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Integrating concepts of landscape ecology with the molecular biology of forest pathogens

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Abstract

Increasingly more research has focused on characterizing diversity within forest pathogen populations using molecular markers but few studies have characterized features of the landscape that help create or maintain this diversity. Forest diseases commonly occur in patchy distributions across natural landscapes which can be reflected in the genetic composition of the fragmented pathogen populations. This metapopulation structure has seldom been examined by forest pathologists but we believe it offers a potential means to understand the genetic ecology of pathogens in natural landscapes. Molecular markers can be used to detect, identify, and measure detailed differences among subpopulations of forest pathogens. Geographical information systems, spatial analysis and modeling, digital imagery of remotely sensed images, and other tools of landscape ecology provide the means to detect and interpret patterns associated with genotypic asymmetry. Integrating the tools and concepts of molecular biology and landscape ecology by focusing on metapopulation disease phenomena offers a way of conceptually linking molecules and ecosystems. Published by Elsevier Science B.V.

Keywords: Forest diseases; Molecular markers; Metapopulations

1. Introduction

Many forest pathogens have the capacity for virtually unlimited genetic diversification, but this rarely, if ever, happens. Spatial structure and heterogeneity of the environment can play primary roles in restricting and directing patterns of genetic diversity but how these patterns develop and persist in forest pathogens populations is not well-understood (Jelinski, 1997). According to Jeger (1988), ''the dynamics of disease in spatially heterogeneous populations is one of the major outstanding problems in plant disease epidemiology''. Forests are uniquely heterogeneous ecosystems.

Landscape ecology and molecular genetics have both been characterized as emerging fields for which tools and concepts are still evolving. Arguably, technology has been the major driving force in the development of both fields; viz. landscape ecology by GIS-dependant spatial imagery and numeric analysis, and molecular biology by genetic marker technology. Whereas, landscape ecology has been adept at developing concepts, molecular biology has emphasized the development of tools. Despite the contrasts between these two fields, we believe that applying and adapting molecular genetics to landscape ecology concepts will offer unique insights into dynamics of diseases in spatially diverse environments. In this paper, we

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briefly consider some synergistic applications of landscape ecology and molecular biology.

2. Landscape ecology

Landscape ecology has been defined as 'the science that studies the development and dynamics of spatial heterogeneity in ecosystems' (Risser et al., 1984). Landscape ecologists view a landscape as a mosaic composed of patches and inter-patch regions called a matrix, and essentially try to answer questions about how landscape structure influences ecological processes and patterns using spatially dependent imagery and statistics. Although landscape ecologists usually make no distinctions among causes of structural changes in the landscape and treat pathogens as genetically uniform populations (Rykiel, 1985), many concepts potentially useful to forest pathology studies have emerged from their work (Forman, 1995; Lidicker, 1995; Turner et al., 1995). In contrast to landscape ecologists, forest pathologists have largely focused on causes and have mostly avoided describing the impact of diseases using the concepts and tools of landscape ecology. Castello et al. (1995) review studies of forest diseases that incorporate landscape approaches.

Landscapes are distinguished by differences in landscape structure based on the physical layout (physiognomy) and relative amounts of each landscape element (composition) (Dunning et al., 1992). Metrics used to characterize physiognomy include interpatch distances, patch size, patch contagion, shape of the patch boundaries, and others that characterize the physical layout of patches in the landscape. Metrics that characterize composition include total number of patches, relative frequencies of different types of patches, patch richness or diversity, and other metrics that characterize frequencies and relative frequencies. The list of metrics and methods used to characterize landscapes is large (Legendre and Fortin, 1989) and rapidly expanding. Consequently, spatial heterogeneity has several meanings, and can be measured in many ways (Li and Reynolds, 1995). Jeger (1988) believes that, 'an aim of spatial analysis should be not only to obtain good statistical descriptions, but also to obtain insight into the mechanisms that generate patterns.'

3. Molecular biology

Molecular biology is a rapidly developing and expanding avenue of research in forest pathology. A review of recent references indicates molecular biology has been used to: (1) identify forest pathogens in the environment (Frontz et al., 1998; Anderson et al., 1998; Fischer and Wagner, 1999; Johannesson and Stenlid, 1999), (2) identify genes responsible for pathogenesis in forest pathogens (Et-Touil et al., 1999), (3) describe patterns of establishment and spread within forest ecosystems (Hamelin et al., 1998b; Pappinen et al., 1996), (4) determine phylogenetic relationships of forest pathogens (Vogler and Bruns, 1998; Terashima et al., 1998), (5) characterize genetic variation among pathogen species (Harrington et al., 1998; Moricca and Ragazzi, 1998; Moricca et al., 1996; White and Morrison, 1999) and pathogen varieties (Dusabenyagasani et al., 1998; Hausner et al., 1999), and (6) characterize genetic variation among pathogen populations (Goggioli et al., 1998; Hamelin et al., 1994; Hamelin et al., 1995; Hantula et al., 1998; Lilja et al., 1998; La Porta et al., 1997; Schulze et al., 1997; Hantula et al., 1998; Högberg et al., 1999; Rogers et al., 1999; Schulze, 1999) and within pathogen populations (Doudrick et al., 1993; Gosselin et al., 1999; Hamelin, 1996; Marçais et al., 1998).

4. Variation within pathogen populations

Most studies in forest pathology that use molecular markers describe genetic variability among pathogens. Molecular markers allow direct assessments of genotypic variability, and have significantly increased the resolution at which genetic differences can be characterized. Each type of molecular marker has different assets and limitations. Restriction fragment length polymorphisms (RFLP), for example, can distinguish between homozygotes and heterozygotes but they are relatively expensive, time-consuming, require relatively large amounts of DNA, and commonly use potentially hazardous radioactive probes. Random amplified polymorphic (RAPD) DNAs are fast, cheap, relatively simple to use, require relatively small amounts of DNA, and require no prior knowledge of the genome sequence. However, RAPDs use amplification of unknown sequences, may not survey all

parts of the genome equally, are not reliably reproducible, cannot distinguish between homozygotes and heterozygotes, and are less effective with large complex genomes. Amplification fragment length polymorphisms (AFLPs) can distinguish between homozygotes and heterozygotes and provide markers in high abundance, but are relatively expensive and require moderate amounts of DNA. Simple sequence repeats (SSR) require only small amounts of DNA, distinguish between homozygotes and heterozygotes, but can require previous knowledge of the DNA sequence to identify SSR regions. New markers and variations on methods using current markers are constantly appearing in the forest pathology literature (e.g. Garbelotto et al., 1996; Hantula et al., 1998).

Selection of the appropriate marker depends on: (1) time, funding, facility, and skill-level constraints, (2) amount of DNA available, (3) number of individuals to be surveyed, (4) pre-existing genome information, and (5) desired level of information. Based on current trends, it appears that molecular marker technology will continue to increase in overall utility and accessibility. Molecular markers are frequently used initially to distinguish pathogen species. With the development of more precise markers, pathogen populations can be characterized below the species level and ultimately a single individual organism can be identified or characterized. Concurrently, DNA sequence information will become more available and eventually entire genome sequences should become available for selected pathogens. As more useful markers are identified over time, such markers can be applied in studies directed toward genetic mapping, population genetics, or assessments of gene flow with forest pathogens. Such developments should generate improved techniques to quantify genetic variation.

Recent reports demonstrate the use of molecular markers to characterize genetic diversity of forest pathogens. For example, Frontz et al. (1998) used RFLPs to show the relative similarity among various species and selections of *Armillaria* spp. isolated from various tree species in Pennsylvania. Hamelin et al. (1998a) used RAPD markers to show that nearly all white pine blister rust cankers contained spermogonia with the same genotype, and thus the fine-level genetic structure of white pine blister rust populations was relatively homogeneous. Goggioli et al., (1998) used isozymes and RAPDs to examine diversity among selections of intersterile groups (ISG) F, S, and P of *Heterobasidion* at various locations throughout Italy. Hamelin et al. (1998b) examined the geographical pattern of genetic diversity of *Gremmeniella abietina* var. *abietina* in Canada and the USA to determine whether scleroderris canker was a result of a single introduction of a European race into North America. La Porta et al. (1997) used RAPDs to describe genetic variation in ISG S isolates of *H. annosum* as a cline across Italy. Milgroom et al. (1996) used RFLPs to characterize the population structure of *Cryphonectria parasitica*, the cause of chestnut blight among isolates from China, Japan, North America, and Europe, and found that this pathogen was probably introduced to North America via Japan not China or Europe as originally thought. RAPD markers were used to localize a pathogenicity gene, Pat1, in *Ophiostoma novo-ulmi* Brasier, a causal agent of Dutch elm disease (Et-Touil et al., 1999). These studies also provided evidence that Pat1 which is associated with aggressiveness was acquired by introgression with *O. ulmi* (Buisman) Nannfeldt. Such studies could also evaluate the potential of a third species, *O. himal-ulmi* Brasier, to contribute pathogenicity genes to North American and European races of *O. novo-ulmi*.

For most forest pathogens, little molecular characterization has been attempted. For those that have been, characterization has been mostly at the species level. Because of technical and practical limitations, it has been difficult to assess relationships of genetic variability with environmental heterogeneity. As the molecular markers become more powerful, we need to give more consideration to environmental heterogeneity. In these studies of genetic diversity, the message is in the spatial context, yet few studies adequately describe the spatial context.

5. Integrating landscape ecology with molecular biology

Although landscape ecology and molecular biology are vastly separated by spatial and temporal scales, the principal genotypic changes, are generated at the molecular level but they are filtered and selected in the ‘environmental context’ suggests that their scales

can overlap (Jelinski, 1997). A major challenge to a landscape pathologist is to define and describe the level at which such scales correspond.

Pathogens show various patterns of spatial variation from no detectable to continuous gradients over extensive regions to distinct patches over small areas. Determining the natural scale is the first step in a landscape ecology study (Wiens, 1989). Thrall and Burdon (1997) suggest that the appropriate spatial scale for studying plant pathogens is the metapopulation. This may be the level at which molecular analyses should focus to characterize the influence of environmental heterogeneity on genetic diversity of pathogens. A metapopulation has been defined as a 'population consisting of spatially-separate subpopulations that are connected by the dispersal of individuals' (Forman, 1995). The concept of metapopulations arises from observations that pathogen populations are seldom uniformly distributed across a landscape but instead often occur as patches. As a consequence of spatial isolation, demographic and genetic features, subpopulations of a metapopulation can change independently, thereby enhancing genetic differences among pathogen populations. According to Thompson (1998), ''metapopulation structure can rapidly shape and reshape the genetic structure of species...''

Population geneticists generally attribute differences in gene frequencies among populations to gene flow. In theory, a randomly mating population will show stable allele frequencies described by the Hardy Weinberg equation. The impact of gene flow is estimated as the effective number of migrants per generation (N_m), which is a function of variance in gene frequencies among populations. These analyses have been mostly based on isozymes. Of the other molecular markers, RFLPs have mostly been used for estimating gene flow (McDermott and McDonald, 1993). Impact of geographic structure is calculated simply as a correlation with distance with assumptions of a uniform environment with a constant population size and dispersal rate (Bossart and Pashley Prowell, 1998). Three results are possible: (1) no *correlation = no* genetic or population structure; (2) no genetic structure but geographic structure; or (3) both genetic and geographic structure (Bossart and Pashley Prowell, 1998). Correlations based on such 'isolation-by-distance' models frequently fail to address complexity of natural systems because they do not take into account spatial heterogeneity.

Metapopulation models are potentially useful for examining the impact of surrounding populations on gene flow in a local population. These models normally estimate colonization or population abundance and probabilities of occurrences using transitional probabilities for movement among groups of sites within spatially explicit landscapes or movement rates calculated from frequency distributions of demographic features of subpopulations (Wiens, 1997). These models can incorporate geographic structure as variations in resistance to movement associated with patch edges, and patch and matrix context. Dunning et al. (1992) present a general classification of landscape processes that apply to forest pathogens (Fig. 1). Landscape ecologists use the term 'connectivity' to

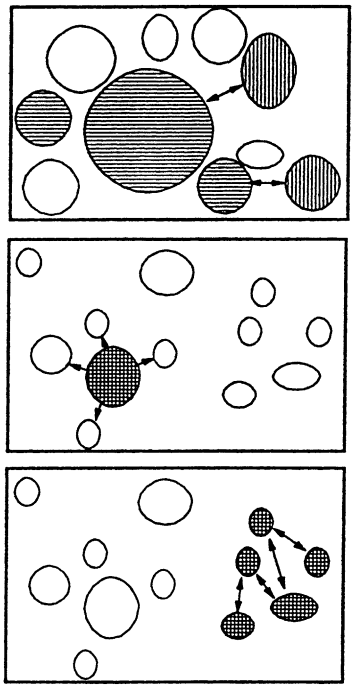


Fig. 1. A hypothetical landscape showing various landscape processes defined by Dunning et al. (1992). Circles represent patches within the surrounding matrix. Top — landscape complementation. Organisms require different resources found in different patches. Center — source and sink. One patch serves as a source to other patches that serve as sinks. Bottom — landscape supplementation. Organisms obtain needed resources by using two or more patches, one patch does not meet requirements.

represent patterns of resistance to movement among patches. According to Wiens (1997), 'through the patterns of connectivity that characterize a landscape, movement pathways are directed in spatially nonrandom manners which can either increase or decrease the likelihood that movement among (subpopulations in a metapopulation) will occur'. Some studies have focused on spatially explicit models in crop pathology (Nelson et al., 1999), but such studies are rare in forest pathology.

Spatial heterogeneity is a necessity for local adaptation (Thompson, 1998). Genotypic variation within pathogen populations results from evolutionary forces like selection, nonrandom mating, mutation, genetic drift, and gene flow. Genetic diversification among populations results from metapopulation dynamics like local extinctions, inter-patch movement, and recolonization. Local adaptations increase with decreasing inter-patch movement. Evolutionary factors and metapopulation dynamics act together to determine genetic diversity and genotypic spatial distributions among subpopulations of a metapopulation. Local extinctions increase with better habitat or greater patch size. Recolonization rate increases by adequate matrix habitat, short inter-patch distance, and corridors (Forman, 1995). How organisms evolve in heterogeneous environments has spawned a rich and prolific literature, from which many terms and concepts can be directly or indirectly applied to forest pathogen metapopulations.

Thrall and Burdon (1997) pointed out that when two species interact, 'population and genetic dynamics may occur over an even broader range of spatial and temporal scales, scales that are likely to differ sharply between the component species within a single association and certainly between different combinations of species.' Disease is a function of the co-occurrence of a virulent pathogen, a susceptible host, and a suitable environment. These three factors independently vary spatially and temporally; sometimes they coincide. Consequently, disease is usually distributed unevenly across the landscape. Thrall and Burdon define different types of 'interaction metapopulations' based on coinciding distributions of hosts and pathogens (Fig. 2). Thrall and Burdon caution that when genetic neutrality is assumed or species interaction are not considered, descriptions of metapopulation dynamics could be wrong.

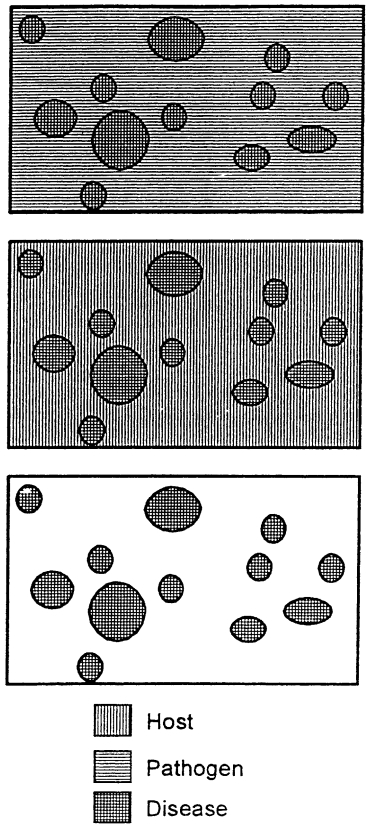


Fig. 2. A hypothetical landscape showing three types of interaction metapopulations defined by Thrall and Burdon (1997). Vertical cross-hatch represents distribution of host. Horizontal cross-hatch represents distribution of pathogen. Top — pathogen is more widely distributed than host. Center — host is more widely distributed than pathogen. Bottom — host and pathogen have similar spatial distributions.

The genetic structure of and gene flow among pathogen metapopulations is of particular interest for sites that contain potential hosts but do not contain pathogen populations. Studies of genetic structure can address whether pathogen absence is due to maladaptation or lack of introduction. Studies of genetic variability and gene flow among pathogen metapopulations may indicate potential mechanisms by which forest pathogen populations could become adapted to a new environment or new hosts. For example, the potential spread of white pine blister rust (caused by *Cronartium ribicola* J.C. Fischer) to drier sites is currently a major concern among forest pathologists and ecologists (G.I. McDonald, pers. comm.). Other

studies are underway to characterize eastern and western populations of *C. ribicola* (R.C. Hamelin, pers. comm.). Avenues of gene flow among populations may also influence pathogen adaptability to new environments (R.C. Hamelin, pers. comm.; G.I. McDonald, pers. comm.). The study of metapopulation genetics of *C. ribicola* would address such issues of site adaptability, host range, and gene flow. Such information would facilitate prediction of pathogen adaptation and spread into new areas.

Recently, *Armillaria* spp. have also become the subject of molecular genetic studies. RFLPs of PCR-amplified intergenic spacer (IGS) have been used to identify species (Harrington and Wingfield, 1995), RAPDS have been used to identify individuals (Guillaumin et al., 1996), and establish phylogenetic relationships (Piercey-Normore et al., 1998). Other studies are ongoing to characterize environmental requirements of diverse *Armillaria* spp. (McDonald, 1998). These studies are attempting to separate *Armillaria* species on the basis of their site requirements. Because ecological roles of these *Armillaria* species can vary from aggressive pathogens to benign saprophytes, it is essential to understand genetic variation within and among *Armillaria* populations to fully assess their role in forest ecology. Using a combination of molecular genetics and landscape ecology, focused studies could be applied to *Armillaria* to help understand the genetic structure and gene flow at the metapopulation level. Using this approach, genetic markers could be identified that correspond to site adaptability or pathogenicity.

6. Case study

To illustrate some of the points presented above, we use as a case study western gall rust on ponderosa pine regeneration in the Upper Pine Creek watershed, a wilderness area of the Black Hills in South Dakota. Digital imagery was used to determine the distribution of canopy openings from aerial photos (Fig. 3 top left and top right) using methods described by Sommerfeld et al. (1998). Canopy density was estimated on a 5 m grid over an area of 400 $m \times 1200$ m, and these data are displayed in a GIS (Fig. 3 bottom right) using methods described by Lundquist (1995). Gaps were defined as clustered cells of low canopy density. The spatial distribution of gaps in the GIS image largely depends on the threshold level of canopy density chosen to represent gaps. The larger the threshold, the higher the proportion gapped canopy. In this case, the threshold was set at density $\leq$40%. To a landscape pathologist, openings in the canopy represent landscape patches, and the closed canopy in between represent landscape matrix.

Spatial distributions of living seedlings, symptomatic living seedlings, and dead seedlings were assessed over the same 5 m grid (Fig. 3 bottom right) as that used to survey canopy density. The resulting images showed that seedlings occurred mostly within canopy gaps, but some occurred also in the forest matrix. The distributions of seedling clusters across the 48 ha plot and the distribution of individual seedlings within seedling clusters (Fig. 3 bottom) show similar patterns. Within many gaps, infected seedlings had died, and in some gaps all seedlings were dead. Because western gall rust requires a living host, it dies too. Local extinction occurs when all seedlings within a patch die (Fig. 4).

Based on an analysis of the distributions, we can make the following observations: (1) the distribution of seedlings is not solely dependent on the presence of a canopy gaps, (2) the distribution of diseased seedlings is not dependent only on the distribution of seedlings, (3) disease does not develop every year, and (4) distribution of diseased seedlings is dependent not only on the distribution of the pathogen. Suitability for seedling establishment and for disease establishment and development vary with space.

The distribution of infected seedlings consisted of subpopulations separated in space which suggests that western gall rust has a metapopulation structure in this watershed. Local extinctions, inter-gap spread of rust spores, and recolonization are potential sources of genetic diversification under these conditions because these mechanisms can cause subpopulations of this metapopulation to demographically and genetically change independent of each other. The extent of diversity within the metapopulation would depend on the abundance of infected and sporulating seedlings within neighboring patches, the distance between them and other patches of susceptible seedlings, and on the resistance to interpatch spread imposed by the matrix between seedling patches. What molecular markers can be used

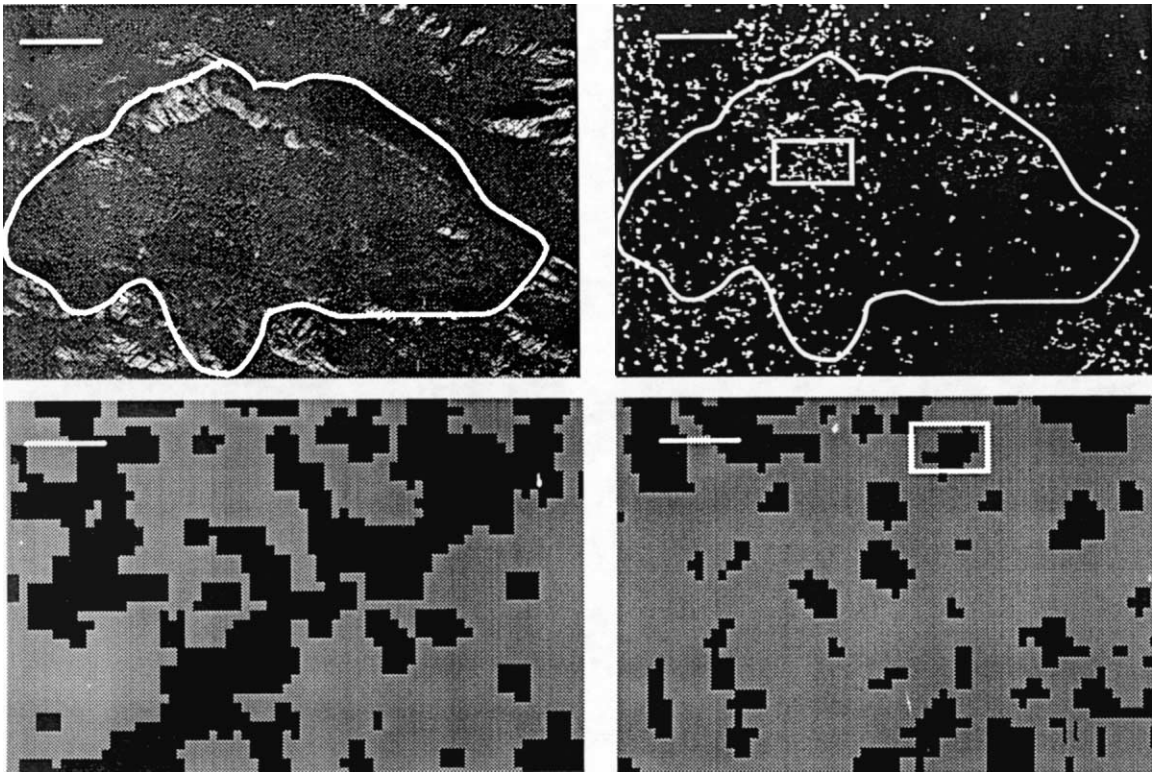


Fig. 3. Distribution of canopy gaps (white patches) and seedlings (black patches) within the Upper Pine Creek watershed as determined by color aerial photography (upper left; white *bar* = 1200 m), digital imagery based on this aerial photo (upper right; *bar* = 1200 m), field assessments of seedling abundance over a 5 m grid on a 400 $m \times 1200$ m plot (lower right; *bar* = 50 m), and a count of individual seedlings within a seedling cluster (lower left; *bar* = 5 m).

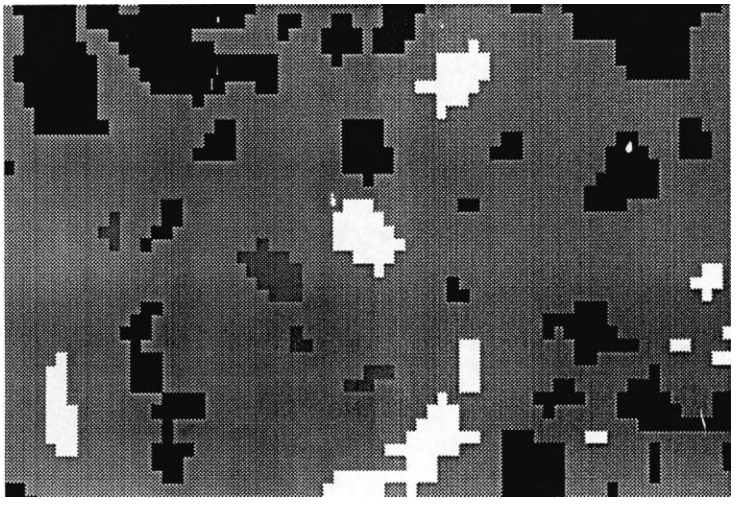


Fig. 4. Distribution of populations of seedlings composed mostly of infected individuals (black regions), healthy seedlings (white), and infected dead seedlings (gray) over a 48 ha plot within ponderosa pine stands in the Upper Pine Creek watershed in the Black Hills of South Dakota.

to show this diversity? Is this genetic diversity ecologically important? If not, then at what scale does such genetic diversity become ecologically important? How does this change with stand manipulation? Western gall rust would serve as a useful model system to examine some aspects of interaction metapopulation dynamics and the evolutionary forces acting to create genetic diversity.

7. Synthesis

With the aim of integrating comments presented above and incorporating them into a wider context, we propose several principles to help integrate concepts of landscape ecology and molecular biology of forest pathogens:

1. The forest landscape is composed of suitable and unsuitable habitat for disease development and persistence.
2. Disease is a function of the co-occurrence of a virulent pathogen, a susceptible host, and a suitable environment, and each varies in time and space. Sometimes they occur together.
3. Diseases are usually distributed unevenly across the landscape. At some scale, most or all diseases have a patchy distribution.
4. Disease distribution partially reflects genetic diversity of the pathogen.
5. Molecular markers greatly expand the capabilities to characterize genetic diversity of forest pathogens, and the spatial distribution of pathogen genotypes.
6. Molecular marker systems differ in their capabilities to characterize pathogen diversity.
7. The spatial scale at which genetic variability contributes to ecological significance varies among pathogens.
8. Genotype changes are generated at the molecular level, but they are filtered and selected in the environmental context. Evolutionary factors and metapopulation dynamics acting together contribute to genetic diversity.
9. Metapopulation structure can influence the spread and distribution of pathogens.
10. Metapopulation structure can rapidly shape and reshape the genetic status of species.
11. Metapopulation models are useful to examine the impact of gene flow in a local pathogen population.
12. Broader genetic diversity may contribute to a wider range of environmental and host conditions in which the pathogen occurs.

8. Conclusions

Forest disease is an ecological phenomenon that is neither a pathogen, nor a host, but a dynamic interaction of both in a suitable, heterogeneous, and changing environment. Forest diseases commonly occur in patchy distributions across natural landscapes which can be reflected in the genetic composition of the fragmented pathogen populations. Genetic diversity is a result of local adaptation caused by evolutionary forces that can act rapidly. Spatial heterogeneity is a prerequisite of local adaptation. Landscape ecologists have made great progress toward refining the art of describing and quantifying heterogeneous landscapes, but organisms are generally treated as having uniform genotypes. Molecular biologists have created an impressive list of molecular markers that can potentially characterize even small differences among pathogen populations but they have rarely correlated these differences with geographic patterns. Detecting genetic diversity depends on the resolving power of the molecular markers. The challenge is to determine what technical resolution and genetic level is meaningful to the landscape/metapopulation scales. The concept of metapopulation is a catalyst for applying many newly developing concepts to forest pathology, and we believe it offers a means to understand the genetic ecology of pathogens in natural landscapes.

"Understanding patterns in terms of the processes that produce them is the essence of science, and is the key to the development of principles for management" (Levin, 1992). Understanding the dynamics of diseases in complex ecosystems and describing their impacts are major issues in forestry. By focusing on the interactions of pathogens and forests, forest pathologists have always dealt with the effects of small and rapidly acting microbes on big, complex, and relatively slow acting forests. This contrast makes forest pathology unique, and has created many conceptual and operational challenges that have changed

little since the beginnings of this field. What has changed, of course, is the technology available to address these challenges, especially in the last 5 or 10 years. Molecular markers can be used to identify and measure detailed differences among subpopulations of forest pathogens. Geographical information systems, spatial analysis and modeling, digital imagery of remotely sensed images, and other tools of landscape ecology provide the means to detect and interpret patterns associated with genotypic asymmetry. Many forest pathologists, and to a large extent, forest ecologists use these tools and concepts but not in an integrated manner as they are presented above. Integrating the tools and concepts of molecular biology and landscape ecology by focusing on metapopulation disease phenomena offers a way of conceptually linking spatial and temporal patterns with the processes that produce them.

References

Anderson, I.C., Chambers, S.M., Cairney, J.W.G., 1998. Use of molecular methods to estimate the size and distribution of mycelial individuals of the ectomycorrhizal basidiomycete *Pisolithus tinctorius*. Mycol Res. 103, 295–300.

Bossart, J.L., Pashley Prowell, D., 1998. Genetic estimates of population structure and gene flow: limitations, lessons and new directions. Tree 13, 202–206.

Castello, J.D., Leopold, D.J., Smallidge, P.J., 1995. Pathogens, patterns and processes in forest ecosystems. Bioscience 45, 16–24.

Doudrick, R.L., Nance, W.L., Nelson, C.D., Snow, G.A., Hamelin, R.C., 1993. Detection of DNA polymorphisms in single urediniospore-derived culture of *Cronartium quercuum* f. sp. *fusiforme*. Phytopathology 83, 388–392.

Dunning, J.B., Danielson, B.J., Pulliam, H.R., 1992. Ecological processes that affect populations in complex landscapes. Oikos 65, 169–175.

Dusabenyagasani, M., Lecours, N., Hamelin, R.C., 1998. Sequence-tagged sites (STS) for studies of molecular epidemiology of scleroderris canker of conifers. Theor. Appl. Genet. 97, 789–796.

Et-Touil, A., Brasier, C.M., Bernier, L., 1999. Localization of a pathogenicity gene in *Ophiostoma novo-ulmi* and evidence that if may be introgressed from *O. ulmi*. Mol. Plant-Microbe Interactions 12, 6–15.

Fischer, M., Wagner, T., 1999. RFLP analysis as a tool for identification of lignicolous basidiomycetes: European polypores. Eur. J. For. Pathol. 29, 295–304.

Forman, R.T.T., 1995. Land Mosaics: The Ecology of Landscapes and Regions. Cambridge University Press, Cambridge.

Frontz, T.M., Davis, D.D., Bunyard, B.A., Royse, D.J., 1998. Identification of *Armillaria* species isolated from bigtooth aspen based on rDNA RFLP analysis. Can. J. For. Res. 28, 141–149.

Garbelotto, M., Ratcliff, A., Bruns, T.D., Cobb, F.W., Otrosina, W.J., 1996. Use of taxon-specific competitive-priming PCR to study host specificity, hybridization, and intergroup gene flow in intersterility groups of *Heterobasidion annosum*. Phytopathology 86, 543–551.

Goggioli, V.P.C., Hamelin, R.C., Vendramin, G.G., 1998. Isozyme and RAPD polymorphisms in *Heterobasidion annosum*. Eur. J. For. Pathol. 28, 63–74.

Gosselin, L., Jobidon, R., Bernier, L., 1999. Genetic variability and structure of Canadian populations of *Chondrostereum purpureum*, a potential biophytocide. Mol. Ecol. 8, 113–122.

Guillaumin, J.J., Anderson, J.B., Legrand, P., Ghahari, S., Berthelay, S., 1996. A comparison of different methods for the identification of genets of *Armillaria* spp. New Phytologist 133, 333–343.

Hamelin, R.C., 1996. Genetic diversity between and within cankers of the white pine blister rust. Phytopathology 86, 875–879.

Hamelin, R.C., Doudrick, R.L., Nance, W.L., 1994. Genetic diversity in *Cronartium quercuum* f. sp. *fusiforme* on loblolly pine in southern US. Curr. Genet. 26, 359–363.

Hamelin, R.C., Beaulieu, J., Plourde, A., 1995. Genetic diversity in populations of *Cronartium ribicola* in plantations and natural stands of *Pinus strobus*. Theor. Appl. Genet. 91, 1214–1221.

Hamelin, R.C., Dusabenyagasani, M., Et-Touil, K., 1998a. Fine-level genetic structure of white pine blister rust populations. Phytopathology 88, 1187–1191.

Hamelin, R.C., Lecours, N., Laflamme, G., 1998b. Molecular evidence of distinct introductions of the European race of *Gremmeniella abietina* into North America. Phytopathology 88, 582–588.

Hantula, J., Maarit Niemi, E., Kaitera, J., Jalkanen, R., Kurkela, T., 1998. Genetic variation of the resin top fungus in Finland as determined by random amplified microsatellites (RAMS). Eur. J. For. Pathol. 28 (361-372).

Harrington, T.C., Wingfield, B.D., 1995. A PCR-based identification method for species of *Armillaria*. Mycologia 87, 280–288.

Harrington, T.C., Steimel, J., Kile, G., 1998. Genetic variation in three *Ceratocystis* species with outcrossing selfing and asexual reproductive strategies. Eur. J. For. Pathol. 28, 217–226.

Hausner, G., Hopkins, A.A., Davis, C.N., Reid, J., 1999. Variation in culture and rDNA among isolates of *Sphaeropsis sapinea* from Ontario and Manitoba. Can. J. Plant Pathol. 21, 256–264.

Högberg, N., Holdenrieder, O., Stenlid, J., 1999. Population structure of the wood decay fungus *Fomitopsis pinicola*. Heredity 83, 354–360.

Jeger, M.J., 1988. The spatial component of plant disease epidemics. In: Jeger, M.J. (Ed.), Spatial Components of Plant Disease. Prentice Hall, Englewood Cliffs, NJ, pp. 1–13.

Jelinski, D.E., 1997. On genes and geography: a landscape perspective on genetic variation in natural plant populations. Landscape and Urban Planning 39, 11–23.

Johannesson, H., Stenlid, J., 1999. Molecular identification of wood-inhabiting fungi in an unmanaged *Picea abies* forest in Sweden. For. Ecol. Manage. 115, 203–211.

La Porta, N.P.C., Kammiovirta, K., Karjalainen, R., Korhonen, K., 1997. Geographical cline of DNA variation within the F intersterility group of *Heterobasidion annosum* in Italy. Plant Pathol. 46, 773–784.

Legendre, P., Fortin, M., 1989. Spatial patterns and ecological analysis. Vegetatio 80, 107–138.

Li, H., Reynolds, J.F., 1995. On definition and quantification of heterogeneity. Oikos 73, 280–284.

Lidicker Jr., W.Z., 1995. Landscape approaches in mammalian ecology and conservation. In: Lidicker Jr., W.Z. (Ed.), The landscape Concept: Something Old, Something New. University of Minnesola Press, Minneapolis, MN, pp. 3–19.

Lilja, A., Karjalainen, R., Parikka, P., Kammiovirta, K., Nuorteve, H., 1998. Pathogenicity and genetic variation of *Phytophthora cactorum* from silver birch and strawberry. Eur. J. Plant Pathol. 104, 529–535.

Lundquist, J.E., 1995. Pest interactions and canopy gaps in ponderosa pine in the Black Hills, South Dakota. For. Ecol. Manage. 74, 37–48.

Marçais, B., Martin, F., Delatour, C., 1998. Structure of *Collybia fusipes* populations in two infected oak stands. Mycol. Res. 102, 361–367.

McDermott, J.M., McDonald, B.A., 1993. Gene flow in plant pathosystems. Ann. Rev. Phytopathol. 31, 353–373.

McDonald, G.I., 1998. Preliminary report on the ecology of *Armillaria* in Utah and the Inland West. In: Trummer, L. (compiler) Proceedings of the 46th Western International Forest Disease Work Conference, 1 October 1998. University of Nevada, Reno, NV, pp. 85–92.

Milgroom, M.G., Wang, K., Zhou, Y., Lipari, S.E., Kaneko, S., 1996. Intercontinental population structure of the chestnut blight fungus *Cryphonectria parasitica*. Mycologia 88, 179–190.

Moricca, S., Ragazzi, A., 1998. Use of RFLP and SSCP analysis to differentiate the pine rusts *Cronartium flaccidum* and *Peridermium pini*. Mycol. Res. 102, 666–670.

Moricca, S., Kasuga, T., Mitchelson, K., Ragazzi, A., Diamandis, S., 1996. Heterogeneity in intergenic regions of the ribosomal repeat of the pine-blister rusts *Cronartium flaccidum* and *Peridermium pini*. Curr. Genet. 29, 388–394.

Nelson, M.R., Orum, T.V., Jaime-Garcia, R., Nadeem, A., 1999. Applications of geographic information systems and geostatistics in plant disease epidemiology and management. Plant Dis. 83, 308–319.

Pappinen, A., Kasanen, R., von Weissenberg, K., 1996. Identification of multiple *Endocronartium pini* infections in Scots pine with PCR. Eur. J. For. Pathol. 26, 183–191.

Piercey-Normore, M.D., Egger, K.N., Bérube, J.A., 1998. Molecular phylogeny and evolutionary divergence of North American biological species of *Armillaria*. Mol. Phylogen. Evol. 10, 49–66.

Risser, P.G., Karr, J.R., Forman, R.T.T., 1984. Landscape ecology: directions and approaches. Illinois Natural History Survey Special Publication, No. 2.

Rogers, S.O., Holdenrieder, O., Sieber, T.N., 1999. Intraspecific comparisons of *Laetiporus sulphureus* isolates from broadleaf and coniferous trees in Europe. Mycol. Res. 103, 1245–1251.

Rykiel, J.R., 1985. Towards a definition of ecological disturbance. Aust. J. Ecol. 10, 361–365.

Schulze, S., 1999. Rapid detection of European *Heterobasidion annosum* intersterility groups and intergroup gene flow using taxon-specific competitive priming PCR (TSCP-PCR). J. Phytopathol. 147, 125–127.

Schulze, S., Bahnweg, G., Moller, E.M., Sandermann Jr., H., 1997. Identification of the genus *Armillaria* by specific amplification of an rDNA-ITS fragment and evaluation of genetic variation within *A. ostoyae* by rDNA-RFLP and RAPD analysis. Eur. J. For. Pathol. 27, 225–239.

Sommerfeld, R.A., Lundquist, J.E., Smith, J., 1998. Characterizing the canopy structure of a disturbed forest using the Fourier transform. In: Proceedings of the First International Conference on Geospatial Information in Agriculture and Forestry, Corando Springs, Buena Vista, FL, 1–3 June 1998.

Terashima, K., Cha, J.Y., Yajima, T., Igarashi, T., Miura, K., 1998. Phylogenetic analysis of Japanese *Armillaria* based on the intergenic spacer (IGS) sequences of their rDNA. Eur. J. For. Pathol. 28, 11–19.

Thompson, J.N., 1998. Rapid evolution as an ecological process. Tree 13, 329–332.

Thrall, P.H., Burdon, J.J., 1997. Host-pathogen dynamics in a metapopulation context: the ecological and evolutionary consequences of being spatial. J. Ecol. 85, 743–753.

Turner, M.G., Gardner, R.H., O'Neill, R.V., 1995. Ecological dynamics at broad scales. Bioscience (Suppl.), 529–535.

Vogler, D.R., Bruns, T.D., 1998. Phylogenetic relationships among the pine stem rust fungi (*Cronartium and Peridermium* spp.). Mycologia 90, 244–257.

White, E., Morrison, D., 1999. DNA testing: an application to *Armillaria* root disease. Technology Transfer Note — Pacific Forestry Centre, Canadian Forest Service No. 18, p. 4.

Wiens, J.A., 1989. Spatial scaling in ecology. Functional Ecol. 3, 385–397.

Wiens, J.A., 1997. Metapopulation dynamics and landscape ecology. In: Hanski, I.A., Gilpin, M.E. (Eds.), Metapopulation Biology: Ecology, Genetics, and Evolution. Academic Press, New York, pp. 43–62.