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 hterozygosity, 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, playnological events, evolutionary relationships, Hymenomycetes, root disease.

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A l'aide de l'analyse des isoenzymes, les auteurs ont étudié des isolats de l'*Heterobasidion* ***annosum*** (Fr.) Bref. representant les groupes d'incompatibilite nord-americains S et P ainsi qu'européens S, P, et F. Les groupes européens S, P, et F montrent plus de variabilité que les groupes nord-americains S et P en termes d'hétérozygocité attendue, de nombre d'allbles par lieu et de pour cent de lieux polymorphes. Contrairement aux groups nord-americains S et P, les groupes d'incompatibilite européens ne peuvent être distingués les uns des autres sur la base des lieux isozymiques individuels, bien qu'il existe des differences significatives dans les fréquences d'allèles entre les groupes europeens S et P. Ceci suggère que l'évolution aurait procédé à des taux différents chez les divers groupes d'interstérilité ou que des barrières d'interstérilité seraient apparues plus tard dans les populations européennes comparativement aux populations nord-américaines de l'*H. annosum.* Les changements de climat et d'association avec les hôtes au cours du tertiaire pourraient avoir joué un role important dans l'évolution des groupe d'incompatibilite chez l'*H. annosum.*

Mots clés : allozymes, arbre forestier hôte, évènements playnologiques, relations Cvolutives, hyménomycètes, maladie racinaire.

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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 P, 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 interfertility 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 manage-

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

ISG and host	No. of isolates	Location	Identity of selected isolates*
European S			
Picea abies	11	Finland	181, 183-186, 401-403, 406, 448, 791205.5.1
Picea abies	3	Italy	429-431
Picea abies	4	Germany	432 -435
European P			
Pinus sylvestris	14	Finland	180, 187, 409–415, 424-427, 750124.2.1
Picea abies	1	Finland	407
Airborn inoculum	1	Finland	791200.1.2
Crataegus spp.	1	Finland	810928.1.1
Pinus sylvestris	1	Switzerland	428
Betula pendula	1	Finland	408
European F			
Abies alba	10	Italy	438 -447
North American S			
Abies concolor	1	Arizona	216
Abies spp.	7	Oregon	217, 219, 221, 222, 225, 228, 229
Abies spp.	23	California	201, 212-214, 230-243, 245, 247, 369, 384, 386
Pinus ponderosa	2	California	302, 322
Pinus ponderosa	1	Oregon	329
Sequoiadendron giganteum	6	California	505-510, 512
North American P			
Pinus ponderosa	4	Montana	111-114
Pinus ponderosa	3	Oregon	104, 323, 327
Pinus ponderosa	12	California	305, 310, 334, 338, 340, 341, 344, 348, 354, 361, 375, 382
Pinus jeffreyi	3	California	387, 390, 398

*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.

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 *Sequoiadendron giganteum* (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 1990*a*; 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 are 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 nun) of Whatman 3 MM filter paper were saturated with the chilled extract, then loaded onto starch

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

Enzyme system	EC No.	Abbr.	Gel buffer system*
Aconitase	4.2.1.3	ACO	E
Alcohol dehydrogenase	1.1.1.1	ADH	A, E
Alpha-esterase	3.1.1.2	AEST	A
Glutamate dehydrogenase	1.4.1.2	GDH	B
Glutamate oxaloacetate transaminase	2.6.1.1	GOT	B
Isocitrate dehydrogenase	1.1.1.42	IDH	D
Leucine aminopeptidase	3.4.11.1	LAP	A
Malate dehydrogenase	1.1.1.37	MDH	D, E
Phosphoglucoisomerase	5.3.1.9	PGI	A
Sorbitol dehydrogenase	1.1.1.14	SRDH	B

*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 intersterility groups (ISG) of *Heterobasidion annosum*

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

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).

gels. The procedures were previously described by Otrosina et al. (1992).

Starch gel electrophoresis was performed on the extracts using four gel buffer systems (Conkle et al. 1982). The four systems, designated A, B, D, and E, were Tris-borate (pH 8.3), Tris-citrate (pH 8.8), morpholine -citrate (pH 8. 1), and morpholine citrate (pH 6. 1), respectively. Running voltages for the A and B gels were 250–320 V at 75 mA and 200-250 V at 70 mA, and for the D and E gels 150-200 V at 60 mA. Electrophoresis was terminated after 4-5 h or after the buffer fronts had migrated 8 cm. The gels were then sliced and stained for enzyme systems (Conkle et al. 1982; Micales et al. 1986). Ten enzyme systems having 12 putative isozyme loci were used in this study. The enzyme systems, Enzyme Commission (EC) numbers, and gel buffer systems are listed in Table 2.

After electrophoresis, gels were scored using the method previously reported (Otrosina et al. 1992). For example, putative allelic variants within an isozyme locus were given alphabetic characters. The designator AA was given, usually for the most common band, for a single banded phenotype presumed to have a homoallelic genotype, BB for the next most common band, etc. Where two and three banded allozyme phenotypes were judged to be heteroallelic, they were designated by the letter given to the two presumed alleles involved, e.g., AB, BC. Enzymes having more than one putative locus were given the numerical designator 2 after the enzyme abbreviation for the most mobile locus and 1 for the more slowly migrating one. Null alleles are defined as those without banding in certain loci relative to our gel systems, staining protocols, and electrophoretic conditions. Null allele frequencies were treated as active alleles for data analysis. Isozyme analyses were performed at least twice on all isolates in this study, and those alleles defined as null were consistent between runs.

For the purposes of genetic and statistical analysis, all isolates were assumed to be dikaryotic. Allele frequencies, genetic distances, percent polymorphic loci, and expected heterozygosity were calculated using STEP SIMDIS and STEP VARIAB in the computer program BIOSYS-1 (Swofford and Selander 1981). Cluster analysis based on calculated Nei (1978) genetic distances using the unweighted pair-group method (Sneath and Sokal 1973) was carried out for the five groups using STEP CLUSTER. The differences between the 95% confidence limits determined for the allele frequencies among the five ISGs were approximated using the Bonferroni inequality with $\alpha = 0.05$. A locus was considered polymorphic if more than one allele was found within a locus for a given population. Also, canonical discriminant analysis (PROC CANDISC) and discriminant analysis with cross validation (PROC DISCRIM) were performed on the allozyme data using SAS (SAS Institute Inc. 1990). Recognizing that some assumptions needed for their application may be violated, we used these statistical programs for their comparative utility.

Results

Number of alleles per locus, percent polymorphic loci, and expected heterozygosities within the groups are given in 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-l*, *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-l* 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

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

Locus and allele	North American S (N = 40)	North American P (N = 22)	European P (N = 19)	European S (N = 18)	European F (N = 10)
ADH					
A	0.04*a*	1.00*b*	0*a*	o*a*	0*a*
B	0.34*a*	0*b*	1 .ooc	0.1 *lab*	O.lOab
c	0.48*a*	0*b*	0*b*	0*b*	0*b*
D	0.14*a*	0*a*	0*a*	0.50*b*	0.60*b*
E	0*a*	0*a*	0*a*	0.22*a*	0*a*
Null	—	—	—	0.17*a*	0.30*a*
GOT					
A	0.90*a*	0*b*	0*b*	0.28*bc*	0.65*ac*
B	0*a*	0.96*bc*	1 .00*b*	0.67*cd*	0.35*ad*
c	0*a*	0.02*a*	0*a*	0.05*a*	0*a*
D	0.10*a*	0.02*a*	0*a*	0*a*	0*a*
MDH-1					
A	1.00*a*	1 .00*a*	1 .00*a*	1.00*a*	1.00*a*
MDH-2					
A	0.99*a*	0*b*	1.00*a*	0.80*a*	1.00*a*
B	0.01*a*	1.00*b*	0*a*	0.06*a*	0 a
c	0*a*	0*a*	0*a*	0.03*a*	0*a*
Null	—	—	—	0.11*	—
PGI					
A	0.97*a*	0*b*	0.21*b*	0*b*	0*b*
B	0*a*	0*a*	0.37*b*	0.61*b*	0.30*ba*
c	0*a*	0*a*	0.16*a*	0*a*	0.10*a*
D	0.03*a*	0*a*	0*a*	0*a*	0*a*
Null	—	1.00*a*	0.26*b*	0.39*bc*	0.60*ba*
GDH					
A	0*a*	1 .00*b*	0*a*	0.17*a*	0*a*
B	0*a*	0*a*	0.906	0.06*a*	0.10*a*
c	0*a*	0*a*	0*a*	0.05*a*	0*a*
Null	1.00*a*	—	O.lOb	0.72*a*	0.90*a*
ACO					
A	0.99*a*	0*b*	0.06*bc*	0.44*d*	0.40*bd*
B	0.01*a*	1.00*b*	0*a*	0.03*a*	0*a*
C	0*a*	0*a*	0*a*	0.41*b*	0.40*ab*
D	0*a*	0*a*	0.89*b*	0.06*a*	0.10*a*
E	0*a*	0*a*	0.05*a*	0*a*	0*a*
Null	—	—	—	0.06*a*	O.lOa
AEST					
A	1 .00*a*	0*b*	0*b*	0.72*ac*	0.50*c*
B	0*a*	1.00*b*	0*a*	0*a*	0*a*
c	0*a*	0*a*	0.84*b*	0.06*a*	0.10*a*
D	0*a*	0*a*	0*a*	0.05*a*	0.30*a*
E	0*a*	0*a*	0.16*a*	0.17*a*	0*a*
Null		—	—	—	0.10*
LAP-1					
A	1.00*a*	0*b*	0.16*bc*	0.44*c*	0*b*
B	0*a*	1.00*b*	0.84*bc*	0.56*c*	0.90*bc*
Null	—		—		0.10"
LAP-2					
A	0.85*a*	0*b*	0.47*c*	0.28*bc*	O.lObc
B	0.15*a*	0*a*	0.16*a*	0.05*a*	0*a*
c	0*a*	0*a*	0.03*a*	0.17*a*	0*a*
D	0*a*	0*a*	0.08*a*	0*a*	0*a*
Null	—	1 .00*a*	0.26*c*	0.50*bc*	0.90*ab*
SRDH					
A	0.05*ac*	0.82*b*	oc	*oc*	*oc*
B	0.92*a*	0*b*	0. 10*b*	0.06*b*	0.30*b*
C	0*a*	0*a*	0.13*ab*	0.50*b*	0.50*b*
D	0.03*a*	0*a*	0.71*b*	0.17*a*	*0*

TABLE 4 *(concluded)*

Locus and allele	North American S (N = 40)	North American P (N = 22)	European P (N = 19)	European S (N = 18)	European F (N = 10)
E	0*a*	0*a*	0*a*	0.11*a*	0.20*a*
Null		0.18*a*	0.05*a*	O.lla	0.20*a*
IDH					
A	0*a*	0.93*b*	0.82*b*	*Oa*	0*a*
B	0.59*a*	0.07*b*	0.18*b*	0.89*a*	0.90*a*
c	0.39*a*	*Obc*	*Obc*	O.llac	*Oa*
D	0.02*a*	*Oa*	0*a*	0*a*	0*a*
Null	—		—	—	0.10*

NOTE: Allele frequencies not followed by the same letter across groups are significantly different from each other at $\alpha = 0.05$ 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

ISG	North American S	North American P	European P	European S	European F
North American S	—				
North American P	2.24	—			
European P	1.00	0.90			
European S	0.41	0.92	0.54	—	
European F	0.49	0.89	0.61	0.08	—

NOTE: Nei (1978) unbiased genetic distance calculated from 12 allozyme loci.

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

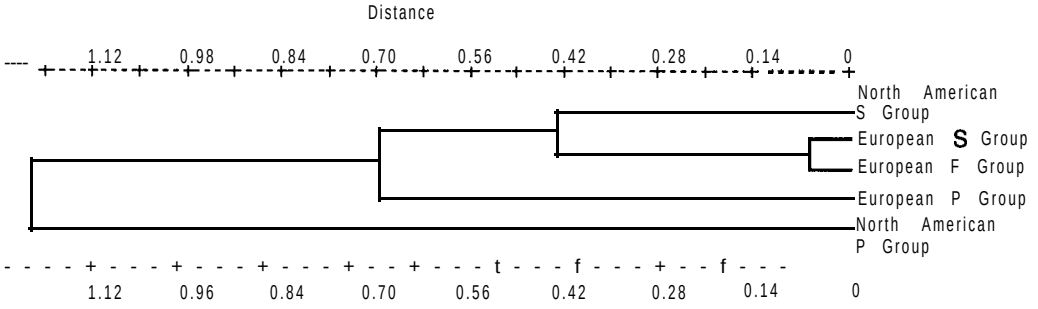

FIG. 1. Dendrogram constructed from cluster analysis of Nei (1978) genetic distances derived from allele frequencies of *H. annosum* allozymes .

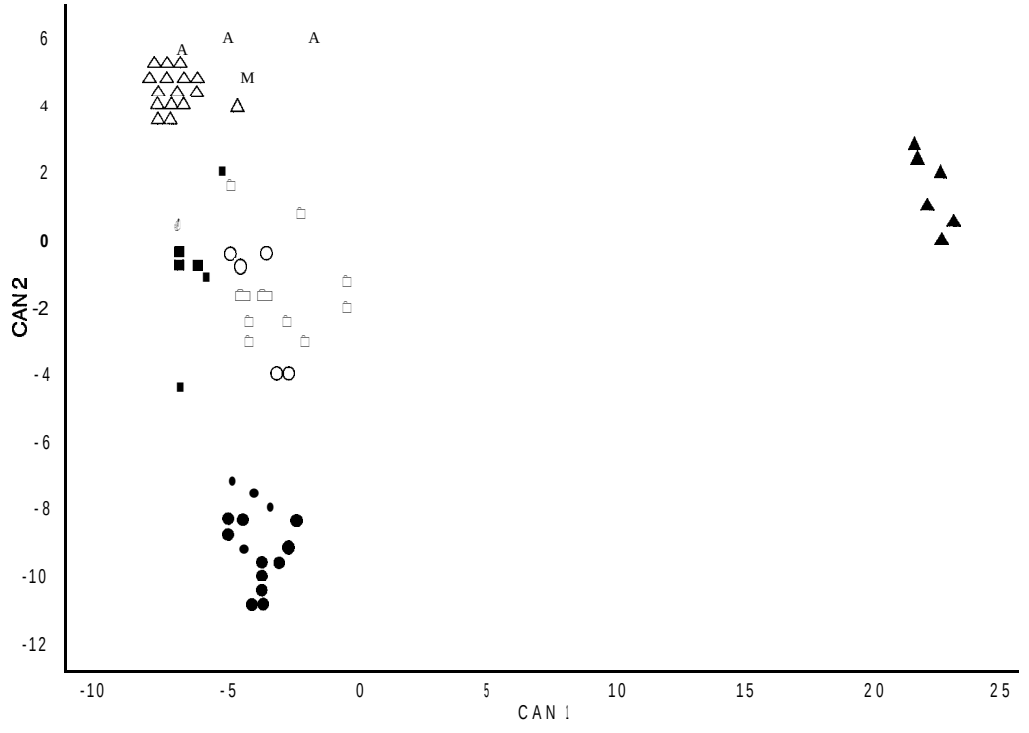

FIG. 2. Plots of first two vectors, CAN 1 and CAN 2, in canonical discriminant analyses of *H. annosum* allozyme data. △, North American S group; A, North American P group; ■ , European F group; o , European S group; ● , European P group. Differences between total number of points plotted and the 109 total isolates analyzed are due to identical canonical scores.

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 pathogenic&y 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 allozyme 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-

TABLE 6. Results of percentage (and number) of ***Heterobasidion annosum*** isolates classified from one intersterility group (ISG) into another by the cross-validation procedure from discriminant analysis of isozyme data

	To ISG					
	European			North American		
From ISG	P	S	F	P	S	Total
European P	94.4 (17)	5.6 (1)	0 (0)	0 (0)	0 (0)	100 (18)
European S	5.9 (1)	52.9 (9)	17.7 (3)	0 (0)	23.5 (4)	100 (17)
European F	10.0 (1)	0 (0)	70.0 (7)	0 (0)	20.0 (2)	100 (10)
North American P	0 (0)	0 (0)	0 (0)	100 (19)	0 (0)	100 (19)
North American S	0 (0)	2.5 (1)	0 (0)	0 (0)	97.5 (39)	100 (40)
% misclassified	5.6	47.1	30.0	0	2.5	

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

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 interfertile with European S group strains from the Alps, indicating a close relationship to the S ISG.

These allozyme 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 1990*b*), 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 allozyme 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, PGI, ADH, ACO,*** and ***LAP-2*** loci. Genetic divergence in isozymes has been shown for certain intercompatible allopatric populations of the *Collybia dryophila* complex between Europe and North America (Vilgalys 199 1).

In sympatric speciation, intersterility barriers may arise abruptly in conspecific subpopulations (Slatkin 1987; Hart1 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. Seedling inoculation experiments (Worrall et al. 1983; Cobb et al. 1989) and field sampling studies employing isozyme analyses (Otrosina 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 fir ***(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 ***(Piceu abies)*** (Stenlid 1987). Seedling inoculation experiments also demonstrated a high capability in P group isolates to infect Norway spruce (Stenlid and Swedjemark 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 allozyme relationships between ISGs from the two continents. During the Tertiary period, homogeneous forests comprising the forerunners 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*, ***Sequoiadendron,*** 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 ***Arctostuphylos*** 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* (Hedgc.) 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

Abies, S. giganteum, and *Tsuga heterophylla* and the P ISG attacking *Pinus, Juniper-us,* and hardwood genera such as *Arctostaphylos, Alnus*, and *Arbutus* in the western United States (Cobb et al. 1989; Worrall et al. 1983). Thus far, the S ISG has not been found on any host in the eastern United States, but the P ISG is a serious problem on various species of *Pinus* there. This is consistent with the fact that the eastern United States forest flora was dominated by hardwoods, some representing P ISG hosts, after the last ice age (Gibbs and Wainhouse 1986).

The mechanisms determining pathogenicity and host specialization in *H. annosum* are not understood. Because *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 is *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 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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