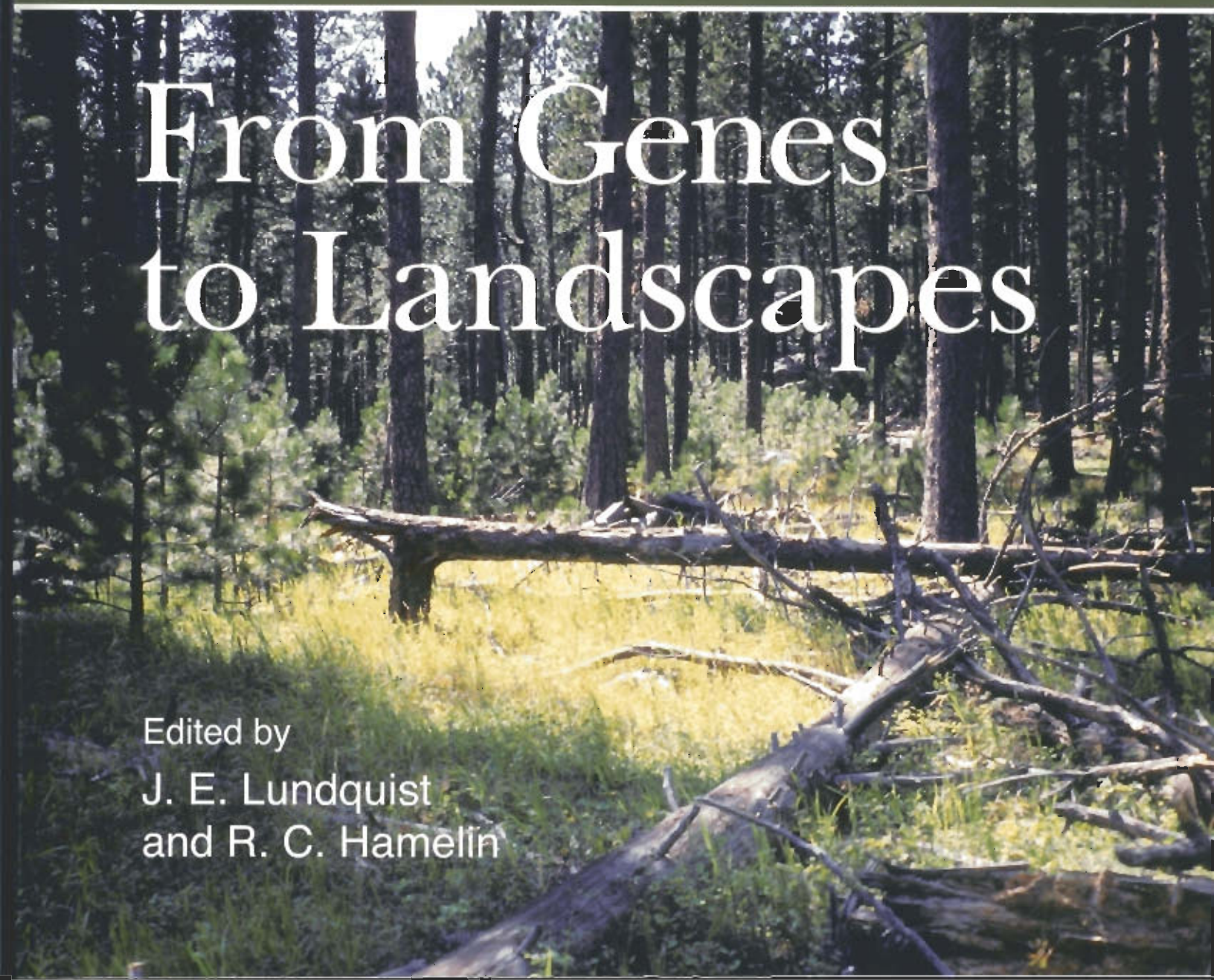
FOREST PATHOLOGY

From Genes to Landscapes



Edited by
J. E. Lundquist
and R. C. Hamelin

Neal B. Klopfenstein

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The American Phytopathological Society
St. Paul, Minnesota U.S.A.

Front cover: root disease pocket in a mature forest in the Black Hills. (Courtesy of John E. Lundquist)

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Assessing Forest-pathogen Interactions at the Population Level

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Introduction

During most of the past century, forest pathologists were limited to the study of pathogen phenotypes, vegetative compatibility, and mating reactions. These studies provided important insights in fungal taxonomy and phylogenetics, reproductive biology, and population genetics. However, these aspects are insufficiently variable or technically unfeasible for making inferences about intraspecific evolutionary processes (i.e., microevolution). Molecular techniques (Kim *et al.*, Chapter 2, this volume) and their application to population genetic analyses have given plant pathologists new tools that allow characterization of pathogens and their hosts at the species, population, and/or individual level. These data have enabled researchers to design experiments and test hypotheses on microevolutionary processes *in situ*. The outcome has been advancement in our basic understanding of organismal interactions from molecular processes to metapopulations and landscape effects.

Forests are among the most biologically complex terrestrial ecosystems. Because of this complexity, forest ecosystems are a rich source of information regarding interactions among the environment, hosts, and pathogens at spatial scales ranging from local to regional or landscape. Frequently, these ecosystems are relatively undisturbed and contain a diverse collection of pathosystems. These plant-pathogen interactions can represent both native, coevolved relationships and exotic introductions spanning wide geographical areas and heterogeneous environments. Interactions among individual hosts and pathogens vary in duration from annual (e.g., leaf spots and some needle casts) to long-lived (e.g., canker diseases and root rots). These relationships also can vary from coevolved obligate relationships (e.g., rusts) to facultative parasites (e.g., *Armillaria* spp.). Thus, forest ecosystems provide a unique opportunity for studies addressing the dynamics within and among host / pathogen populations and the forces that shape genetic structure.

Population genetics is the study of the organization of genetic variation among individuals within a species (Hartl and Clark 1997). The evolutionary forces of gene flow, genetic drift, mutation, recombination, and natural selection influence the genetic organization or structure of populations. Each of these components can drive genetic change (e.g., differences in gene frequencies) resulting in either the differentiation or homogenization of allele frequencies among populations. Many pathosystems are thought to have metapopulation dynamics in which populations (demes) are connected by gene flow. These populations may be ephemeral, with extinction and recolonization occurring constantly (Hanski and Gilpin 1997, Fig. 3.1). Estimates of

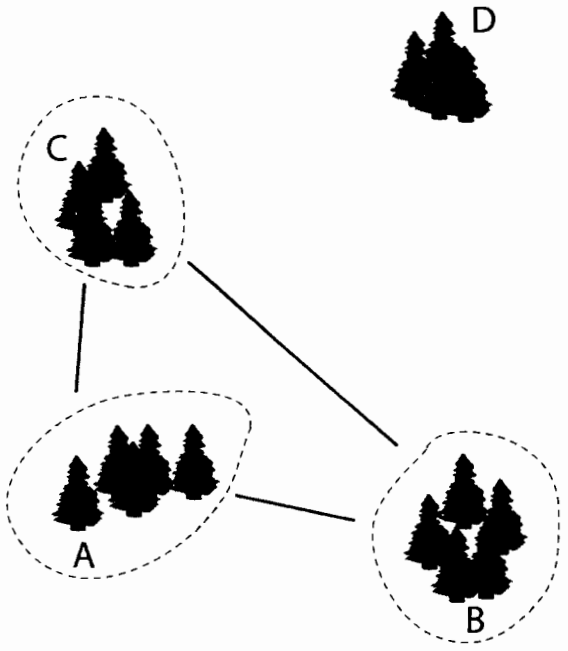


Figure 3.1. Hypothetical forest tree and pathogen metapopulation. Gene flow occurs among host and pathogen populations (A, B, C) symbolized by dotted outline. Extinction and recolonization occurs periodically. Population D is unsuitable for the pathogen establishment.

genetic structure, diversity, and a thorough understanding of the biology of the organism(s) can provide insights into broader questions across disciplines. These insights could include elucidating interactions between genetic structure and the environment, defining the role of pathogen races in the epidemiology of a disease, or the incorporation of genetic diversity in the development and implementation of disease resistance breeding programs. Thus, populations are the fundamental unit to reveal the effects of evolutionary forces, while providing critical information for forest disease management. To better understand the complex evolutionary processes of forest pathosystems, future work must be directed toward defining populations and applying population genetic analyses to various host-pathogen interactions.

Processes Affecting Population Dynamics

Interactions of pathogen, host, and environment in space and time affect the population dynamics of forest pathosystems. Assessing these dynamics remains a difficult task that requires knowledge of the disease process, life history, and genetic characters of both the host and pathogen populations that are applied at the appropriate spatial scale (Worrall 1999). Presently, empirical data that address population dynamics in natural pathosystems are limited. One of the best-understood natural pathosystems is the autoecious flax rust, *Melampsora lini,* and its host, the perennial herb *Linum marginale* occurring in southeastern Australia. This pathosystem exhibits a gene-for-gene interaction (see Flor 1955) and metapopulation dynamics (Thrall and Burdon 2000; Thrall *et al.* 2001). These data illustrate that patterns of disease incidence can vary considerably across space and time. The largest difference in frequency of resistance and virulence phenotypes occurred among environmental gradients correlated with distinct genetic structure in host populations. Thrall and Burdon (2003) found a positive correlation in highly virulent rust (i.e., pathogens with the ability to infect multiple resistance genotypes of the host) and high resistance in the host population. They proposed that aggressive races (i.e., those with ability to produce a greater amount of inoculum) are favored in susceptible populations, and virulent races are favored in resistant populations.

Pathogens vary considerably in their reproductive processes and capabilities, which can greatly influence their evolutionary potential. Asexual and inbreeding pathogens have lower evolutionary potential because there is little or no sexual recombination, whereas highly outcrossing pathogens create recombinant progeny. This principle led McDonald and Linde (2002) to propose that outcrossing pathogens have a greater capacity to overcome resistance in the host. Other important factors for evolutionary potential include gene flow, effective population size, and mutation rate. Evolutionary potential enables a pathogen to adapt more rapidly to local hosts or environments, possibly resulting in increased genetic differentiation among populations due to selection (reviewed in Kaltz and Shykoff 1998). This higher evolutionary potential among certain pathogens is supported by empirical data. Melampsora rust (caused by *Melampsora epitea*) of willow (*Salix* spp.) has recently become established on once-resistant, clonal willow in the British Isles. Over the course of one season, the same clone became heavily infected with Melampsora rust at three different sites. Amplified fragment length polymorphisms (AFLPs) were used to characterize rust samples at each site. Pei *et al.* (2000) found substantial genetic differentiation among rust populations at each site, which may indicate separate introductions. Their study suggests that virulence had developed at each site through independent processes of sexual recombination. Comparable studies with other pathosystems could elucidate the role of the differing disease interactions (e.g., gene-for-gene versus horizontal resistance) and life history characteristics (e.g., simple versus compound interest diseases) on population genetic dynamics.

Many studies have implicated climate change or other disturbances as major forces in shaping population genetic structure of forest trees (e.g., Walter and Epperson 2001; Petit *et al.* 1997; Sinclair *et al.* 1999). Glacial and interglacial periods that changed biogeographical ranges and host population dynamics have also affected the associated pathogen populations. Populations of forest trees generally show low levels of population differentiation relative to other plant species with most genetic variation occurring within rather than among populations. These patterns may be attributable to lifecycle characteristics including long, overlapping generations and a prolonged juvenile stage, which are typical for most forest trees. A prolonged juvenile stage may reduce inbreeding by increasing the time for additional migrants to enter a founder population. Models have shown these characteristics to be critical in preserving genetic diversity and lessening the impact of founder effects (Austerlitz *et al.* 2000). Despite the typically low genetic differentiation among populations relative to other organisms, genetic structure could correspond to important evolutionary adaptation to a particular environment or pathogen. For example, a low estimate of population genetic structure based on neutral markers may not reflect real differences in functional genes, some of which could be associated with fitness or pathogen resistance. To date, genetic studies of forest trees have provided insights into the roles of gene flow via pollen and seed, hybridization between species, and sites of glacial refugia in shaping extant populations (e.g., Latta and Mitton 1997; Oddou-Muratorio *et al.* 2001; Richardson *et al.* 2002). These processes have been investigated using uniparentally and biparentally inherited organellar DNA. An understanding of population structure, genetic diversity, and the driving forces of host populations is critical for understanding pathogen

populations and gaining further insights into host-pathogen interactions.

Currently, only a few studies have attempted to compare population genetic structure of forest trees in association with historical and contemporary disease pressure. Molecular markers, such as AFLPs, can be utilized to assess the genetic relationships and diversity of populations under different selection pressures. Using AFLP markers, Kim *et al.* (2003) correlated the genetic diversity of western white pine (*Pinus monticola*, WWP) populations grown under high and low pressure from blister rust (caused by *Cronartium ribicola*). They found that genetic diversity of WWP was lower within local populations relative to regional sampling, regardless of blister-rust hazard. However, the local population surviving under high blister-rust pressure was more genetically similar to a composite population originating from a rust-resistance breeding program.

The effect of disease pressure on host genetic structure has been reported for other pathosystems. The oak wilt fungus *Ceratocystis fagacearum*, is believed to be an introduced species with low levels of genetic diversity (Kurdyla *et al.* 1995). McDonald *et al.* (1998) studied the genetic structure of a pre- and post-epidemic stand of live oak (*Quercus fusiformis*). Changes in allele and genotype frequencies between pre- and post-epidemic trees led them to propose that disease pressure has had an important role in the genetic structure of this stand.

The diverse structure in forest ecosystems provides niches for an assortment of plant-pathogen interactions that range in time from seasonal to decades or centuries and that cover areas from microsites to vast expanses. The duration and nature of the disease process and the life-history traits of the host and pathogen play important roles in population dynamics. Below, we discuss specific interactions and contrast the role of diverse processes in shaping host-pathogen populations.

Armillaria Species

Basidiomycete plant pathogens, symbionts, and saprophytes of roots pose an intriguing challenge for understanding population dynamics. Organisms such as *Armillaria* spp. typically display a wide host range and can exist as genets (vegetative clones) that can occupy up to 15 ha or more and exist on a site for at least 1,500 years (Smith *et al.* 1992). Asexual propagules have not been documented for *Armillaria* spp., but this aspect of the lifecycle is currently being studied (P. J. Zambino, per. comm.). Spread of individual genets is believed to occur by vegetative growth (via rhizomorphs), over distances of a few meters a year (Shaw and Roth 1976, van der Kamp 1993), and separate *Armillaria* genets rarely overlap within a site (Legrand *et al.* 1996). *Armillaria* spp. can undergo sexual recombination via basidiospore production and mating; however, the frequency of sexual reproduction and dispersal distances of basidiospores are unknown.

Several studies have used molecular markers to infer species relationships and population genetic structure at various spatial scales. Coetzee *et al.* (2000) used sequences of intergenic spacer (IGS) and internal transcribed spacer (ITS) regions of rDNA to assess phylogenic relationships within *A. mellea*. Four lineages of *A. mellea* were found that correlated to geographic origin: Asia, western North America, eastern North America, and Europe. At a regional level, Smith *et al.* (1994) used mitochondrial and nuclear DNA restriction fragment markers to examine relatedness and spatial distribution of *A. ostoyae* genets infecting red pine (*Pinus resinosa*) in northern Michigan. These studies indicated that *Armillaria* genets were established by sexual mating events, and that the breeding population of *A. ostoyae* extended beyond 1 km at the study site. Saville *et al.* (1996) used seven nuclear loci to analyze genotype frequencies of 121 genets of *A. gallica* within four regions of eastern North America. Because genotype frequencies were not significantly different from Hardy-Weinberg expectations in that study, it appears that gene flow was sufficient to prevent local differentiation of genetic structure. In another study, no correlation was found between geographic and genetic distances among *A. ostoyae* genets (Dettman and van der Kamp 2001). In addition, interspecific hybridization can apparently occur within *Armillaria* spp. (Kim *et al.* 2001).

Even though *Armillaria* genets appear to be extremely long-lived, populations are dynamic. The genetic and species composition is likely influenced by environmental factors such as host species composition, fire disturbance regimes, moisture, and management practices. For example, Dettman and van der Kamp (2001) used Random Amplified Polymorphic DNA (RAPD) analysis to examine population structure of *A. ostoyae* in British Columbia at two sites that differed in stand age and time since disturbance. They found that *A. ostoyae* genets were much smaller in the more recently disturbed, younger tree stand than in the mature tree stand. Earlier studies by Rizzo (1995) support these results. They proposed that the differences in genet size could be influenced by environmental factors associated with the disturbance regimes and stand structure, as well as competition and selection among genets. More studies are needed to assess relationships among environmental parameters, host composition, and population structure of *Armillaria* spp.

Currently, studies are underway to characterize *Armillaria* populations using AFLPs (Figure 3.2; M.-S. Kim, per. comm.). These studies will look for relationships among population structure, environmental parameters (e.g., habitat types, soil nutrients), and ecological behavior (e.g., saprophytic or pathogenic; Klopfenstein *et al.* 2001). A better understanding of these relationships will allow predictions of ecological behavior and foster the development of appropriate forest management practices to reduce *Armillaria* damage. In theory, once relationships between the

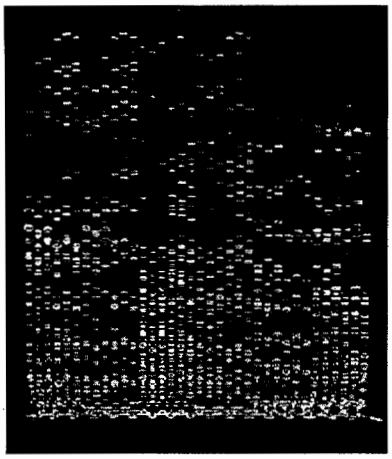


Figure 3.2. AFLP gel image from *Armillaria* species showing a wide array of banding patterns that distinguish individual genets.

environment and intra- and interspecific composition are better understood, several disease management objectives could be implemented. Management may involve establishing a predictive model of *Armillaria* species occurrence and density and dictate a silvicultural treatment to favor saprophytic species.

Cronartium ribicola

Rust fungi possess unique biological attributes that can have significant effects on population structure. Population studies of heteroecious rusts must consider the host range in conjunction with the alternate host range. Many rust diseases are endemic, while others are introduced. Selection pressure can vary greatly in relation to rust virulence and aggressiveness on hosts, and host resistance to rust pathogens. Some rust spores (e.g., aeciospores and urediniospores) apparently have the capacity for long distance dispersal, but the mating systems of most rusts are not well characterized.

White pine blister rust (caused by *Cronartium ribicola*) was introduced into North America in the early 1900's (Spaulding 1916) and has subsequently spread to most areas supporting five-needle pines (Smith and Hoffman 2000; McDonald and Hoff 2001). Information on the population structure of *C. ribicola*, hosts, and alternate hosts (e.g., *Ribes* spp.) are necessary to develop management practices and tools, such as deployment or regeneration of resistant host materials, efficient rust-resistance screening, and predicting infection rates. Previous studies on the infection of pine and *Ribes* spp. indicate that genetic variation in *C. ribicola* occurs over space and time (Hoff and McDonald 1993; Kinloch *et al.* 1998; McDonald 2000). Blister rust epidemics among five-needle pine have varied in severity. In some stands where mortality has exceeded 95%, WWP appears to be in the process of recovery (Fig. 3.3). At the smallest scale, within cankers, RAPD markers of haploid monokaryotic spermogonia and IGS sequences of haploid cultures showed that genotypic variation exists among aecia from putatively homogenous, haploid, monokaryotic cankers (Hamelin 1996; White *et al.* 1996; Hamelin *et al.* 1998a). Sexual recombination in the teliospores is evident from the equal segregation of basidiospore genotypes

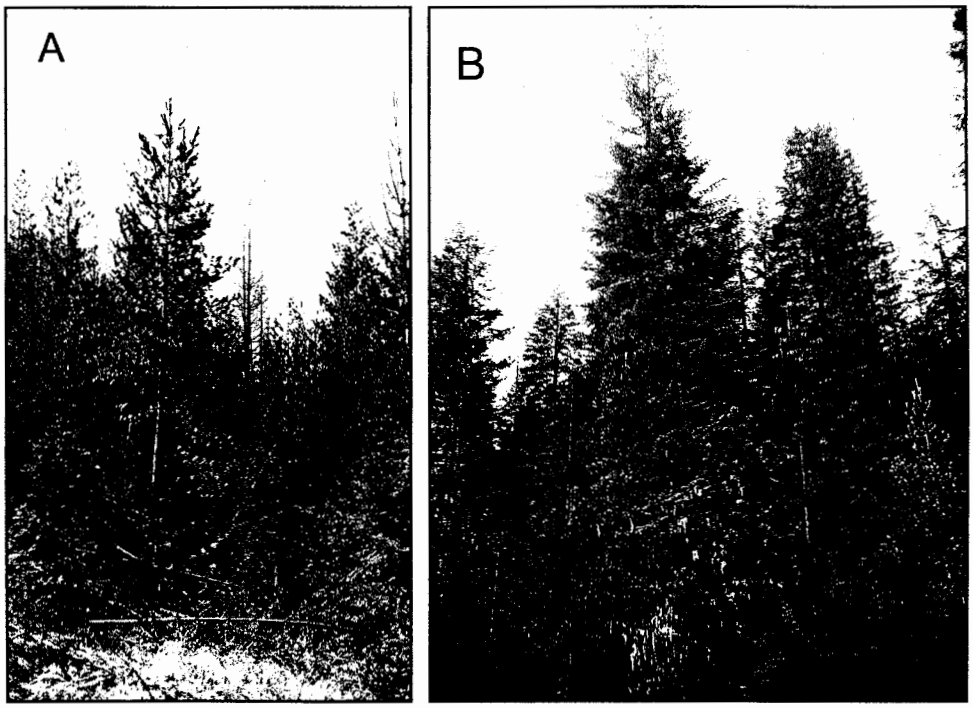


Figure 3.3. *Pinus monticola* at the Emerald Creek, Idaho population. Photo A shows blister rust resistant tree 57 in 1951 along with severe mortality in background. Photo B shows present day tree 57 shown as the tallest in background with encroachment of climax spp. and regeneration in foreground.

(Gittzendanner *et al.* 1996). The outcrossing exhibited by *C. ribicola* likely increases its evolutionary potential. This may have provided the genetic variation that allowed this pathogen to colonize multiple host species in North America and in environments ranging from relatively mesic (e.g., *P. monticola* stands) to xeric high-elevation (e.g., *P. flexilis* and/or *P. albicaulis* stands).

Population genetic studies of *C. ribicola* have found varying degrees of genetic structure that may depend on geographical areas sampled and the detection methods. Using isozyme, RAPD, and restriction fragment length polymorphism (RFLP) markers, Kinloch *et al.* (1998) found differentiation among populations of *C. ribicola* in western North America; however, genetic distances were not correlated to geographic distances. The largest genetic distance was found between the Happy Camp population (northern California) and the other populations sampled in western North America. The Happy Camp population of *C. ribicola* posseses the vcr1 genotype, a virulent race of otherwise resistant trees carrying a hypersensitive gene (Cr1). Hamelin *et al.* (1995) and Et-touil *et al.* (1999) found a somewhat different population structure existing in eastern Canada. RAPD markers revealed that most genetic diversity was found within populations. Low-level genetic differentiation was observed among populations within a region, but genetic differentiation was not evident among regions (e.g., provinces) or type of stand. Subsequent studies by Hamelin *et al.* (2000) used similar RAPD markers to demonstrate that *C. ribicola* populations are distinct in eastern and western North America. Further work using codominant markers suggest that a potential barrier to gene flow has existed between these regions, yet local populations within each region were in Hardy-Weinberg equilibrium (Hamelin, pers. comm.).

The population structure of hosts, alternate hosts, and other interacting microorganisms raise several provocative issues regarding forces that shape *C. ribicola.* Nearly 100 years have passed since the introduction of *C. ribicola* into North America and range expansion is still occurring in Wyoming and northeastern Colorado (Johnson and Jacobi 2000). Does a rust population on the leading edge of spread tend to be more aggressive and genetically uniform? Have local *C. ribicola* populations that exist near sites of introduction become less aggressive and more genetically diverse over time?

Phylogenetic analyses of pine stem rusts (*Cronartium* and *Peridermium* spp.) using ITS sequences have provided support for host-specific evolution to the alternate (i.e., telial) host (Vogler and Bruns 1998). The interaction between host tissues and *C. ribicola* has important implications regarding the population genetic structure and evolution. Canker longevity depends on the tree size, site of infection, and host / pathogen interaction. Considerable variation has been noted in canker latency and expansion rates (Hunt 1997), and this variation likely impacts epidemiological processes. Are characteristics such as aggressive colonization of pine tissue and fast production of aeciospores only favored during early stages of epidemics? Are less aggressive rust populations favored over extended time periods? Some cankers are known to be active for more than 30 years, while other infections cause tree mortality in a few years (Fig. 3.4). Aggressiveness and lifetime reproductive



Figure 3.4. A) a white pine blister rust canker that is >10 years old and has completely colonized circumference of the small diameter stem, killing the host (*P. monticola*). B) a slow growing canker of blister rust. The host, *P. monticola* from F2 resistance material, is approx. 30 years old and the canker is approx. 25 years old.

success are likely important components in the epidemiology and population genetics of *C. ribicola* (McDonald *et al.*, Chapter 5, this volume). Once environmental influences on aggressiveness are better understood, management practices can be developed to favor less aggressive rust populations.

Host specificity is another possible force shaping populations. *C. ribicola* is widespread on five species of five-needle pine in North America and many *Ribes* spp. McDonald (2000) measured infection efficiency and incubation period on inoculated leaf discs of two clones of five *Ribes* spp. using inoculum from four rust sources. Infection efficiency of rust sources varied considerably among *Ribes* clones. For example, some rust sources produced little or no infection on certain *Ribes* clones, whereas other sources produced high levels of infections on the same *Ribes* clone. In addition, populations of the rust vary in virulence among WWP populations (McDonald *et al.* 1984). Sniezko *et al.* (2001) demonstrated that the WWP Cr2 genotype, conferring a hypersensitive reaction to *C. ribicola*, co-occurs in distribution with the vcr2 genotype of the rust, which is virulent against the Cr2 genotype. These data suggest that populations of *C. ribicola* are under local stabilizing selection and could become adapted to particular hosts and alternate hosts. A more thorough understanding of the genetic structure in relation to the biological and / or environmental gradients in *C. ribicola* can provide inferences into the evolutionary forces shaping this pathosystem.

Development of suitable genetic marker systems (e.g., microsatellites, RAPD, AFLP, single nucleotide polymorphisms, etc.) will allow critical studies on the population structure of pathogens, hosts, alternate hosts, and other related organisms within the white pine blister rust pathosystem. Continued investigation of the changing genetic structure of the blister rust pathosystems should generate significant insights to help manage and monitor disease progress. If systems can be developed to successfully manage rust populations, stable ecosystems containing five-needle pines may return to the landscape on a wide scale.

Gremmeniella spp.

Scleroderris canker, caused by *Gremmeniella abietina* (Lagerberg) Morelet var. *abietina* Petrini *et al.*, is a serious disease of conifers in Asia, Europe, and North America (Manion 1984). *G. abietina* is a haploid, ascomycetous fungus that produces sexual (apothecia) and asexual (pycnidia) fruiting bodies (Donaubauer 1972). Several studies have used DNA sequences and markers to examine taxonomic and genetic relatedness of diverse *G. albietina* isolates (Hamelin and Rail 1997; Hamelin *et al.* 1993, 1996; Hansson *et al.* 1996; Hantula and Müller 1997; Hellgren and Högberg 1995). A study in Sweden detected high genetic variability in *G. abietina* and little gametic disequilibrium, suggesting a high degree of random mating among a population founded by many unrelated genotypes (Wang 1997). Additional studies determined that identical genotypes occurred at low frequency and existed only on the same tree or nearby tree, thereby indicating limited spatial dispersal of asexual spores (Wang *et al.* 1997). RAPD marker analyses indicate that North America has experienced multiple introductions of the European race of *G. abietina* (Hamelin *et al.* 1998b).

In northern Sweden, an introduced host, lodgepole pine (*Pinus contorta* Dougl. ex Loud.), has experienced severe damage from endemic pathogens (Karlman *et al.* 1994). The transfer of exotic host material can be of concern to the stability of endemic host-pathogen populations. Ennos (2001) proposed that the introduction of low-resistance host material, such as *P. contorta*, could shift the adaptive processes of the native pathogen populations. In theory, an epidemic on an exotic, highly susceptible host could select for individuals with an increased aggressiveness in the pathogen population. In addition, a highly susceptible host could result in a large number of spores, increasing the probability of mutation and infection of a new host species (Roy 2001). Additional studies are needed to test this hypothesis.

Cryphonectria parasitica

Chestnut blight is a fungal disease of American chestnut (*Castanea dentata*) and European chestnut (*C. sativa*) caused by *Cryphonectria parasitica* (formerly *Endothia parasitica*). A chestnut blight epidemic swept through North America and Europe after C. *parasitica* was introduced from Asia in the early 1900's. Previous work has demonstrated that *C. parasitica* can harbor a cytoplasmic hypovirulence factor that can be transmitted among vegetatively compatible strains (Anagnostakis 1987). The hypovirulence factor has been shown to be double-stranded RNAs (dsRNAs) that have recently been classified in a new virus family, the Hypoviridae (Hillman *et al.* 1995). Hypovirus-infected isolates of *C. parasitica* display characteristics of reduced fitness (e.g., slower canker growth, reduced conidia production, and inhibition of female fertility; Nuss 1992). Hypoviruses are apparently transmitted through asexual spores (conidia), but not through sexual spores (ascospores), of *C. parasitica* (Anagnostakis 1987; Griffin 1986; Van Alfen *et al.* 1978). Hypoviruses can be spread cytoplasmically among vegetatively compatible strains, but their spread *in vitro* is impeded between vegetatively incompatible strains (Anagnostakis 1987). Further work has demonstrated that this transmission barrier is not absolute. A quantitative negative relationship exists between the number of polymorphic vegetative incompatibility (*vic)* loci and rates of transmission; however, each *vic* locus varies in its ability to inhibit viral transmission (Liu and Milgroom 1996).

Naturally established populations that display hypovirulence are correlated with a low diversity of vegetative

compatibility (vc) types (Anagnostakis *et al.* 1986; Cortesi *et al.* 1996; Heiniger and Rigling 1994; Liu *et al.* 1996). Similarly, biocontrol efforts with hypoviruses have been successful in populations of *C. parasitica* in Europe and Michigan, which display lower diversity of vc types than that found in eastern North America (Fulbright *et al.* 1983; Heiniger and Rigling 1994). Higher diversity in vegetative incompatibility types is found in eastern North America, where hypovirulence is not well established (Anagnostakis 1987; MacDonald and Fulbright 1991). The high diversity of vc types in eastern North America may represent the most important barrier to successful transmission of hypoviruses within *C. parasitica* populations, which may prevent hypoviruses from controlling chestnut blight in that region (Anagnostakis 1987; MacDonald and Fulbright 1991).

Vegetative incompatibility in European populations of *C. parasitica* is controlled by six unlinked, biallelic *vic* loci (Cortesi and Milgroom 1998). Individuals are vegetatively incompatible when alleles are different at one or more *vic* loci. Allele frequencies at *vic* loci were used to analyze genetic structure of 13 European populations and three North American populations of *C. parasitica*. European populations were found to have less vegetative incompatibility diversity, presumably due to lower *vic*-allele diversity and limited recombination (Milgroom and Cortesi 1999).

DNA fingerprinting and vegetative compatibility were used to examine the genetic structure of three newly established populations of *C. parasitica* located in northern Switzerland, outside the primary range of European chestnut (Hoegger *et al.* 2000). The newly established populations were nearly clonal, with only one or two vegetative incompatibility types, whereas another population within the main range of chestnut exhibited much higher diversity in DNA fingerprints and vegetative incompatibility type. Hoegger *et al.* (2000) concluded that *C. parasitica* had been disseminated primarily by asexual reproduction in the newly established populations.

One hypothesis concerning the evolution of vegetative incompatibility systems in fungi is that they may limit the spread of deleterious entities such as viruses (Caten 1972; Malik and Vilgalys 1999). This protective mechanism also may limit the spread of an introduced biological control agent, such as a hypovirus, to a single vc type. Because hypovirus infection reduces fitness of *C. parasitica*, this process could exert frequency-dependent selection on vegetative compatibility types, where rare vegetative compatibility types have a selective advantage by escaping hypovirus infection. However, a study of *vic* allele frequencies in Europe did not support this hypothesis. Relatively recent founder events of the fungus or other factors may also be influencing *vic* allele frequencies (Milgroom and Cortesi 1999).

An understanding of vc diversity within a *C. parasitica* population is essential when considering biological control options that are based on hypovirus transmission. Conventional wisdom predicts that biological control via hypovirus transmission would be less successful and more difficult to apply within *C. parasitica* populations that contain a high diversity in vegetative compatibility types. However, inoculations with hypovirus-containing isolates of three vc types resulted in hypovirus spread to 45 vc types over a 17-year period (Hogan and Griffin 2002). This result suggests that hypovirus transmission may be more effective *in vivo* than *in vitro* tests suggest. Continued studies are necessary to fully understand the genetic structure of *C. parasitica* populations and the genetic controls involved with transmission before effective strategies can be developed and implemented to control chestnut blight.

In the past, the phenotype of vegetative incompatibility has been frequently used to distinguish groups of fungi; however, little is known about the genetic mechanisms involved. Knowledge of the genetic control in vc and mating types can be a useful tool in understanding the genetic dynamics of pathogen populations. The number of *vic* loci and the diversity of alleles at a locus can influence the genetic diversity with populations. Characterization of the *vic* profiles can provide information about population genetic structure and gene flow, as well as the population biology and capacity for recombination (Milgroom 1996).

Other Pathosystems

Genetic diversity of the pitch canker fungus, *Fusarium subglutinans* f. sp. *pini*, was examined using vc types and RFLPs of mitochondrial DNA (Correll *et al.* 1992). Results of this study indicate that the California pathogen population was asexually reproducing and/or was recently introduced. Swiss needle cast, caused by *Phaeocryptopus gaeumannii*, is a major needle blight disease on coastal Douglas-fir, *Pseudotsuga menziesii* var. *menziesii*. Using single-strand conformational polymorphisms (SSCP) from 5 amplified genes, Winton (2001) found two sympatric lineages of *P. gaeumannii* in Oregon and Washington, U.S.A. Genotypic and genetic diversity within the lineages was low, indicating a predominantly asexual lifecycle. Only one lineage was associated with the epidemic levels of disease.

Arbitrary signatures from amplification profiles (ASAP) were used to distinguish isolates of *Discula destructiva*, the cause of dogwood anthracnose, from various regions of North America (Caetano-Anollés *et al.* 1996). The ASAP analyses also revealed fine population structure, and indicated that eastern and western populations of *D. destructiva* may have resulted from recent and separate introductions. Recently, Zhang and Blackwell (2002) performed additional studies on the population structure of *D. destructiva* using AFLP and sequences of intergenic spacer (IGS) of nuclear ribosomal DNA, translation elongation factor (EF-1α), and the β-tubulin gene. Their studies also indicated that *D. destructiva* exists as two distinct, disjunct groups corresponding to eastern and western North America. Further analyses indicated that this pathogen was still under

intense selection pressure; however, transition to a less virulent, heterogeneous population may have begun near a possible epidemic center in the eastern U.S.A.

General Considerations

Complex interactions among hosts, pathogens, associated organisms, and the physical environment are critical forces that shape host and pathogen populations in forest ecosystems. Such processes vary with pathosystem, site, and time. Forces shaping host/pathogen populations may vary substantially, even within the same locale. Studies of the population genetics of hosts / pathogens offer exciting new approaches to understanding the dynamic interactions among trees and microorganisms. Such approaches are particularly attractive because dynamic biological properties are inherently related to changing genetic structure. Molecular tools allow a high-resolution characterization of biological populations, and tools to monitor environmental parameters provide detailed information on abiotic factors.

Widely ranging interactions across forest ecosystems can be addressed by incorporating biological and physical information into Geographic Information Systems (GIS). Such approaches will assist our understanding of population structure at the landscape level and help determine the appropriate geographic scale for population studies of specific pathosystems (Klopfenstein *et al.* 2001; Lundquist and Klopfenstein 2001). Available molecular tools and new methods in genome-wide expression using microarray technology will certainly become important in understanding natural population dynamics, thereby enabling researchers to ask more specific questions about population genetic dynamics (e.g., Cowen *et al.* 2002). The integration of population genetics at the landscape level will provide great insight into forces driving dynamic interactions among biological organisms and the abiotic environment across forest landscapes. However, it is essential that representative samples are collected and archived at continuing time points to observe changes in host/pathogen populations. This process is especially important for pathosystems that are changing rapidly due to strong selection pressure.

The application of population genetics to forest management is critical to understanding processes of disease stabilization and developing effective disease prediction and management systems. The spatial patterns of pathogen populations can help identify infection sources, predict disease spread, and the study of local extinction and reintroduction (Real and McElhany 1996). When population structure is better understood, forest managers and breeders can tailor their practices and programs to reflect the populations of forest trees and associated pathogens. This process should allow more effective prediction of infection rates and more efficient improvement in genetic resistance and disease stability based on the properties of the host and pathogen population.

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