus formosus*) individuals using co-dominant microsatellite loci. *Molecular Ecology*.

Epperson BK (1993) Recent advances in correlation studies of spatial patterns of genetic variation. *Evolutionary Biology*, **2**,: 95-155.

Epperson BK (1995) Spatial distributions of genotypes under isolation by distance. *Genetics*, **140**, 1431-1440.

Epperson BK (1997) Gene dispersal and spatial genetic structure. *Evolution*, **51**, 672-681.

Epperson BK, Huang Z, Li TQ (1999) Measures of spatial structure in samples of genotypes for multiallelic loci. *Genetical Research*, **73**, 251-261.

Epperson BK, Li TQ (1996) Measurement of genetic structure within populations using Moran's spatial autocorreation coefficient. *Proceedings of the National Academy of Science USA*, **93**, 10528-11032.

Farnet AM, Roux M, Le Petit J (1999) Genotypic variations among isolates of *Marasmius quercophilus*, a white-rot fungus isolated from evergreen oak litter. *Canadian Journal of Botany*, **77**, 884-890.

Feibelman T, Bayman P, Cibula WG (1994) Length variation in the internal transcribed spacer of ribosomal DNA in chanterelles. *Mycological Research*, **98**, 614-618.

Feibelman TP, Doudrick RL, Cibula WG, Bennett JW (1997) Phylogenetic relationships within the Cantharellaceae inferred from sequence analysis of the nuclear large subunit rDNA. *Mycological Research,* **101**, 1423-1430.

Felsenstein J (1978) Cases in which parsimony or compatibility methods will be positively misleading. *Systematic Zoology,* **27**, 401-410.

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* **39,** 783-791.

Field D, Wills C (1998) Abundant microsatellite polymorphism in *Saccharomyces cerevisiae*, and the different distributions of microsatellites in eight prokaryotes and *S. cerevisiae*, result from strong mutation pressures and a variety of selective forces. *Proceedings of the National Academy of Science* U.S.A., **95**, 1647–1652.

Fiore-Donno A-M, Martin F (2001) Populations of ectomycorrhizal *Laccaria amethystina* and *Xerocomus* spp. show contrasting colonization patterns in a mixed forest. *New Phytologist,* **152**, 533-542.

Fisher MC, Koenig GL, White TJ, Taylor JW (2000) A test for concordance between the multilocus genealogies of genes and microsatellites in the pathogenic fungus *Coccidioides immitis. Molecular Biology and Evolution,* **17**, 1164-1174.

Fisher MC, Koenig GL, White TJ, Taylor JW (2002) Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognized as the non-California population of *Coccidioides immitis*. *Mycologia,* **94**, 73-84.

Fleischer RC, Loew S (1995) Construction and screening of microsatellite-enriched genomic libraries. In: *Molecular Zoology: Advances, Strategies, and Protocols* (eds. Ferraris J, Palumbi S) pp. 459-468. Wiley-Liss, New York.

Fortin M-J, Drapeau P, Legendre P (1989) Spatial autocorrelation and sampling design in plant ecology. *Vegetatio*, **83**, 209-222.

Franklin JF (1979) Vegetation of the Douglas-fir region. Pp. 93-112. In: *Forest soils of the Douglas-fir region.* Eds., PE Heilman, HW Anderson, DM Baumgartner. Washington State University, Cooperative Extension, Pullman.

Franklin JF, Bledsoe CS, Callahan JT (1990) Contributions of the Long-Term Ecological Research Program. *Bioscience,* **40**, 509-523.

Franklin JF, Dyrness CT (1973) *Natural vegetation of Oregon and Washington.* USDA Forest Service Gen. Tec. Rep. PNW-8. Pacific Northwest Forest and Range Exp. Station, Portland, Oregon.

Franklin JF; Forman RTT (1987) Creating landscape patterns by forest cutting: ecological consequences and principles. *Landscape Ecology*, **1**, 518.

Franklin JF, Spies TA (1991) *Composition, function, and structure of old-growth Douglas-fir forests.* In Wildlife and Vegetation of Unmanaged Douglas-Fir Forests. (eds. Ruggiero LF, Aubry KB, AB, Huff MH) USDA Forest Service General Technical Report PNW-GTR-285. 474pp.

Fukuda M, Nakai YF, Hibbett DS, Matsumoto T, Hayashi Y (1994) Mitochondrial DNA restriction fragment length polymorphisms in natural populations of *Lentinula edodes. Mycological Research,* **98**, 169-175.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes applications to the identification of mycorrhizae and rusts. *Molecular Ecology,* **2**, 113-118.

Gardes M, Bruns TD (1996) ITS-RFLP matching for identification of fungi. *Methods in Molecular Biology,* **50**, 177-186.

Garza JC, Slatkin M, Freimer NB (1995) Microsatellite allele frequencies in humans and chimpanzees, with implications for constraints on allele size. *Molecular Biology and Evolution,* **12**, 594-603.

Gherbi H, Delaruelle C, Selosse M-A, Martin F (1999) High genetic diversity in a population of the ectomycorrhizal basidiomycete *Laccaria amethystina* in a 150 year-old beech forest. *Molecular Ecology,* **8**, 2003-2013.

Goffeau A, Barrell BG, Bussey H, *et al.* (1996) Life with 6000 genes. *Science,* **274**, 563-567.

Greenland D (1994) The Pacific Northwest regional context of the climate of the HJ Andrews Experimental Forest. *Northwest Science*, **69**, 81-96.

Gregory PH (1973) *Microbiology of the atmosphere*, 2nd edn. Leonard Hill Books, Aylesbury, UK.

Gryta H, Debaud J-C, Effosse A, Gay G, Marmeisse R (1997) Fine-scale structure of populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum* in coastal sand dune forest ecosystems. *Molecular Ecology,* **6**, 353-364.

Gryta H, Debaud J-C, Marmeisse R (2000) Population dynamics of the symbiotic mushroom *Hebeloma cylindrosporum*: mycelial persistence and inbreeding. *Heredity,* **84**, 294-302.

Guidot A, Debaud JC, Marmeisse R (2001) Correspondence between genet diversity and spatial distribution of above-and below-ground populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum. Molecular Ecology,* **10**, 1121-1131.

Guidot A, Gryta H, Gourbière F, Debaud J, Marmeisse R. (2002) Forest habitat characteristics affect balance between sexual reproduction and clonal propagation of the ectomycorrhizal mushroom *Hebeloma cylindrosporum. Oikos,* **99**, 25-36.

Gulden G, Dunham S, Stockman J (2001) DNA studies in the *Galerian marginata* complex. *Mycological Research,* **105**, 432-440.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics,* **48**, 361-372.

Hancock JM (1999) Microsatellites and other simple sequences: genomic context and mutational mechanisms. In *Microsatellites: evolution and applications*. (ed. Goldstein DB, Schlötterer C. Oxford University Press, Oxford. pp. 1–9.

Harmon ME, Sexton J (1995) Water balance of conifer logs in early stages of decompositon. *Plant and Soil,* **172**, 141-152.

Harrington FA, McNew DL (1998) Partial interfertility among the *Ceratocystis* species on conifers. *Fungal Genetics and Biology,* **25**, 44-53.

Harrington FA, Potter D (1997) Phylogenetic relationships within *Sarcoscypha* based on nucleotide sequences of the internal transcribed spacer of nuclear ribosomal DNA *Mycologia,* **89**, 258-267.

Harrington TC, Rizzo DM (1999) Defining species in the fungi. pages 43-71 *In*: Worrall JJ (ed.) *Structure and Dynamics of Fungal Populations*. Kluwer Press. Dordrecht, The Netherlands.

Harrington TC, Steimel J, Wingfield MJ, Kile GA (1996) Isozyme variation and species delimitation in the *Ceratocystis coerulescens* complex. *Mycologia,* **88**, 104-113.

HarringtonTC, Wingfield BD (1995) A PCR-based identification method for species of *Armillaria. Mycologia,* **87**, 280-288.

Harris LD (1984) *The Fragmented Forest*. University of Chicago Press, Chicago, IL.

Hartmann S, Nason JD, Bhattacharya D (2001) Extensive ribosomal DNA genic variation in the columnar cactus *Lophocereus*. *Journal of Molecular Evolution*, **53**, 124-134.

Harvey AE, Jurgensen MF, Larsen MJ (1978) Seasonal distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. *Forest Science*, 24, 203-208.

Harvey AE, Jurgensen MF, Larsen MJ (1980a) Clearcut harvesting and ectomycorrhizae: survival of activity on residual roots and influence on bordering forests stance in western Montana. *Canadian Journal of Forest Research*, **10**, 300-303.

Harvey AE, Larsen MJ, Jurgensen MF (1976) Distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. *Forest Science*, **22**, 393-398.

Harvey AE, Larsen MJ, Jurgensen MF (1980b) Early effects of partial cutting on the number and distribution of active ectomycorrhizae in steep, rocky soils of Douglas-fir/larch forests in western Montana. *Canadian Journal of Forest Research*, **10**, 436-440.

Hawksworth DL (1991) The fungal dimension of biodiversity: its magnitude and significance. *Mycological Research*, **95**, 441-456.

Haynes RW (1986) *Inventory and value of old-growth in the Douglas-fir region*. USDA Forest Service Research Note PNW-437, 18p.

Heywood JS (1991) Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics*, **22**, 335-355.

Høiland K, Holst-Jensen A (2000) *Cortinarius* phylogeny and possible taxonomic implications of ITS rDNA sequences. *Mycologia*, **92**, 694-710.

Horton TR (2002) Molecular approaches to ectomycorrhizal diversity studies: Variation in ITS at a local scale. *Plant and Soil* **244**, 29-39.

Horton TR & Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black box. *Molecular Ecology*, **10**, 1855-1871.

Horton TR, Cazares E, Bruns TD (1998) Ectomycorrhizal vesicular-arbuscular and

dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first five months of growth after wildfire. *Mycorrhiza*, 8, 11-18.

Hosford D, Pilz D, Molina R, Amaranthus M (1997) *Ecology and Management of the Commercially Harvested American Matsutake Mushroom*. General Technical Report. PNWGTR-412. US Department of Agriculture, Forest Service, Pacific Northwest Research Station: Portland, Oregon.

Huelsenbeck JP (1997) Is the Felsenstein zone a fly trap? *Systematic Biology,* **46**, 69-74.

Hughes KW, Mcghee LL, Methven AS, Johnson JE, Petersen RH (1999) Patterns of geographic speciation in the genus *Flammulina* based on sequences of the ribosomal ITS1-5.8S-ITS2 area. *Mycologia,* **91**, 978-986.

Hughes CR, Queller DC (1993) Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Molecular Ecology,* **2**, 131-137.

Hutchinson A (1989) Absence of conidia as a morphological character in ectomycorrhizal fungi. *Mycologia,* **81**, 587-594.

Ingold CT (1971) *Fungal spores: Their liberation and dispersal*. Clarendon Press, Oxford.

James TY, Vilgalys R (2001) Abundance and diversity of *Schizophyllum commune* spore clouds in the Caribbean detected by selective sampling. *Molecular Ecology*, **10**, 471-479.

Jandel Scientific (1993) *SigmaPlot Version 2.01 Scientific Graphing Software*: User's Manual. San Rafael, CA.

Jany J-L, Garbaye J, Martin F (2002) *Cenococcum geophilum* populations show a high degree of genetic diversity in beech forests. *New Phytologist*, **154**, 651-659.

Johannesson H, Ryman S, Lundmark H, Danell E (1999) *Sarcodon imbricatus* and *S. squamosus* – two confused species. *Mycological Research,* **103**, 1447-1452.

Johansson JF (2002) Belowground ectomycorrhizal community structure along a local nutrient gradient in a boreal forest in Northern Sweden. MS Thesis, Swedish University of Agricultural Sciences, Uppsala.

Jonsson L, Dahlberg A, Nilsson M-C, Zackrisson O, Kårén O (1999) Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. *Molecular Ecology*, **8**, 205-215.

Kanchanaprayudh J, Lian C, Zhou Z, Hogetsu T, Sihanonth P. (2002) Polymorphic microsatellite markers of a *Pisolithus* sp. from Eucalyptus plantation. *Molecular Ecology Notes,* **2**, 263-264.

Kelly KL, Judd DB (1976) Color-universal language and dictionary of names. US Department of Commerce, National Bureau of Standards, NBS Special Publication 440. US Government Printing Office, Washington, DC.

Kohn LM (1992) Developing new characters for fungal systematics: An experimental approach for determining the rank of resolution. *Mycologia,* **84**, 139-153.

Koufopanou V, Burt A, Taylor JW (1997) Concordance of gene genealogies reveals reproductive isolation in the pathogenic fungus *Coccidioides immitis. Proceeding of the National Academy of Science, USA,* **94**, 5478-5482.

Koufopanou V, Burt A, Szaro T, Taylor JW (2001) Gene genealogies, cryptic species, and molecular evolution in the human pathogen *Coccidioides immitis* and relatives (Ascomycota, Onygenales). *Molecular Biology and Evolution,* **18**, 1246-1258.

Kretzer AM, Molina R, Spatafora JW (2000) Microsatellite markers for the ectomycorrhizal basidiomycete *Rhizopogon vinicolor. Molecular Ecology* **9**, 1171-1193.

Kubis S, Schmidt T, Heslop-Harrison JS (1998) Repetitive DNA elements as a major component of plant genomes. *Annals of Botany,* **82**(Suppl. A), 45–55.

Lacey J (1996) Spore dispersal – its role in ecology and disease: the British contribution to fungal aerobiology. *Mycological Research*, **100**, 641-660.

Langrell SRH, Lung-Escarmant B, Decroocq S (2001) Isolation and characterization of polymorphic simple sequence repeat loci in *Armillaria ostoyae. Molecular Ecology Notes* **1**, 305-307.

Largent DL, Johnson D, Watling R (1977) *How to identify mushrooms to genus III. Microscopic features*. Mad River Press Inc., Eureka, California. 86 pp.

Largent DL, Sime AD (1995) A preliminary report on the phenology, sporulation, and lifespan of *Cantharellus cibarius* and *Boletus edulis* basidiomes at Patrick's Point State Park. In: Adams DH, Rios JE, Stoere AJ (eds.) Proc. Symp. Of 43rd Annual Meeting of the California Forest Pest Council, November 16-17 1994. Ranco Cordova, CA pp. Appendix xxxii-xliv.

Leake JR, Read DJ (1990) Proteinase activity in mycorrhizal fungi. I. The effect of extracellular pH on the production and activity of proteinase by ericoid endophytes from soils of contrasting pH. *New Phytologist,* **155**, 243-250.

Lehmann T, Hawley WA, Collins FH (1996) An evaluation of evolutionary constraints on microsatellite loci using null alleles. *Genetics,* **144**, 1155-1163.

Lehmkuhl JF, Ruggiero LF (1991) Forest fragmentation in the Pacific Northwest and its potential effects on wildlife. In Wildlife and Vegetation of Unmanaged Douglas-Fir Forests. (eds. Ruggiero LF, Aubry KB, AB, Huff MH) USDA Forest Service General Technical Report PNW-GTR-285. 474pp.

Liegel L, Ed. (1998) *The biological, socio-economic, and managerial aspects of chanterelle mushroom harvesting: the Olympic Peninsula, Washington State, USA.* AMBIO Special Report No. 9, 35 pp.

Liu Y, Ammirati JF, Rogers SO (1997) Phylogenetic relationships in *Dermocybe* and related *Cortinarius* taxa based on nuclear ribosomal RNA internal transcribed spacers. *Canadian Journal of Botany,* **75**, 519-532.

Liu G, Chambers SM, Cairney JWG (1998) Molecular diversity of ericoid mycorrhizal endophytes isolated from *Woollsia pungens. New Phytologist,* **140**, 145-153.

LoBuglio KF, Rogers SO, Wang CJK (1991) Variation in ribosomal DNA among isolates of the mycorrhizal fungus *Cenococcum geophilum. Canadian Journal of Botany,* **69**, 2331-2343.

Lodge DJ, Cantrell S (1995) Fungal communities in wet tropical forests: variation in time and space. *Canadian Journal of Botany,* 73 (Suppl), S1391-S1398.

Lodge DJ, McDowell WH, McSwiney CP (1994) The importance of nutrient pulses in tropical forests. *Trends in Ecology and Evolution,* **9**, 384-387.

Longato S, Bonfante P (1997) Molecular identification of mycorrhizal fungi by direct amplification of microsatellite regions. *Mycological Research,* **101**, 425-432.

Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics*, **15**, 65-95.

Luoma DL, Frenkel R, Trappe JM (1991) Fruiting of hypogeous fungi in Oregon Douglas-fir forests: seasonal and habitat variation. *Mycologia*, **83**, 335-353.

Magnussen S (1993). Bias in genetic variance estimates due to spatial autocorrelation. *Theoretical Applied Genetics,* **86**, 349-355.

Maniatis T, Fritsch EF, Sambrook J (1982) *Molecular cloning. A laboratory manual.* Cold Spring Harbor Laboratory, Cold Springs: Harbor, New York.

Manly BFJ (1997) *Randomization, Bootstrap and Monte Carlo Methods in Biology.* Chapman & Hall, London.

Martin FN (1990) Variation in the ribosomal DNA repeat unit within single-oospore isolates of the genus *Pythium*. *Genome,* **33**, 585-591.

Mayr E (1940) Speciation phenomena in birds. *American Naturalist,* **74**, 249-278.

Mayr E (1963) *Populations, Species, and Evolution*. Harvard University Press, Cambridge, Massachusetts.

McDonald BA (1997) The population genetics of fungi: tools and techniques. *Phytopathology,* **87**, 448-453.

Miller RM, Lodge DJ (1997) Fungal Responses to Disturbance: Agriculture and Forestry. In *The Mycota IV: Environmental and Microbial Relationships* (Eds. Wicklow DT, Söderström B) Springer-Verlag, Berlin.

Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In. Allen M (ed.) *Mycorrhizal functioning, and integrative plant-fungal process*. New York, Chapman and Hall. Pp. 357-423.

Molina R, O'Dell T, Luoma D, Amaranthus M, Castellano M, Russell K (1993) *Biology, Ecology, and Social Aspects of Wild Edible Mushrooms in the Forests of the Pacific Northwest: A Preface to Managing Commercial Harvest.* General Technical Report PNWGTR-309. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station: Portland, Oregon.

Molina R, O'Dell T, Luoma D *et al.* (1993) Biology, ecology, and social aspects of wild edible mushrooms in the forests of the Pacific Northwest: a preface to managing commercial harvest. General Technical Report PNW-GTR-309. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 42p.

Molina R, Pilz D, Smith J, Dunham S, Dreisbach T, O'Dell T, Castellano M (2001) Conservation and management of forest fungi in the Pacific Northwestern United States: an integrated ecosystem approach. In Moore D, Nauta MM, Evans SE, Rotheroe M. *Fungal Conservation: Issues and Solutions*. Cambridge University Press.

Molina R, Trappe JM (1982) Patterns of ectomycorrhizal host specificity and potential among Pacific Northwest conifers and fungi. *Forest Science,* **28,** 423-458.

Moritz C (1994) Defining "evolutionarily significant units" for conservation. *Trends in Ecology & Evolution*, **9**, 373-375.

Morkkynen T, Weissenbergh KV, Pappine A (1997) Estimation of dispersal gradients of S- and P-type basidiospores of *Heterobasidion annosum. European Journal of Forest Pathology,* **27**, 291-300.

Morrison PM (1988) Old growth in the Pacific Northwest: a status report. Washington, DC: *The Wilderness Society*, 46 p.

Morrison P, Swanson FJ (1990) *Fire history and pattern in a Cascade Range landscape.* U.S. Forest Service General Technical Report PNW-GTR-254. Pacific Northwest Research Station, Portland Oregon, USA.

Mui D, Feibelman T, Bennett JW (1998) A preliminary study of the carotenoids of some North American species of Cantharellus. *International Journal of Plant Science,* **159**, 244-248.

Munsell Color Company Inc. (1966) *Munsell book of color*, Munsell Color Company, Baltimore, Maryland. 22pp. with approximately 1,500 color standards.

Murrill WA (1912) The Agaricaceae of the Pacific Coast – I. *Mycologia,* **4**, 205-217.

Newton AC, Allnutt TR, Gillies ACM, Lowe AJ, Ennos RA (1999) Molecular phylogeography, intraspecific variation and the conservation of tree species. *Trends in Ecology and Evolution,* **14**, 140-145.

Norvell L, Roger J, Lindgren J, Kopecky F (1996) Oregon Cantharellus Study Project 1986-96. In: Pilz D, Molina R, eds. *Managing Forest Ecosystems to Conserve Fungus Diversity and Sustain Wild Mushroom Harvests*. General Technical Report PNW-GTR-371. US Department of Agriculture, Forest Service. Pacific Northwest Research Station, Portland, OR, pp. 69-72.

O'Dell TE, Luoma DL, Molina RJ (1992) Ectomycorrhizal fungal communities in young, managed, and old-growth Douglas-fir stands. *Northwest Environmental Journal*, **8**, 166-168.

O'Dell TE. Ammirati JF, Schreiner EG (1999) Species richness and abundance of ectomycorrhizal basidiomycete sporocarps on a moisture gradient in the *Tsuga heterophylla* zone. *Canadian Journal of Botany,* **77**, 1699-1711.

Oden NL (1984) Assessing the statistical significance of a spatial correlogram. *Geographical Analysis,* **16**, 1-16.

O'Donnell K (1992) Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). *Current Genetics,* **22**, 213-220.

Parks JC, Werth CR (1993) A study of spatial features of clones in a population of bracken fern, *Peteridium aquilinum* (Dennstaedtiaceae). *American Journal of Botany,* **80**, 537-544.

Peakall R, Smouse PE (2001) GenAlEx V5: Genetic Analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia. http://www.anu.edu.au/BoZo/GenAlEx/

Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology*, **4**, 249-252.

Perry DA, Molina R, Amaranthus MP (1987) Mycorrhizae, mycorrhizospheres, and reforestation: current knowledge and research needs. *Canadian Journal of Forest Research*, **17**, 929-940.

Perry DA, Amaranthus MP, Borchers JG, Borchers SL, Brainerd RE (1989) Bootstrapping in ecosystems. *Bioscience*, **39**, 230-237.

Perry DA, Margolis H, Choquette C, Molina R, Trappe JM (1992) Ectomycorrhizal mediation of competition between coniferous tree species. *New Phytologist,* **112**, 501-511.

Petersen RH (1979) Notes on cantharelloid fungi. IX. Illustrations of newly or poorly understood taxa. *Nova Hedwigia,* **31**, 1-23.

Petersen RH, Hughes KW (1999) Species and speciation in mushrooms: Development of a species concept poses difficulties. *Bioscience,* **49**, 440-452.

Pilz D, Molina R (2002) Commercial harvests of edible mushrooms from the forests of the Pacific Northwest United States: issues, management, and monitoring for sustainability. *Forest Ecology and Management,* **155**, 3-16.

Pilz D, Perry DA (1984) Impact of clearcutting and slash burning on ectomycorrhizal associations of Douglas-fir seedlings. *Canadian Journal of Forest Research,* **14**, 94-100.

Pine EM, Hibbett DS, Donoghue MJ (1999) Phylogenetic relationships of cantharelloid and clavarioid Homobasidiomycetes based on mitochondrial and nuclear rDNA sequences. *Mycologia,* **91**, 944-963.

Primmer CR, Raudsepp T, Chowdhary BP, Møller AP, and Ellegren H (1997) Low frequency of microsatellites in the avian genome. *Genome Research,* **7**, 471–482.

Pugh GJF, Boddy L (1988) A view of disturbance and life strategies in fungi. *Proceedings of the Royal Society Edinburgh*, **94B**, 3-11.

Ramírez L, Larraya LM, Pisabarro AG (2000) Molecular tools for breeding basidiomycetes. *International Microbiology,* **3**, 147-152.

Raymond M, and Rousset F (1995) GENEPOP v. 3.1c: Population genetics software for exact tests and ecumenicism. *Journal of Heredity,* **86**, 248-249.

Read DJ, Francis R, Finlay RD (1985) Mycorrhizal mycelia and nutrient cycling in plant communities. In: Fitter AH, Atkinson D, Read DJ, Usher MB (eds.). *Ecological interactions in soils*. Oxford, Blackwell Scientific Publications. pp. 193-218.

Redecker D, Szaro TM, Bowman RJ, Bruns TD (2001) Small genets of *Lactarius xanthogalactus, Russula cremoricolor* and *Amanita francheti* in late-stage ectomycorrhizal successions. *Molecular Ecology,* **10**, 1025-1034.

Redhead SA, Norvell LL, Danell E (1997) *Cantharellus formosus* and the Pacific Golden Chanterelle harvest in Western North America. *Mycotaxon,* **65**, 285-322.

Reusch TBH, Stam WT, Olsen JL (1999b) Size and estimated age of genets in eelgrass *Zostera marina* L. assessed with microsatellite markers. *Marine Biology,* **133**, 519-525.

Ridgway R (1912) *Colour standards and colour nomenclature.* Washington DC.

Robbins WG (1988) Hard times in paradise, Coos Bay, Oregon 1850-1986. University of Washington Press, Seattle, WA.

Ruckelshaus MH (1998) Spatial scale of genetic structure and an indirect estimate of gene flow in eelgrass, *Zostera marina. Evolution,* **52**, 330-343.

Rydin H, Martin D, Hallingbäck T (1997) Biological characteristics, habitat

associations, and distribution of macrofungi in Sweden. *Conservation Biology*, **11**, 628-640.

Sambrook J, Fritsh EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual* 2nd edn. Cold Springs Harbor Laboratory Press, New-York.

Sanderson MJ (1989) Confidence limits on phylogenies: The bootstrap revisited. *Cladistics,* **5**, 113-129.

Sanderson MJ, Doyle JJ (1992) Reconstruction of organismal and gene phylogenies from data on multigene families concerted evolution homophasy and confidence. *Systematic Biology,* **41**, 4-17.

Sawyer NA, Chambers SM, Cairney JWG (1999) Molecular investigation of genet distribution and genetic variation of *Cortinarius rotundisporus* in eastern Australian sclerophyll forests. *New Phytologist,* **142**, 561-568.

Schlosser WE, Blatner KA (1995) The wild edible mushroom industry of Washington, Oregon and Idaho. *Journal of Forestry,* **93**, 31-36.

Seifert KA, Wingfield BD, Wingfield MJ (1995) A critique of DNA sequence analysis in the taxonomy of filamentous Ascomycetes and ascomycetous anamorphs. *Canadian Journal of Botany,* **73** (Suppl. 1), 760-767.

Selosse M-A, Jacquot D, Bouchard D, Martin F, Le Tacon F (1998) Temporal persistence and spatial distribution of an American inoculant strain of the ectomycorrhizal basidiomycete *Laccaria bicolor* in European forest plantations. *Molecular Ecology,* **7**, 561-573.

Selosse M-A, Martin F, Bouchard D, Le Tacon F (1999) Structure and dynamics of experimentally introduced and naturally occurring *Laccaria* sp. discrete genotypes in a Douglas fir plantation. *Applied and Environmental Microbiology,* **65**, 2006-2014.

Selosse M-A, Martin F, Le Tacon F (2001) Intraspecific variation in fruiting phenology in an ectomycorrhizal *Laccaria* population under Douglas fir. *Mycological Research,* **105**, 524-531.

Shinohara ML, LoBuglio KF, Rogers SO (1996) Group-1 intron family in the nuclear ribosomal RNA small subunit genes of *Cenococcum geophilum* isolates. *Current Genetics,* **29**, 377-387.

Shinohara ML, Lobuglio KF, Rogers SO (1999) Comparison of ribosomal DNA ITS

regions among geographic isolates of *Cenococcum geophilum. Current Genetics,* **35**, 527-535.

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R (1997) Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature,* **388**, 579-582.

Sime AD, Petersen RH (1999) Intercontinental interrelationships among disjunct populations of *Melanotus* (Strophariaceae, Agaricales). *Mycotaxon,* **71**, 481-492.

Simpson GG (1951) The species concept. *Evolution,* **5**, 285-298.

Simpson GG (1961) *Principles of Animal Taxonomy*. Columbia University Press, New York.

Sirjusingh C, Kohn LM (2001) Characterization of microsatellites in the fungal plant pathogen, *Sclerotinia sclerotiorum. Molecular Ecology Notes* **1**, 267-269.

Slatkin M, Barton NH (1989). A comparison of three indirect methods for estimating average levels of gene flow. *Evolution,* **4**, 1349-1368.

Smith AH (1968) The Cantharellaceae of Michigan. *Michigan Botanist,* **7**, 143-183.

Smith AH, Morse EE (1947) The genus *Cantharellus* in the western United States. *Mycologia,* **39**, 497-534.

Smith HV, Smith AH (1973) *How to know the non-gilled fleshy fungi*. Wm. C. Brown Co., Dubuque. 402 pp.

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd ed. Academic Press.

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity,* **82**, 561-573.

Sokal RR, Jacquez GM (1991) Testing inferences about micro-evolutionary processes by means of spatial autocorrelation analysis. *Evolution*, **45**, 152-168.

Sokal RR, Oden NL (1978) Spatial autocorrelation in biology 1. *Methodology Biological Journal of the Linnean Society,* **10**, 199-228.

Sokal RR, Oden NL, Thomson BA (1997) A simulation study of microevolutionary inferences by spatial autocorrelation analysis. *Biological Journal of the Linnean Society*, **65**, 41-62.

Sokal RR, Wartenberg DE (1983) A test of spatial autocorrelation analysis using an isolation-by-distance model. *Genetics,* **105**, 219-237.

Sork VL, Nason J, Campbell DR, Fernandez JF (1999) Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology & Evolution*, **14**, 219-224.

Spies TA, Franklin JF (1988) Old-growth and forest dynamics in the Douglas-fir region of western Oregon and Washington. *Natural Areas Journal,* **8**, 190-201.

Spies TA, Franklin JF (1989) Gap characteristics and vegetation response in coniferous forests of the Pacific Northwest. *Ecology*, **70**, 543-545.

Spies TA, Franklin JF (1991) The structure of natural young, mature, and old-growth Douglas-fir forests in Oregon and Washington. In *Wildlife and vegetation of unmanaged Douglas-fir forests*. Technical Coordinators, Ruggiero LF, Aubry KB, Carey AB, Huff MH USDA Forest Service General Technical Report PNW-GTR-285. pp 91-109.

Spies TA, Franklin JF, Thomas TB (1988) Coarse woody debris in Douglas-fir forests of western Oregon and Washington. *Ecology*, **69**, 1689-1702.

Spies TA, Ripple WJ, Bradshaw GA (1994) Dynamics and pattern of a managed coniferous forest landscape. *Ecological Applications,* 4, 555-568.

Swofford DL (1999) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).* Version 4. Sinauer Associates: Sunderland, Massachusetts.

Taylor DL, Bruns TD (1999) Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. *Molecular Ecology,* **8**, 1837-1850.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology,* **31**, 21-32.

Thiers HD (1985) *The Agaricales (Gill Fungi) of California. 2. Cantharellaceae.* 34 pgs. Mad River Press, Eureka.

Thomas JW, Raphael MG, Anthony RG, Forsman ED, Gunderson AG, Holthausen RS, Marcot BG, Reeves GH, Sedell JR, Solis DM (1993) *Viability assessments and management considerations for species associated with late-successional*

and old-growth forests of the Pacific Northwest. Portland, Oregon. USDA Forest Service. 523 p.

Todd NK, Rayner ADM (1980) Fungal Individualism. *Scientific Program at Oxford,* **66**, 331-354.

Tóth G, Gáspári Z, Jurka J (2000) Microsatellites in Different Eukaryotic Genomes: Survey and Analysis. *Genome Research,* **10**, 967-981.

Trappe J (1977) Selection of fungi for ectomycorrhizal inoculation in nurseries. *Annual Review of Phytopathology,* **15**, 203-222.

Ueno S, Tomaru N, Yoshimaru H, Manabe T, Yamamoto S (2000) Genetic structure of *Camellia japonica* L. in an old-growth evergreen forest, Tsushima, Japan. *Molecular Ecology,* **9**, 647-656.

US Department of Agriculture, Forest Service; US Department of the Interior, Bureau of Land Management (1994a) *Final Supplemental Environmental Impact Statement on Management of Habitat for Late-successional and Old-growth Forest Related Species Within the Range of the Spotted Owl.* Vol. 1, US Department of Agriculture, Forest Service; US Department of the Interior, Bureau of Land Management: Washington, DC.

US Department of Agriculture, Forest Service; US Department of the Interior, Bureau of Land Management (1994b) *Record of Decision for Amendments to Forest Service and Bureau of Land Management Planning Documents Within the Range of the Northern Spotted Owl.* US Department of Agriculture, Forest Service; US Department of the Interior, Bureau of Land Management: Washington, DC.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA form several *Cryptococcus* species. *Journal of Bacteriology,* **172**, 4238-4246.

Visser S (1995) Ectomycorrhizal fungal succession in jack pine stands following wildfire. *New Phytologist,* **129**, 389-401.

Vogt KA, Grier CC, Vogt DJ (1986) Production, turnover and nutrient dynamics of above- and belowground detritus of world forests. *Advances in Ecological Research,* **15**, 303-377.

Vogt KA, Bloomfield J, Ammirati JF, Ammirati SR (1992) Sporocarp production by Basidiomycetes, with emphasis on forest ecosystems. In Carroll GC, Wicklow DT (eds.) *The Fungal Community: Its Organization and Role in the*

Ecosystem, 2nd Edition. Marcel Dekker, New York, NY, pp. 563-581.

Wachtel SS, Tiersch TR (1993) Variations in genome mass. *Comparative Biochemistry and Physiology,* **104B**, 207–213.

Waddell KL, Oswald DD, Powell DS (1989) *Forest statistics of the United States, 1987.* Pacific Northwest Forest and Range Experiment Station, U.S. Department of Agriculture Forest Service, Resource Bulletin PNW-RB-168.

Wallin DO, Swanson FJ, Marks B, Cissel JH, Kertis J (1996) Comparison of managed and pre-settlement landscape dynamics in forests of the Pacific Northwest, USA. *Forest Ecology and Management,* **85**, 291-309.

Waters JR, McKelvey KS, Luoma DL, Zabel CJ (1997) Truffle production in old-growth and mature fir stands in northeastern California. *Forest Ecology and Management*, **96**, 155-166.

Waters JR, McKelvey KS, Zabel CJ, Oliver WW (1994) The effects of thinning and broadcast burning on sporocarp production of hypogeous fungi. *Canadian Journal of Forest Research*, **24**, 1516-1522.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution,* **38**, 1358-1370.

Weir BS (1990) *Genetic data analysis*. Sinauer Publ., Sunderland, MA

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications* (ed. Innis M), pp. 315-322. Academic Press, CA.

Wiens JA (1989) Spatial scaling in ecology. *Functional Ecology,* **3**, 385-397.

Wiley EO (1978) The evolutionary species concept reconsidered. *Systematic Zoology,* **27**, 17-26.

Witthuhn RC, Wingfield BD, Wingfield MJ, Wolfaard M, Harrington TC (1998) Monophyly of the conifer species in the *Ceratocystis coerulescens* complex using DNA sequence data. *Mycologia,* **90**, 96-101.

Wolfenbarger DO (1946) Dispersion of small organisms. *American Midland Naturalist*, **35**, 1-152.

Wright S (1951). The genetical structure of populations. *Annual Eugenetics,* **15**, 323-354.

Wright S (1969) *Evolution and the Genetics of Populations*. Vol. 2. The Theory of Gene Frequencies. University of Chicago Press, Chicago.

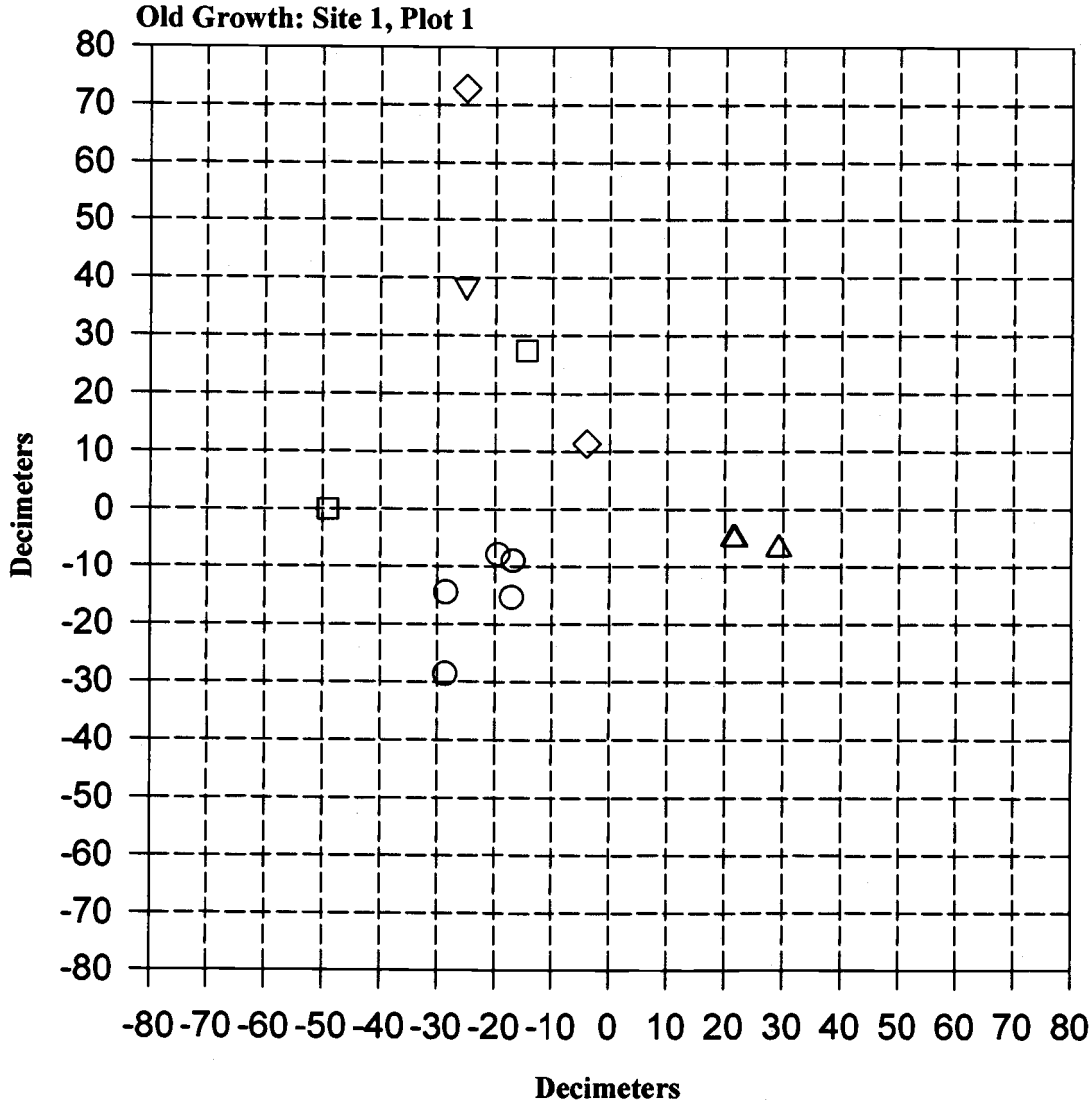
Yan ZH, Rogers SO, Wang CJK (1995) Assessment of *Phialophora* species based on ribosomal DNA internal transcribed spacers and morphology. *Mycologia,* **87**, 72-83.

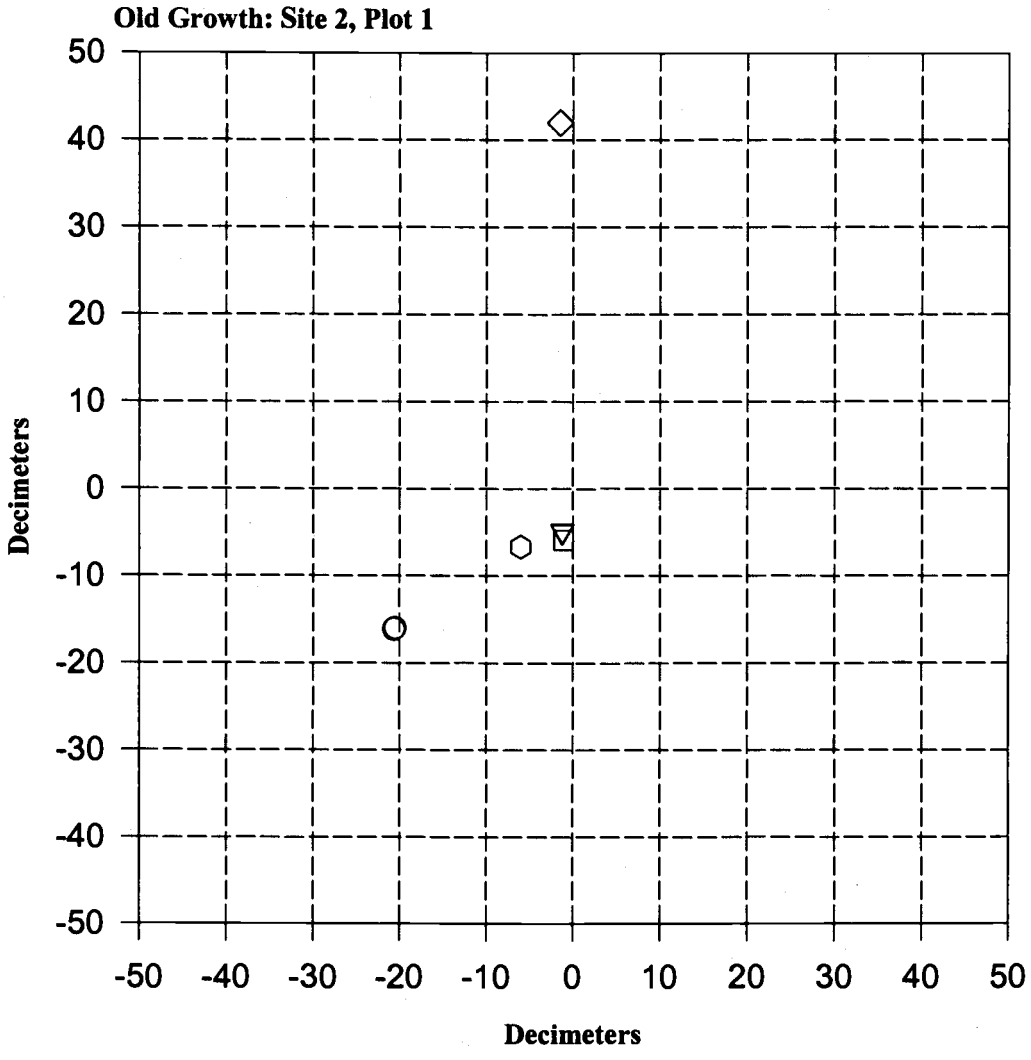
Zhou Z, Miwa M, Hogetsu T (1999) Analysis of genetic structure of a *Suillus grevillei* population in a *Larix kaempferi* stand by polymorphism of inter-simple sequence repeat (ISSR). *New Phytologist,* **144**, 55-63.

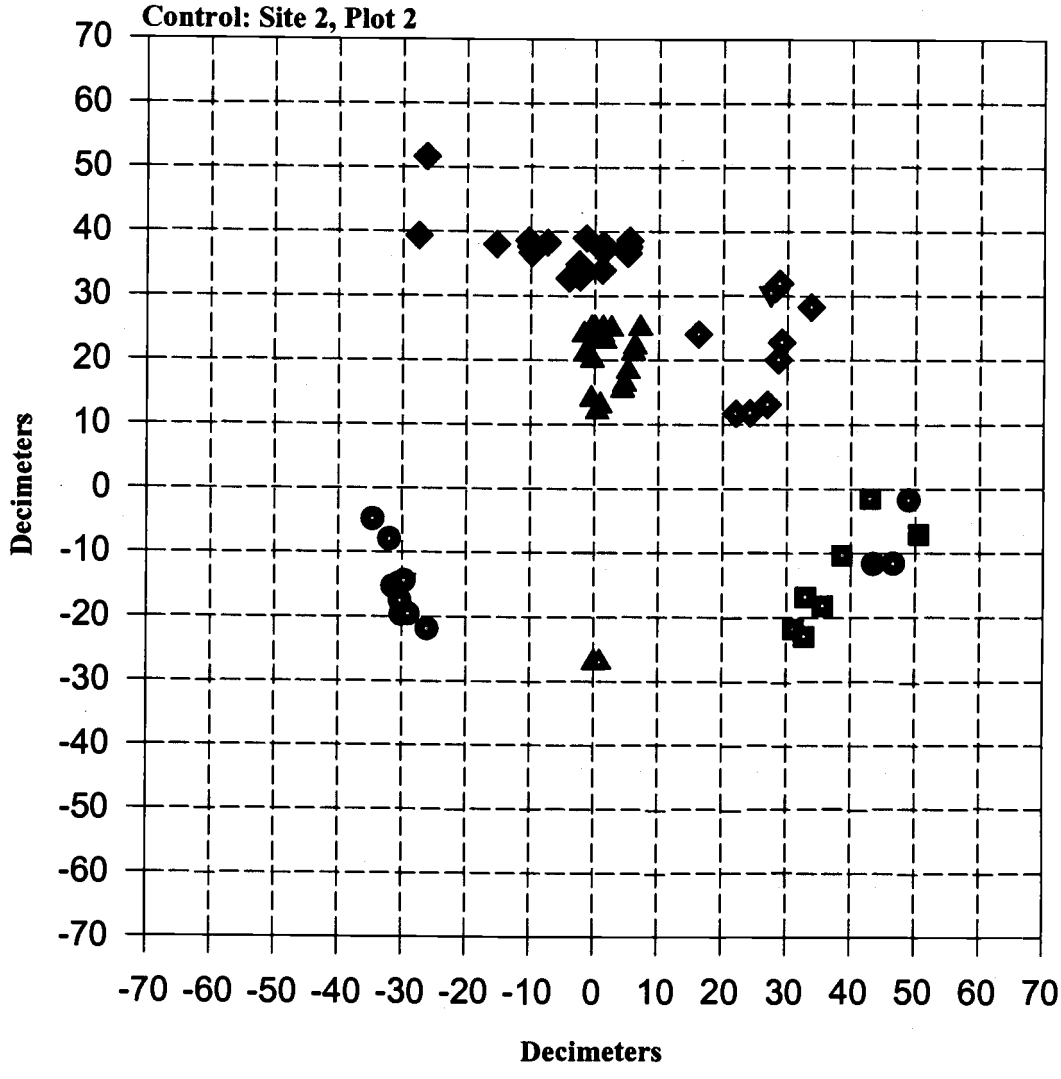
Zhou Z, Miwa M, Hogetsu T (2001) Polymorphism of simple sequence repeats reveals gene flow within and between ectomycorrhizal *Suillus grevillei* populations. *New Phytologist,* **149**, 339-348.

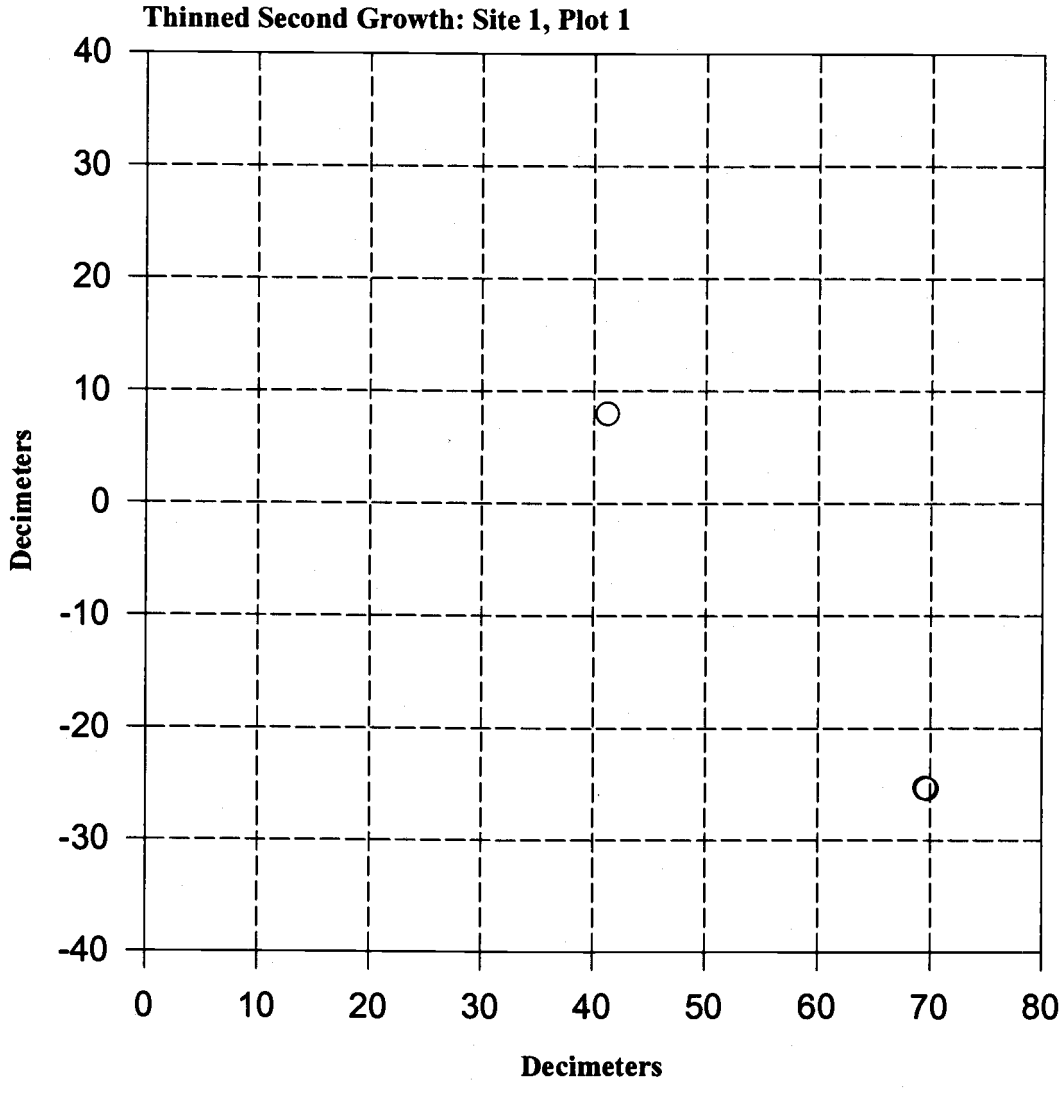
APPENDIX

Appendix A. Additional Genet Plot Maps not Shown in Chapter 2. Plot headings correlate with plot designations given in Table 2.4 on page 34. Each symbol marks the location of a single chantrelle fruit-body. Filled symbols represent C. formosus fruit-bodies and open symbols represent ITS-RFLP type B fruit-bodies. Multiple like symbols on each plot delineate the extent of putative genets measured against a 10 dm grid superimposed on the plot map. Like symbols on different plots do not represent identical multilocus genotypes. Plot sampling boundaries change from plot to plot to improve genet resolution.

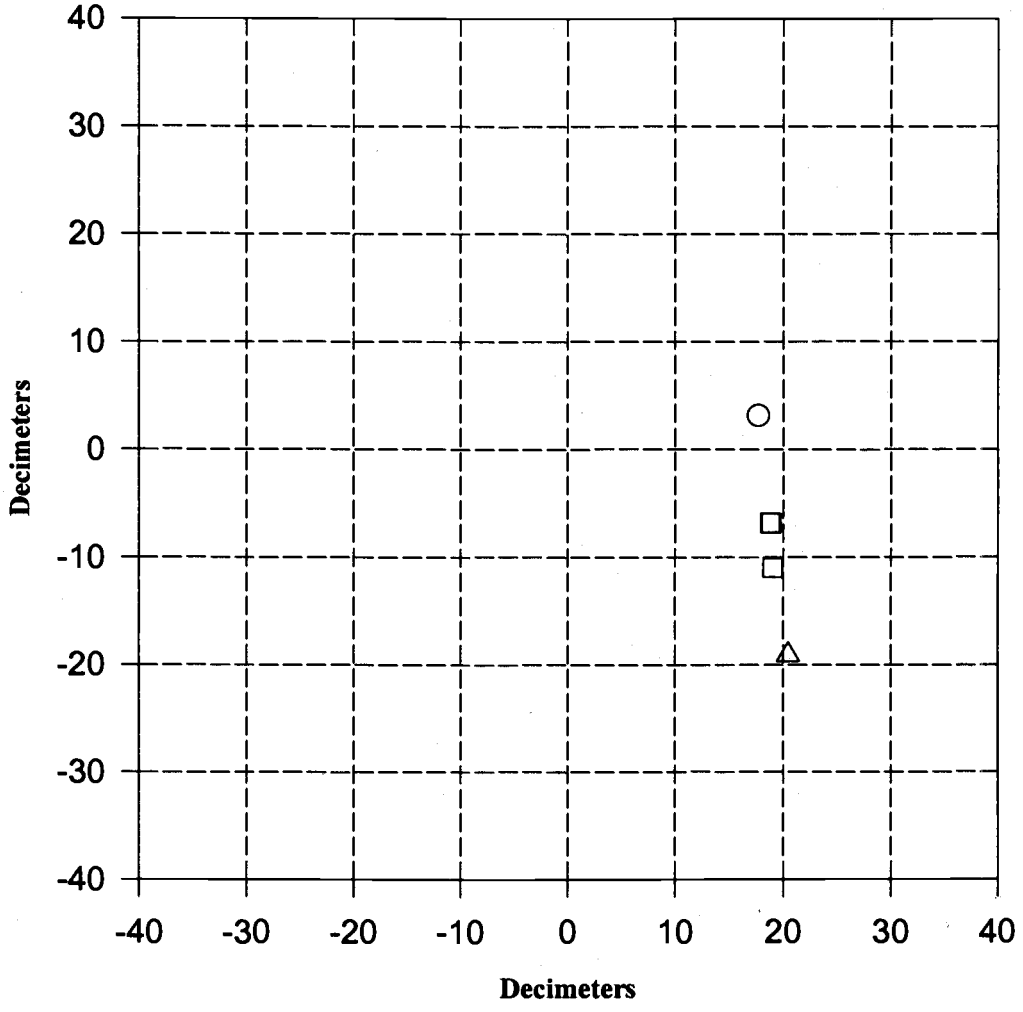

Old Growth: Site 1, Plot 1
Decimeters
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-80 -70 -60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60 70 80
Decimeters

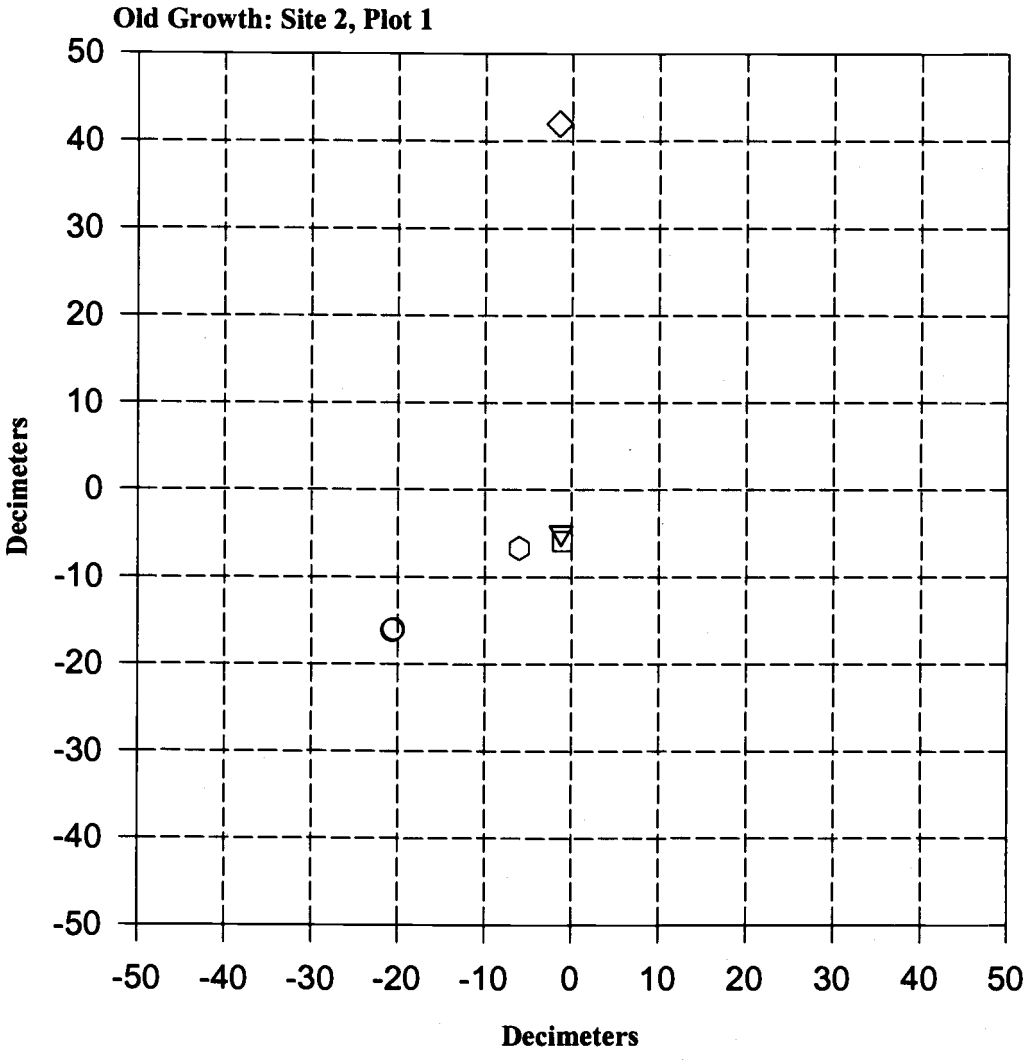

Old Growth: Site 2, Plot 1
Decimeters
50
40
30
20
10
0
-10
-20
-30
-40
-50
-50 -40 -30 -20 -10 0 10 20 30 40 50
Decimeters

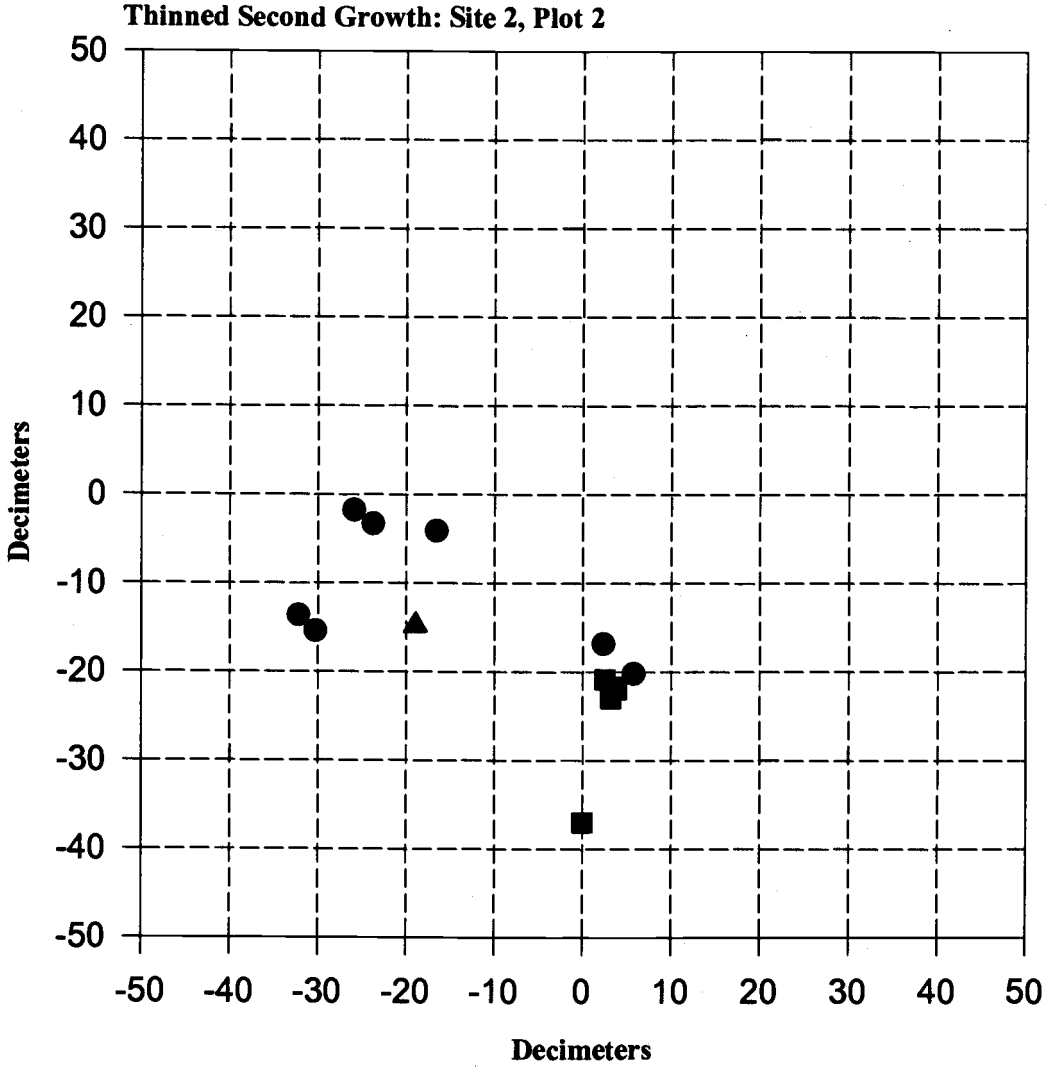

Control: Site 2, Plot 2
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
Decimeters
-70 -60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60 70
Decimeters


Thinned Second Growth: Site 1, Plot 1
40
30
20
10
0
-10
-20
-30
-40
Decimeters
0
10
20
30
40
50
60
70
80
Decimeters

Thinned Second Growth: Site 1, Plot 2




Old Growth: Site 2, Plot 1
Decimeters
50
40
30
20
10
0
-10
-20
-30
-40
-50
-50 -40 -30 -20 -10 0 10 20 30 40 50
Decimeters


Thinned Second Growth: Site 2, Plot 2
50
40
30
20
10
0
-10
-20
-30
-40
-50
Decimeters
-50 -40 -30 -20 -10 0 10 20 30 40 50
Decimeters