Phylogeography of *Armillaria ostoyae* in the western United States

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Abstract

Using a direct-PCR method, sequences of the nuclear ribosomal DNA (i.e., large subunit, internal transcribed spacer, 5.8S, and intergenic spacer) were obtained from *Armillaria ostoyae* genets collected from the western United States. Phylogenetic analyses using Bayesian methods defined several groups of *A. ostoyae*. Analysis of *A. ostoyae* from outside the western United States indicates the presence of a circumboreal group of *A. ostoyae* that also occurs in Utah. Phylogeographically unique groups were also present in the Rocky Mountain and Pacific Northwest regions. Other *Armillaria* species were used as outgroups to examine evolutionary relationships among the groups of *A. ostoyae*. The occurrence of these groups allows inferences about paleogeographic and paleoclimatic influences on phylogeography of *A. ostoyae*. Additionally, hybridization has occurred among groups that may have been previously isolated for millions of years. Hybridization has potential implications on species evolution and could contribute to variation in pathogenicity and virulence.

Introduction

Throughout its circumboreal distribution, *Armillaria ostoyae* (Romagnesi) Herink is a principal cause of Armillaria root rot disease (Guillaumin et al. 1989, Morrison and Pellow 2002). In western North America, it adversely impacts commercial timber production by causing significant tree mortality and a reduction in tree growth (Williams et al. 1989). Distinct differences in *A. ostoyae* epidemiology have been noted among coastal and interior regions of western North America (McDonald 1990, Goheen and Otrosina 1998, Morrison and Pellow 2002) and studies have shown that genets of *A. ostoyae* can show varying levels of pathogenicity and virulence (Omdal et al. 1995, Morrison and Pellow 2002). A variety of ecophysiological factors associated with host and pathogen have been hypothesized as contributing to differences in pathogenicity and epidemiology of *A. ostoyae* (McDonald 1990, Morrison and Pellow 2002). Little is known about the contribution of intraspecific genetic diversity within *A. ostoyae* to these phenomena, although genetic variability within *A. ostoyae* has been demonstrated among genets from various geographic locations (Anderson and Stasovski 1992, Piercey-Normore et al. 1997, Schulze et al. 1997, Chillali et al. 1998, Terashima et al. 1998, White et al. 1998, Sicoli et al. 2003).

Objectives

Assess genetic diversity among genets of *A. ostoyae* to examine intra- and interspecific phylogeographic relationships. Investigation of genetic diversity may be important to understand:

1) **varying levels of pathogenicity and virulence within *A. ostoyae*,**

2) **phylogeographic relationships among *A. ostoyae* genets and genets of other *Armillaria* species, and**

3) **adaptation to diverse environmental factors.**

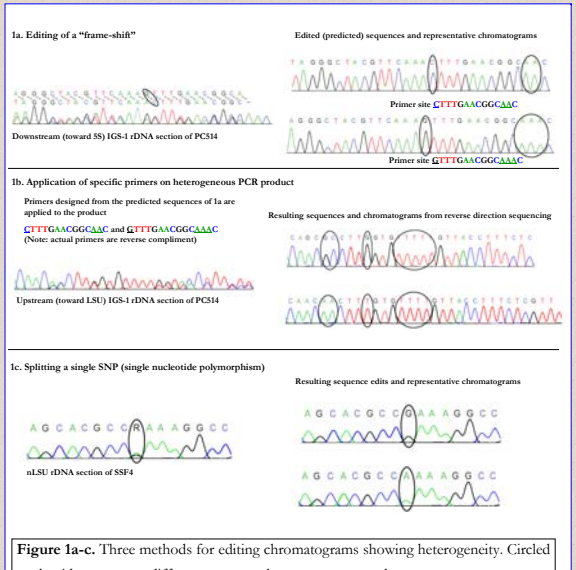
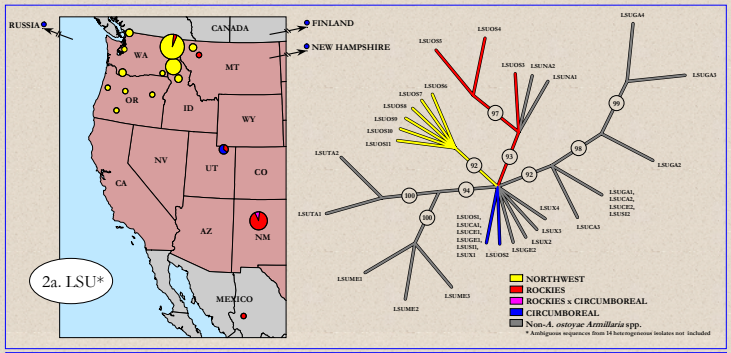
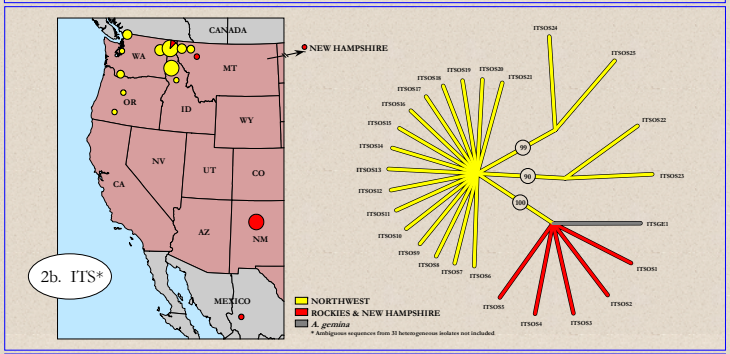
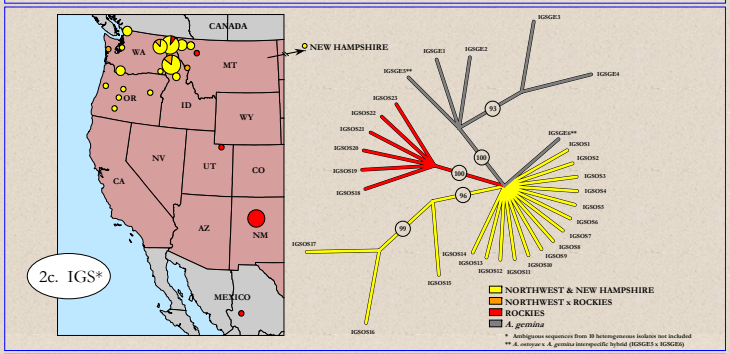


Figure 1a-c. Three methods for editing chromatograms showing heterogeneity. Circled nucleotides represent differences among heterogeneous product.







Figures 2a-c. Phylogeographic distribution of *A. ostoyae* genets in relation to major clades produced from radial 90% majority rule consensus trees; 2a – nuclear large ribosomal subunit (LSU), 2b – internal transcribed spacer and 5.8S rDNA (ITS), and 2c – intergenic spacer (IGS). Numbers between clades indicate estimated posterior probability.

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Table 1. Summary of *Armillaria* species and genets used in phylogeographic analysis

Species	Origin	Number of isolates[a]	Collector(s) or Reference
A. calvescens	Michigan, USA	2	M.T. Banik
	Quebec, Canada	1	J.A. Berubé
A. cepistipes	British Columbia, Canada	2	D.J. Morrison
	Washington, USA	1	H.H. Burdsall Jr.
A. gallica	British Columbia, Canada	1	D.J. Morrison
	Michigan, USA	1	M.T. Banik
	Wisconsn, USA	1	M.T. Banik
A. gemina	New York, USA	2	J.J. Worrall
	West Virginia, USA	1	M.E. Mielke
A. mellea	New Hampshire, USA	1	T.C. Harrington
	Virginia, USA	1	G.F. Bills
	Wisconsn, USA	1	M.T. Banik
A. nabsnona	Alaska, USA	1	C.G. Shaw III
	British Columbia, Canada	1	D.J. Morrison
	Idaho, USA	1	G.I. McDonald
A. ostoyae	Chihuahua, Mexico	1	C.G. Shaw III
	Eastern, Finland	1	Unknown
	Idaho, USA	23	IFTNC', G.I. McDonald
	Montana, USA	8	B.A. Fergason, G.I. McDonald
	New Hampshire, USA	1	T.C. Harrington
	New Mexico, USA	14	G.I. McDonald, Omdal et al. 1995
	Oregon, USA	6	Ferguson et al. 2003, G.I. McDonald
	Primorye, Russia	1	G.M. Filip
	Utah, USA	3	G.I. McDonald
	Washington, USA	19	J.F. Ammirati, IFTNC', M.-S. Kim, G.I. McDonald
A. sinapina	British Columbia, Canada	1	D.J. Morrison
	Michigan, USA	1	M.T. Banik
	Washington, USA	1	J.F. Ammirati
A. tabescens	Georgia, USA	2	G. Schnabel
	South Carolina, USA	1	G. Schnabel
NABS X[b]	Idaho, USA	3	G.I. McDonald

[a] Each isolate represents a distinct genet
[b] NABS = North American Biological Species
[c] Intermountain Forest Tree Nutrition Cooperative, Department of Forest Resources, University of Idaho, Moscow, ID 83844

Materials and Methods

PCR products from ribosomal DNA (rDNA) including nuclear large ribosomal subunit (LSU), internal transcribed spacer and 5.8S rDNA (ITS), and intergenic spacer one (IGS) were obtained from 104 genets of *Armillaria* by direct-PCR method (mycelium scraped from pure culture was added directly to a PCR-reaction mixture). Seventy-three of these genets were *A. ostoyae* from the western United States. Thirty other genets, including four *A. ostoyae* genets from other geographical regions and 27 genets of other *Armillaria* species, were used for phylogenic comparisons (Table 1). PCR products were purified and sequenced at Davis Sequencing, Inc. (Davis, CA). The sequences were edited by hand/eye with BioEdit software (Hall 1999) and manually aligned. When possible, chromatograms showing heterogeneous PCR product were split by one of the methods described in Figure 1 into homogenous sequence representations before phylogenetic analysis. The method described in Figure 1b is similar to that of the mismatch amplification mutation assay (MAMA) method (Cha et al. 1992, Rauscher et al. 2002). Remaining ambiguous sequences from heterogeneous PCR product were excluded from the datasets to reduce ambiguity of phylogenetic signal.

Phylogenetic analysis was performed for each DNA region using MrBayes v3.0B4 (Huelsenbeck and Ranquist 2001) for Bayesian inference of phylogeny. Indels were treated as a single event and coded using the simple gap coding method (Simmons and Ochoterena 2000). To select a model for use in Bayesian inference, we used MrModeltest 1.0b (Nylander 2003). Four chains were run for $3x10^6$ generations generating files with 30,001 trees, the first 6,000 of these trees were discarded as the "burnin" of the chains. The remaining trees were used to make majority rule consensus trees using PAUP* (4.0b10) (Swofford, D.L. 2001).

Results and Discussion

Phylogenetic results from the LSU region show three distinct groups (circumboreal, Rockies, and Northwest) of *A. ostoyae* present in the western United States (Figure 2a). Phylogenetic results of ITS and IGS regions confirm distinct Rockies and Northwest groups (Figures 2b and 2c). It has been hypothesized that the origin of *Armillaria* species, *A. novae-zelandiae* and *A. luteobubalina* of the Southern Hemisphere may precede the breakup of the supercontinent Gondwanaland (Coetzee et al. 2003). In the Northern Hemisphere, the presence of a circumboreal group of *A. ostoyae* occurs on three continents, and this group shares identical LSU sequences with several other *Armillaria* species. This pattern indicates possible sequence conservancy for millions of years with an early origin that may precede Pangea. More studies are needed to determine when the Rockies and Northwest groups may have diverged from this original circumboreal group. It seems likely that the formation of these groups is related to historical paleographic and paleoclimatic events.

Direct PCR has been previously shown to detect 90% of the heterogeneous rDNA products in an individual and the relative peak height seems to reflect relative copy number (Rauscher et al. 2002). In this study, heterogeneous products indicating intraspecific and intragenomic variation were common in all regions analyzed. Figure 2a. shows the presence of a Rockies x circumboreal hybrid and Figure 2c shows the presence of Northwest x Rockies hybrids. Hybridization may influence species evolution and has potential for contributing heterosis. These groups and hybrids may also contribute to differences in pathogenicity and epidemiology among individuals.

Continued studies are underway to analyze possible contribution of these phylogeographically distinct groups to differences in pathogenicity and epidemiology, while determining relationships to historical paleogeographic and paleoclimatic events.