Population structure of *Heterobasidion annosum* from North America and Europe

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Isolates of *Heterobasidion annosum* (Fr.) Bref. representing North American S and P and European S, P, and F intersterility groups were subjected to isozyme analysis. European S, P, and F groups had more variability than the North American S and P groups in expected heterozygosity, number of alleles per locus, and percent polymorphic loci. In contrast with the North American S and P groups, the European intersterility groups could not be distinguished from each other on the basis of individual isozyme loci, although significant differences in allele frequencies exist between European S and P groups. This suggests that evolution proceeded at different rates in the intersterility groups, or intersterility barriers appeared later in the European populations relative to the North American populations of *H. annosum*. Changes in climate and host species associations during the Tertiary may have been a major factor in evolution of *H. annosum* intersterility groups.

**Key words:** allozymes, forest tree hosts, evolutionary events, evolutionary relationships, Hymenomycetes, root disease.


A l’aide de l’analyse des isoenzymes, les auteurs ont examiné des isolats de *Heterobasidion annosum* (Fr.) Bref. représentant les groupes d’incompatibilité nord-américains S et P ainsi que les groupes d’incompatibilité européens S, P, et F. Les groupes d’incompatibilité européens S, P, et F montrent plus de variabilité que les groupes nord-américains S et P en termes de variabilité d’hétérozygotes attendue, de nombre d’alleles par lieu et de pour cent de lieux polymorphes. Contrairement aux groupes nord-américains S et P, les groupes d’incompatibilité européens ne peuvent être distingués les uns des autres sur la base des lieux isozymiques individuels, bien qu’il existe des différences significatives dans les fréquences d’alleles entre les groupes européens S et P. Ceci suggère que l’évolution aurait procédé à des vitesses différentes chez les divers groupes d’incompatibilité, ou que des barrières d’incompatibilité se seraient apparues plus tard dans les populations comparativement aux populations nord-américaines de *H. annosum*. Les changements climatiques et d’association avec les hôtes au cours du tertiaire pourraient avoir joué un rôle important dans l’évolution des groupes d’incompatibilité chez *H. annosum*.

**Mots clés:** allozymes, arbre forestier hôte, événements playnologiques, relations évolutives, Hyménomycètes, maladie racinaire.

**Introduction**

*Heterobasidion annosum* (Fr.) Bref, is an important pathogen of conifers in temperate zone forests of the northern hemisphere (Otrosina and Scharpf 1989). The fungus causes root rot, butt rot, and extensive mortality in affected stands.

The fungus was regarded by forest pathologists as a genetically and pathologically uniform organism until Korhonen (1978) delimited two intersterility groups (ISGs) of the fungus. One group, designated S, has a host preference for *Pinus* species, whereas the other or S group, has a preference for *Picea* species. Recently, Chase (1985) and Chase and Ullrich (1990b) showed that at least five genes determine intersterility in *H. annosum*. Homozygosity for a "+" allele at any of the five loci results in the formation of a dikaryon.

There is considerable polymorphism for the genes that regulate interfertility in the North American populations (Chase and Ullrich 1990b). Chase et al. (1989) reported approximately 18% interfertility in laboratory mating experiments between North American S and P group homokaryons. This contrasts with the 4% interfertility reported for European S and P groups (Korhonen 1978). Allozyme analyses of the North American ISGs of *H. annosum* indicated that the S and P groups from North America are highly diverged and little or no gene flow is occurring between ISGs in natural populations (Otrosina et al. 1992). Isozymes of polygalacturonase and pectin esterase, which may be involved in pathogenesis of *H. annosum* (Johansson 1988), have been used to distinguish between European and North American ISGs (Karlsson and Stenlid 1991). These results suggest considerable intercontinental genetic differences between the ISGs, although these enzymes should not be considered neutral markers because of their role in pathogenicity. Because there is considerable information on the genetics of the mating system in *H. annosum* and its relationship to pathogenicity, this fungus serves as a model system for the study of population structure and evolution of wood-decaying Hymenomycetes. Knowledge of population structure and evolution would be valuable in our understanding the responses of forest tree root pathogens to changes in tree species associations brought about by management...
ment regimes and would clarify our understanding of host specialization.

This paper reports a study of allozyme analyses of neutral metabolic enzymes. The purpose was to determine the genetic relationships between the European and North American ISGs of *H. annosum*.

Materials and methods

Western United States S group and P group isolates of *H. annosum* were obtained in California, Arizona, Oregon, and Montana from stumps or diseased trees of *Pinus ponderosa* Douglas ex P. Laws. & C. Laws., *Pinus jeffreyi* E. Murray, *Abies concolor* (Gordon 8z Glend.) Lindl. ex Hildebr., or *Sequoia/Spruce* (Lindl.) Buchholz. European isolates of the P, S, and F groups were obtained from the collection of Korhonen. These isolates were obtained mainly from *Pinus sylvestris* L., *Picea abies* (L.) Karst., and *Abies alba* Mill. Determinations of intersterility groups were carried out by pairing homokaryons with tester strains as previously described (Chase and Ullrich 1990a; Korhonen 1978). A listing of the ISGs, host trees, country of origin, and the 109 isolates is in Table 1. The isolates used in this study were deposited in culture collections at the USDA Forest Service, Pacific Southwest Research Station, Albany Calif., and at the USDA Forest Service, Forestry Sciences Laboratory, Athens, Ga.

These isolates do not necessarily represent a random sample of defined populations; however, they do represent a sample over extensive geographic areas, and randomness will be assumed for statistical treatment of the data.

*Mycelia* of *H. annosum* were grown in 250-ml Ehrlenmeyer flasks containing 100 ml liquid potato dextrose broth. The flasks were incubated at room temperature for 3 weeks, then the *mycelia* were vacuum harvested in Buchner funnels over Whatman No. 4 filter paper. *Mycelia* were placed in a mortar with 0.2 M phosphate buffer amended with bovine serum albumin, frozen with liquid nitrogen, and ground. Paper wicks (3 x 10 mm) of Whatman 3 MM filter paper were saturated with the chilled extract, then loaded onto starch

### Table 1. Intersterility groups (ISG), hosts, country of origin, and isolate identities for *Heterobasidion annosum* isolates used in isozyme study

<table>
<thead>
<tr>
<th>ISG and host</th>
<th>No. of isolates</th>
<th>Location</th>
<th>Identity of selected isolates*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>European S</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Picea abies</em></td>
<td>11</td>
<td>Finland</td>
<td>181, 183-186, 401-403, 406, 448, 791205.5.1</td>
</tr>
<tr>
<td><em>Picea abies</em></td>
<td>3</td>
<td>Italy</td>
<td>429-431</td>
</tr>
<tr>
<td><em>Picea abies</em></td>
<td>4</td>
<td>Germany</td>
<td>432-435</td>
</tr>
<tr>
<td><strong>European P</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pinus sylvestris</em></td>
<td>14</td>
<td>Finland</td>
<td>180, 187, 409-415, 424-427, 750124.2.1</td>
</tr>
<tr>
<td><em>Picea abies</em></td>
<td>1</td>
<td>Finland</td>
<td>407</td>
</tr>
<tr>
<td>Airborn inoculum</td>
<td>1</td>
<td>Finland</td>
<td>791200.1.2</td>
</tr>
<tr>
<td><em>Crataegus</em> spp.</td>
<td>1</td>
<td>Finland</td>
<td>810928.1.1</td>
</tr>
<tr>
<td><em>Pinus sylvestris</em></td>
<td>1</td>
<td>Switzerland</td>
<td>428</td>
</tr>
<tr>
<td>Betula <em>pendula</em></td>
<td>1</td>
<td>Finland</td>
<td>408</td>
</tr>
<tr>
<td><strong>European F</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Abies alba</em></td>
<td>10</td>
<td>Italy</td>
<td>438-447</td>
</tr>
<tr>
<td><strong>North American S</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Abies concolor</em></td>
<td>1</td>
<td>Arizona</td>
<td>216</td>
</tr>
<tr>
<td><em>Abies</em> spp.</td>
<td>7</td>
<td>Oregon</td>
<td>217, 219, 221, 222, 225, 228, 229</td>
</tr>
<tr>
<td><em>Abies</em> spp.</td>
<td>23</td>
<td>California</td>
<td>201, 212-214, 230-243, 245,</td>
</tr>
<tr>
<td><em>Pinus ponderosa</em></td>
<td>2</td>
<td>California</td>
<td>302, 322</td>
</tr>
<tr>
<td><em>Pinus ponderosa</em></td>
<td>1</td>
<td>Oregon</td>
<td>329</td>
</tr>
<tr>
<td><em>Sequoia/Spruce</em> giganteanum</td>
<td>6</td>
<td>California</td>
<td>505-510, 512</td>
</tr>
<tr>
<td><strong>North American P</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pinus ponderosa</em></td>
<td>4</td>
<td>Montana</td>
<td>111-114</td>
</tr>
<tr>
<td><em>Pinus ponderosa</em></td>
<td>3</td>
<td>Oregon</td>
<td>104, 323, 327</td>
</tr>
<tr>
<td><em>Pinus ponderosa</em></td>
<td>12</td>
<td>California</td>
<td>305, 310, 334, 338, 340, 341, 344,</td>
</tr>
<tr>
<td><em>Pinus jeffreyi</em></td>
<td>3</td>
<td>California</td>
<td>387, 390, 398</td>
</tr>
</tbody>
</table>

*Accession numbers for *H. annosum* isolates linked by a dash (-) are in numerical sequence. Numbers conform to culture collections on deposit at the USDA Forest Service, Pacific Southwest Research Station, 800 Buchanan St., Albany, CA 94701, U.S.A., and USDA Forest Service, Southeastern Forest Experiment Station, Green Street, Athens, GA 30602, U.S.A.

### Table 2. Enzyme systems and gel buffer systems employed in isozyme analysis of *Heterobasidion annosum* isolates

<table>
<thead>
<tr>
<th>Enzyme system</th>
<th>EC No.</th>
<th>Abbr.</th>
<th>Gel buffer system†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aconitase</td>
<td>4.2.1.3</td>
<td>ACO</td>
<td>E</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>1.1.1.1</td>
<td>ADH</td>
<td>A, E</td>
</tr>
<tr>
<td>Alpha-esterase</td>
<td>3.1.1.2</td>
<td>AEST</td>
<td>A</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>1.4.1.2</td>
<td>GDH</td>
<td>B</td>
</tr>
<tr>
<td>Glutamate oxaloacetate</td>
<td>2.6.1.1</td>
<td>GOT</td>
<td>B</td>
</tr>
<tr>
<td>transaminase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>1.1.1.42</td>
<td>IDH</td>
<td>D</td>
</tr>
<tr>
<td>Leucine aminopeptidase</td>
<td>3.4.1.11</td>
<td>LAP</td>
<td>A</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>1.1.1.37</td>
<td>MDH</td>
<td>D, E</td>
</tr>
<tr>
<td>Phosphoglucoisomerase</td>
<td>5.3.1.9</td>
<td>PGI</td>
<td>A</td>
</tr>
<tr>
<td>Sorbitol dehydrogenase</td>
<td>1.1.1.14</td>
<td>SRDH</td>
<td>B</td>
</tr>
</tbody>
</table>

*Gel buffer systems prepared following Conkle et al. (1982). A, Tris-borate; B, Tris-citrate; D and E, morpholine citrate.
TABLE 3. Summary of genetic variability parameters for European and North American interserility groups (ISG) of Heterobasidion annosum.

<table>
<thead>
<tr>
<th>ISG</th>
<th>No. of isolates</th>
<th>Mean no. of alleles per locus</th>
<th>% polymorphic loci</th>
<th>Expected heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>North American S</td>
<td>40</td>
<td>2.0 (0.3)</td>
<td>41.7</td>
<td>0.15 (0.06)</td>
</tr>
<tr>
<td>North American P</td>
<td>22</td>
<td>1.3 (0.2)</td>
<td>25.7</td>
<td>0.04 (0.03)</td>
</tr>
<tr>
<td>European P</td>
<td>19</td>
<td>2.3 (0.4)</td>
<td>66.7</td>
<td>0.26 (0.07)</td>
</tr>
<tr>
<td>European S</td>
<td>18</td>
<td>3.3 (0.4)</td>
<td>91.7</td>
<td>0.47 (0.06)</td>
</tr>
<tr>
<td>European F</td>
<td>10</td>
<td>2.4 (0.3)</td>
<td>83.3</td>
<td>0.37 (0.08)</td>
</tr>
</tbody>
</table>

Note: Numbers in parentheses are standard errors.

* A locus was considered polymorphic if more than one allele was detected.

**Expected heterozygosity by Hardy-Weinberg law (Nei 1978).**

Table 3. Of the 12 allozyme loci analyzed, 11 were polymorphic among the five groups in the collection. The MDH-1 locus was monomorphic for all isolates, and no cathodal bands were observed in any enzyme system among the ISGs. The European S group was the most variable population, with an average of 3.3 alleles per locus, 91.7% polymorphic loci, and expected heterozygosity of 0.47. In contrast, the North American P group was the least variable, with 1.3 alleles per locus, 25% polymorphic loci, and heterozygosity of 0.04. Using these criteria, isolates of H. annosum from the European S, P, and F ISGs were more variable overall than the North American S or P ISGs (Table 3).

Allele frequencies for the 12 allozyme loci analyzed in the five groups are presented in Table 4. There are more shared alleles between the European S, P, and F group isolates than between the North American S and P group isolates. As reported previously, there is nearly complete fixation for alternative alleles between the North American S and P groups such that loci such as MDH-2, ADH, PGI, GDH, LAP-1, LAP-2, and AEST can be reliably used for diagnostics (Otrosina et al. 1992). Based upon 95% confidence limits, differences in allele frequencies exist between the European S and P groups, but fixation is not evident among the European ISGs compared with the North American ISGs. On a locus by locus basis, there are significant differences between the European P ISG and the North American P ISG in allele frequencies within the ADH, ACO, SRDH, LAP-2, MDH-2, GDH, and AEST loci. Also, significant differences in allele frequencies are present between the European S and North American S groups in the ADH, PGI, GOT, ACO, LAP-1, LAP-2, and SRDH loci. European S and F ISGs share common alleles in most allozyme loci, and only one statistically significant difference in frequency was apparent within the LAP-1 locus for allele A.

Nei (1978) genetic distances and cluster analysis based upon allele frequencies among the five groups indicate considerable genetic divergence between European and North American H. annosum and between European S and P ISGs and North American S and P ISGs (Table 5; Fig. 1). The European F ISG clustered with the European S ISG at a relatively short distance, indicating a closer genetic relationship between them relative to the other groups.

Canonical discriminant analysis of the allozyme data indicated the relative positions of isolates with respect to their clustering within the groups (Fig. 2). The vectors CAN 1 and CAN 2 were highly significant (p = 0.0001) and explained 91% of the variability in the data set. The North American P group isolates clustered far apart from the other four groups and have the least variability relative to number of multilocus...
The results indicate considerable differentiation among *H. annosum* ISGs. The European S and P groups, having the most variability with respect to the number of alleles per locus and expected heterozygosity, contrast with the North American S and P groups, which have the least variability with genotypes associated with them. The North American S group, the European S group, and the European P group isolates formed distinct clusters as plotted on these vectors. The European S and F groups present a more diffuse clustering pattern, although they appear to be distinguishable from the other groups. The F group isolates lie closest to the European S group.

Results from the cross-validation procedure of the discriminant analysis indicate the European S group and the F group have the highest errors of classification compared with the other groups, with 47 and 30% of the isolates being misclassified, respectively (Table 6). The North American P group and S group yielded 0 and 2.5% misclassifications, respectively. The European P group had a 5.6% rate of misclassification. The European S group had the majority of misclassifications placed in the F group or North American S group. The F group, on the other hand, had two isolates classified into the North American S group and one in the European P group. The European P groups and the North American S group each had one isolate misclassified into the European S group. The overall rate for misclassifications of the five groups of *H. annosum* isolates was 17.0%.

### Discussion

The results indicate considerable differentiation among *H. annosum* ISGs. The European S and P groups, having the most variability with respect to the number of alleles per locus and expected heterozygosity, contrast with the North American S and P groups, which have the least variability with

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**Table 4. Allele frequencies for 12 allozyme loci analyzed among the North American and European S, F, and P intersterility groups of *Heterobasidion annosum***

<table>
<thead>
<tr>
<th>Locus and allele</th>
<th>North American</th>
<th>European</th>
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<tbody>
<tr>
<td></td>
<td>(N = 40)</td>
<td>(N = 22)</td>
</tr>
<tr>
<td>ADH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.04a</td>
<td>1.00b</td>
</tr>
<tr>
<td>B</td>
<td>0.34a</td>
<td>0b</td>
</tr>
<tr>
<td>c</td>
<td>0.48a</td>
<td>0b</td>
</tr>
<tr>
<td>D</td>
<td>0.14a</td>
<td>0a</td>
</tr>
<tr>
<td>E</td>
<td>0a</td>
<td>0a</td>
</tr>
<tr>
<td>Null</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GOT</td>
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<td></td>
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<tr>
<td>A</td>
<td>0.90a</td>
<td>0b</td>
</tr>
<tr>
<td>B</td>
<td>0.96bc</td>
<td>1.00b</td>
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<tr>
<td>c</td>
<td>0a</td>
<td>0.02a</td>
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<tr>
<td>D</td>
<td>0.10a</td>
<td>0.02a</td>
</tr>
<tr>
<td>MDH-1</td>
<td></td>
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<tr>
<td>A</td>
<td>1.00a</td>
<td>1.00a</td>
</tr>
<tr>
<td>B</td>
<td>0.99a</td>
<td>0b</td>
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<tr>
<td>c</td>
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<tr>
<td>PGI</td>
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<td>A</td>
<td>0.97a</td>
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<td>c</td>
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<td>0a</td>
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<tr>
<td>D</td>
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</tr>
<tr>
<td>GDH</td>
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<td>A</td>
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<td>1.00b</td>
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<td>D</td>
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**Table 4 (concluded)**

<table>
<thead>
<tr>
<th>Locus and allele</th>
<th>North American</th>
<th>European</th>
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<tr>
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<td>(N = 40)</td>
<td>(N = 22)</td>
</tr>
<tr>
<td>IDH</td>
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<tr>
<td>A</td>
<td>0.9a</td>
<td>0.93b</td>
</tr>
<tr>
<td>B</td>
<td>0.59a</td>
<td>0.07b</td>
</tr>
<tr>
<td>c</td>
<td>0.39a</td>
<td>0bc</td>
</tr>
<tr>
<td>D</td>
<td>0.02a</td>
<td>0a</td>
</tr>
<tr>
<td>Null</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Note:** Allele frequencies not followed by the same letter across groups are significantly different from each other at α = 0.05 using the Bonferroni inequality. Explanation of enzyme system abbreviations is given in Table 2.

* Null alleles found only in one population in a given locus.

**Table 5. Genetic distances between *Heterobasidion annosum* ISGs from North America and Europe**

<table>
<thead>
<tr>
<th>ISG</th>
<th>North American</th>
<th>European</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>P</td>
</tr>
<tr>
<td>North American S</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>North American P</td>
<td>2.24</td>
<td>-</td>
</tr>
<tr>
<td>European S</td>
<td>1.00</td>
<td>0.90</td>
</tr>
<tr>
<td>European S</td>
<td>0.41</td>
<td>0.92</td>
</tr>
<tr>
<td>European F</td>
<td>0.49</td>
<td>0.89</td>
</tr>
</tbody>
</table>

**Note:** Nei (1978) unbiased genetic distance calculated from 12 allozyme loci.
regard to these parameters. The allele frequencies and genetic distances (Nei 1978) indicate a high degree of divergence between these populations. In a previous study on western North American S and P groups of *H. annosum*, Otrosina et al. (1992) found nearly complete fixation for alternative alleles in 8 of 10 allozyme loci and were able to use these loci to differentiate ISGs in the North American collection. Two additional loci, *SRDH* and *IDH*, not included in that study but analyzed in this study, yielded similar results relative to the North American S and P groups.

In an electrophoretic study of pectic isozymes in *H. annosum*, Karlsson and Stenlid (1991) found significantly less variability in polygalacturonase isozymes in European S group isolates than in the European P group and were able to use these enzyme markers to distinguish between European and North American ISGs. Because of their involvement in pathogenicity of *H. annosum*, pectic enzymes should not be considered as neutral markers with respect to isozyme variation (Johansson 1988). In our study, we assume that the loci analyzed are neutral markers and represent a sample of the genome with respect to isozyme variation (Ayala et al. 1974).

The European S, P, and F groups have considerable amounts of shared alleles among them with respect to different loci, and consequently no single locus stands out as diagnostic. The genetic distances (Nei 1978) between the North American and European ISGs (Table 5) and cluster analysis (Fig. 1) indicate a closer relationship among the European S, P, and F groups than between the North American S and P groups. The European S and F groups are apparently closely related and may represent a more recent stage of speciation than either the European S and P ISGs or the North American S and P ISGs; the latter group is highly diverged (Otrosina et al. 1992). The plots of the first two canonical vectors (Fig. 2) also indicate that the F group may be in the early stages of allozyme differentiation. Capretti et al. (1990) recently described the F ISG, which is known only in association with *A. alba* in the Apen-
nine Mountains in Italy and in the Italian Alps. The F group is intersterile with European S isolates from the Italian Alps. The F group is interfertile with northern European S testers (Capretti et al. 1990) that are intersterile with European S group strains from the Alps, indicating a close relationship to the S ISG.

These allosyme data in conjunction with the known mating compatibilities among these groups suggest an emerging pattern of speciation in _H. annosum_. There appear to be elements of both allopatric and sympatric speciation processes involved in this pattern. Because of the obvious geographic separation between the European S, P, and F groups and the North American S and P groups, allopatric processes play a major role in the observed differentiation between these groups. The respective S and P ISGs from both continents have a high degree of within-group interfertility (Chase and Ullrich 1990b), and the F group also has a high level of interfertility with the North American S group (Capretti et al. 1990). These characteristics are consistent with the apparent lack of abrupt development of intersterility barriers associated with allopatric speciation (Brasier 1987). On the other hand, our allosyme data indicate a high degree of genetic divergence between European and North American S and P group populations of _H. annosum_, particularly in the SRDH, PG1, ADH, ACO, and LAP-2 loci. Genetic divergence in isozymes has been shown for certain incompatible allopatric populations of the _Collybia dryophil a_ complex between Europe and North America (Vilgalys 1991).

In sympatric speciation, intersterility barriers may arise abruptly in conspecific subpopulations (Slatkin 1987; Hartl and Clark 1989). For plant pathogenic fungi, these subpopulations can be associated with certain hosts, in which development of reproductive isolation confers a competitive advantage by being linked to traits that increase pathological adaptations of the fungus to those hosts (Brasier 1987). Thus, host interactions may be a component of the evolution of the S and P ISGs of _H. annosum_ in Europe and North America. Seeding inoculation experiments (Worrall et al. 1983; Cobb et al. 1989) and field sampling studies employing isozyme analyses (Ootrosina et al. 1992) provided evidence of host specificity in _H. unnosum_ from North America. We conducted extensive field sampling in North America of symptomatic pines (_Pinus_ spp.) and true firs (_Abies_ sp.). Isolates of _H. annosum_ from these symptomatic hosts were always of the P group from pine species and S group from true firs. In Scandinavia, however, P group isolates of _H. annosum_ can cause butt rot of Norway spruce (_Picea abies_) (Stenlid 1987). Seeding inoculation experiments also demonstrated a high capability in P group isolates to infect Norway spruce (Stenlid and Svendjemark 1988). F group isolates from Italy may be host specific with _A. alba_, even though the S group is widespread on _Picea_ in the Alps (Capretti et al. 1990).

Thus, differing host-species associations may partially account for the apparent differential pathologies and allosyme relationships between ISGs from the two continents. During the Tertiary period, homogeneous forests comprising the fore-runners of the modern genera _Abies, Pinus, Betula, and Populus_ were present in the Arctic Circle region. At this time, a land bridge existed between Asia and North America and some degree of continuity was present among northern Europe, Iceland, Greenland, and North America (Gibbs and Wainhouse 1986). South of the Arctic Circle, forerunners of the genera _Abies, Picea, Pseudotsuga, Chamaecyparis, and Tsuga_ were also present (Daubenmire 1978). During the latter Tertiary period, separate western and eastern forests developed in the North American continent and in Eurasia. The western United States became dominated by conifer species such as _Pinus, Sequoiodendron_, and _Abies_ while hardwood species rose to dominance in the east (Gibbs and Wainhouse 1986; Axelrod 1977). Hardwood genera such as _Betula, Alnus, Arbutus, and Arctostaphylos_ were reported as hosts to the P ISG of _H. annosum_ (Korhonen 1978; Otrosina et al. 1992; Smith et al. 1966). We postulate that _H. annosum_ may have begun evolving host specialization during the Tertiary, when a more or less contiguous trans-Arctic forest existed. Fossil evidence indicates that _Pinus_ was in existence and was dominant during the late Mesozoic, whereas other modern genera of the Pinaceae, such as _Picea_ and _Abies_, did not appear until the early to mid-Tertiary (Miller 1976, 1988). It is therefore possible that _H. annosum_ may have been a pathogen on _Pinus_ and related premodern genera as early as the late Mesozoic. Interestingly, Creber and Ash (1990) report decay in fossilized wood from Upper Triassic trees in the southwestern United States that resembles decay caused by _Oligoporus amarus_ (Hedge.) Gilbn. & Ryv. and _H. annosum_. The southward retreat of forests during the late Tertiary, driven by colder climatic conditions, with concomitant changes in forest tree species associations, may have resulted in the present-day host specialization relationships between _H. annosum_ ISGs. These relationships are characterized by the S ISG attacking

<table>
<thead>
<tr>
<th>From ISG</th>
<th>European</th>
<th>North American</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>S</td>
<td>F</td>
</tr>
<tr>
<td>European P</td>
<td>94.4 (17)</td>
<td>5.6 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>European S</td>
<td>5.9 (1)</td>
<td>52.9 (9)</td>
<td>17.7 (3)</td>
</tr>
<tr>
<td>European F</td>
<td>10.0 (1)</td>
<td>0 (0)</td>
<td>70.0 (7)</td>
</tr>
<tr>
<td>North American P</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>North American S</td>
<td>0 (0)</td>
<td>2.5 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>% misclassified</td>
<td>5.6</td>
<td>47.1</td>
<td>30.0</td>
</tr>
</tbody>
</table>

Note: Analysis of the isozyme data was performed using SAS (SAS Institute Inc., 1990). Values are percentages classified into ISGs, with numbers of isolates in parentheses.
H. annosum is a white-rot fungus, it must degrade chemically complex lignin in addition to other constitutive and induced host defense chemicals. Research on how H. annosum interacts with these compounds relative to various hosts may yield insights into the mechanisms that form the basis for host specialization between the S and P ISGs. For example, hardwood lignins are apparently easier to biodegrade than those of conifers (Highley and Kirk 1979), and the reason why the P ISG attacks certain hardwoods may be that the S and P ISGs have differing mechanisms of lignin degradation.

In Scandinavia and northern Europe, modern-day coniferous forests are dominated by Picea and Pinus. It is thought that Picea evolved after Pinus and that they are more closely related to each other than are Abies, which is believed to have evolved more recently than either (Miller 1977; Hawley and DeHayes 1985). Evolutionary relationships among these major host genera, with Pinus being more closely related to Picea than to Abies, may partially explain crossover infections of Picea by the P ISG, whereas there is no evidence of crossover between this ISG and Abies in the field.

Our allozyme data indicate that there are more shared alleles between the European S, P, and F ISGs than between the North American S and P ISGs, where there is a high degree of fixation for alternative alleles in several loci. This may indicate that intersterility barriers arose later in the European ISGs relative to the North American ISGs, or evolution took place at differing rates between the ISGs in the two continents. Thus, the allozyme relationships among the European ISGs may be evidence of relative evolutionary recency of genetic isolation in this group compared with the North American ISGs. Interestingly, the S ISGs and the F ISG appear to be more closely related to each other than do the P ISGs (Figs. 1 and 2), suggesting relative evolutionary recency, paralleling the supposed evolutionary recency of Picea and Abies.

Although the number of isolates we analyzed does not permit absolute statements about total amounts of genetic diversity relative to allozymes in each population, the extensive geographic areas represented by our collections do reflect relative amounts of diversity present in the ISGs. The apparently greater genetic diversity, as measured by expected heterozygosity (Table 3), in the European ISGs versus the North American ISGs may reflect the origins of genetic isolation in H. annosum in the Arcto-Tertiary flora. The European ISGs may have retained some of the genetic diversity present at the latter stages of evolution in this fungus because host species associations and species migrations in Europe and Scandinavia proceeded differently from that of North America. One major reason for this could be the different orientations of mountain range axes of the North American and European continents. During the last glacial age (Wisconsin), forest tree species populations in the western United States became fragmented and responded to climatic changes along elevational and latitudinal gradients, with some host tree species such as ponderosa pine becoming relatively rare and restricted in distribution (Spaulding 1984; Critchfield 1984). This process may account for the relatively low amount of allozyme variability in the North American P group.

In Europe, because of differing orientations of mountain ranges, responses of forest tree species to glaciation events may not have resulted in comparable fragmentation. Although considerable numbers of plant species became extinct, there is evidence that, at the height of the last European glaciation event, belts of cold-resistant conifer species existed within broad central Eurasian steppe regions (Frenzel 1968). Forests dominated by mixed conifer species also existed along the southern edges of the European land mass bordering the Mediterranean and extending into regions bordered by the southern portions of the Black Sea and Caspian Sea (Frenzel 1968). Some host species distributions may have resulted in preservation of genetic variability in the fungus through the glaciation event.

Because of the magnitude of time involved, many alternative hypotheses exist relative to evolutionary relationships between host specificity, genetic variability, and intersterility. For example, the lower genetic variability in the North American S and P groups relative to the European S, P, and F groups may indicate that H. annosum was recently introduced on the North American continent, possibly after the last ice age. Also, the P ISG may not have evolved on Pinus, and its population structure may be the consequence of recent forest management practices. Other tree species such as western juniper (Juniperus occidentalis Hook) and eastern red cedar (Juniperus virginiana L.) may have served as primary hosts to the P ISG. Our field observations indicate western juniper is apparently highly susceptible to the P ISG, exhibiting mortality around infected pine stumps often before disease symptoms appear on adjacent pine. Heterobasidion annosum was also observed fruiting on diseased eastern red cedar on sites where no previous management activities could be linked to the presence of H. annosum root disease (F.W. Cobb, unpublished data). Instead of being passively influenced by interactions of climate and forest vegetation, H. annosum may be a primary ecological force as an agent determining species composition and stand structure.

Detailed experiments designed to study host specificity at the cellular and biochemical levels need to be conducted using genetically defined isolates and hosts. Understanding how hosts respond to infection by H. annosum within and between ISGs will provide insights into the origins of host specificity. Also, research on infection biology that addresses questions regarding relationships between basidiospore and conidiospore inoculum and disease development on various hosts, such as juniper species, may shed light on how populations of H. annosum were maintained prior to forest management practices. In addition, studies of local populations of H. annosum relative to various host ranges by using isozymes and DNA analyses will further elucidate evolutionary pathways in this important forest pathogen. These studies should employ planned sampling designs with samples stratified to represent various host types and geographic areas to that inferences can be made relative to genetic differentiation of those populations.

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