Genetics

Allozyme Differentiation of Intersterility Groups of *Heterobasidion annosum* Isolated from Conifers in the Western United States

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ABSTRACT

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Allozyme analysis was conducted on 64 isolates from basidiocarps of *Heterobasidion annosum*. The isolates belonged to the "S" and "P" intersterility groups and were collected from five conifer species found in the western United States. Ten allozyme loci distributed among eight enzyme systems were examined. Intersterility groups differed at nine loci. Few alleles were common to both intersterility groups, and only one locus, MDH-1, was monomorphic. Several loci, including MDH-2, GDH, and PGI, were diagnostic for the S or P intersterility groups, suggesting fixation for alternative or null alleles. Distribution of alleles between intersterility groups indicates an extremely high degree of genetic divergence; Nei's genetic distance was equal to 0.926. Isolates from diseased pine trees had only alleles consistent with those of P group, and isolates from diseased true fir had S group alleles, indicating a complete association among intersterility groups, allozyme profiles, and host species. These data support a hypothesis of genetic isolation between intersterility groups in nature. Allozyme genotypes provide a rapid test for identifying intersterility groups of the fungus in North America.

Additional keywords: Basidiomycetes, biological species, isozymes.

Heterobasidion annosum (Fr.:Fr.) Bref. (=*Fomes annosus* (Fr.:Fr.) Cooke) is a destructive pathogen that causes root and butt rot of coniferous tree species in temperate zone forests throughout the world. In California alone, about 3.3 million hectares of commercial forest lands is estimated to be affected by this pathogen (32).

In pine species, the fungus colonizes recently cut stumps via airborne deposition of basidiospores. The fungus then colonizes adjacent healthy trees through root contacts and grafts. In addition to stump infections, basal wounds and other infection courts may account for infection of *Abies* spp. and possibly other nonpine species. Once present in a stand, the fungus can survive for decades in resinous woody tissues and can cause continued mortality via spread through root contacts to adjacent trees and in new seedling reproduction (21).

Until recently, *H. annosum* was regarded by forest pathologists as a single taxonomic unit. Korhonen (17), however, demonstrated two intersterility groups (ISGs) or biological species of the fungus. He designated these "S" and "P". The S group was isolated primarily from butt-rotted *Picea abies* (L.) H. Karst. and *Pinus sylvestris* L. saplings. The P group was isolated from *P. sylvestris* and a variety of other conifers and hardwood species. Later, Chase (6) and Chase and Ullrich (9) showed that both the S and P ISGs are present in the western United States. Similar results were obtained by Harrington et al (15). Numerous field observations and some experimental inoculation studies with seedlings of different host species (11,35) indicate considerable differentiation in the fungus relative to host range or host specialization.

Allozyme analyses have been used by many researchers to determine inter- and intraspecific variation in various fungal species (e.g., 18,19,37). However, very few studies have dealt with interrelationships between intersterility barriers and allozyme differentiation. In the genus *Pleurotus*, intersterility studies have demonstrated complete reproductive isolation between morphologically indistinguishable or barely distinguishable species (3). Relationships between intersterility barriers and isozymes have been studied in the genus *Armillaria*, a taxon containing important forest tree root pathogens that have undergone comparatively little morphological differentiation (22). Similarly, the S and P ISGs of *H. annosum* in the United States are not easily distinguishable on the basis of macroscopic criteria, and further comparative morphological studies remain to be done. May and Royse (18) employed allozyme analysis to separate isolates of *Pleurotus*

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species into specific classes, and they also have provided evidence of species misidentification among certain groups of this fungus.

This study examines the relationships between ISGs of *H. annosum* and allozyme structure of populations of this fungus in California and Oregon, where there are numerous conifer hosts of this pathogen. Preliminary results of this work have been published (10,27).

MATERIALS AND METHODS

Fungus isolates and hosts. Collections were made from disease centers in both pine and true fir stands and in other host tree species in the area ranging from the Fremont National Forest in southern Oregon to the San Bernardino National Forest in southern California. A small number of isolates was obtained from sites in Montana and Arizona. Host tree species included Jeffrey pine (*Pinus jeffreyi* E. Murray), ponderosa pine (*Pinus ponderosa* Douglas ex P. Laws. & C. Laws), white fir (*Abies concolor* (Gordon & Glend.) Lindl ex Hildebr.), red fir (*Abies magnifica* Andr. Murray), Coulter pine (*Pinus coulteri* D. Don), single-leaf pinyon (*Pinus monophylla* Torr. & Frém.), giant sequoia (*Sequoiadendron giganteum* (Lindl.) Buchholz), western juniper (*Juniperus occidentalis* Hook.), incense cedar (*Libocedrus decurrens* Torr.), and manzanita (*Arctostaphylos uva-ursi* (L.) Spreng.). Isolates of *H. annosum* were obtained from the context tissue of basidiocarps or from infected root tissue of symptomatic seedlings, larger trees, or stumps. In a few instances, polybasidiospore isolates were obtained from spore castings of individual basidiocarps. Geographic origins, host substrate, and number of isolates obtained for both allozyme analysis and intersterility testing are given in Table 1.

TABLE 1. Conifer hosts, sample origins, numbers of isolates identified by intersterility groups used in isozyme study, and MDH-2 diagnostic alleles used in the study of *Heterobasidion annosum*[a]

Host substrate	Sample origin[b]	Number of isolates	MDH-2 allele
S group isolates			
White fir stumps	ENF	5	A
	FNF	7	A
	PNF	4	A
	SBNF	4	A
	SNP	5	A
	ARZ	1	A
Red fir stumps	PNF	1	A
	SNP	2	A
Sequoia stumps or downed stems	SNP	5	A
Ponderosa pine stumps	FNF	1	A
	LNF	2	A
	SBNF	1	A
Jeffrey pine stumps	SBNF	4	A
P group isolates			
Ponderosa pine stumps	IFG	1	B
	LNF	1	B
	FNF	2	B
	MNF	8	B
	YNP	1	B
	SBNF	1	B
Ponderosa pine roots[c]	MONT	4	B
	ORE	1	B
Jeffrey pine stumps	SBNF	3	B

[a] Isozyme profile of MDH-2 locus indicating putative alleles A and B associated with the S and P intersterility groups, respectively.
[b] ENF = Eldorado National Forest, CA; FNF = Fremont National Forest, OR; PNF = Plumas National Forest, CA; SBNF = San Bernardino National Forest, CA; ARZ = Catalina Mountains, AR; SNP = Sequoia National Park, CA; LNF = Lassen National Forest, CA; YNP = Yosemite National Park, CA; MNF = Modoc National Forest, CA; ORE = central Oregon; MONT = Montana; IFG = Institute of Forest Genetics, Placerville, CA.
[c] Isolates from the collection of Chase and Ullrich (9).

Careful observations were made at the time of sporophore collection from stumps for evidence of pathogenic activity. Among the criteria employed to determine pathogenic activity were the presence of resinosis in the stump root traces, existence of decay columns present before felling, and dead or dying seedlings and saplings near the stumps.

Dikaryotic cultures of *H. annosum* were obtained by plating pieces of the context tissue of basidiocarps of the fungus onto 1.25% malt extract agar or by plating pieces of infected root tissue on this medium. Before being plated, surfaces of basidiocarps and root tissue were swabbed with 95% ethanol. Single basidiospore isolates (homokaryons) were collected from each actively sporulating basidiocarp for intersterility experiments as previously described (8,9). Most basidiocarps yielded spores. A minimum of 25 monosporus isolates were obtained from each basidiocarp.

Intersterility experiments. The homokaryons were classified as belonging to the S or P ISGs by pair-mating experiments using tester strains of *H. annosum* (9). A minimum of five homokaryons from each collection were screened.

Preparation and extraction of mycelia for electrophoresis. Agar plugs from margins of actively growing colonies of each isolate of the fungus were inoculated into 250-ml Erlenmeyer flasks containing 100 ml of potato-dextrose broth. Flasks were incubated at 22 C for 2.5–3 wk. All flasks were swirled briskly for a few seconds every other day to provide aeration. Tests demonstrated that yields of enzymes were optimal under these growth conditions (25).

After incubation, mycelium in the flasks was harvested in a Buchner funnel by vacuum filtration over Whatman No. 4 paper disks and was rinsed under vacuum with approximately 50 ml of distilled water to remove excess medium. The resultant mycelial mats were then transferred to microtiter wells (28) or to Eppendorf microfuge tubes, sealed, and stored at −70 C until they were extracted for electrophoresis.

Protocol for processing the mycelium stored in the microtiter wells was performed as described previously (28). The following extraction procedure was used for the mycelium in Eppendorf tubes. Six-tenths of a milliliter of 0.2 M phosphate buffer, pH 7.5, amended with 1 mg/ml of bovine serum albumin was added to approximately 2 g (fresh weight) of mycelium in a 100-ml mortar. Sufficient liquid nitrogen was added to freeze the mycelial plug, which was then ground with a pestle until a paste was formed. No differences in results were obtained between the two storage and extraction methods. A square of Whatman No. 4 filter paper approximately 2 × 2 cm was placed on the paste and became saturated with extract. Wicks made of 3- × 10-mm strips of Whatman 3 MM chromatography paper were then placed atop the saturated filter paper to absorb the extract. The extract in the mortars and in the wicks was kept on ice throughout the procedure. The saturated wicks were then placed in clean microtiter wells, sealed, and stored overnight at −70 C.

Electrophoresis. Horizontal starch gel slabs were prepared the day before electrophoresis following the procedures of Conkle

TABLE 2. Enzyme and gel buffer systems used for allozyme analysis of S and P intersterility groups of *Heterobasidion annosum*

Enzyme system	EC number	Abbreviation	Gel buffer system[a]
Alcohol dehydrogenase	1.1.1.1	ADH	A,E
Glutamate oxaloacetate transaminase	2.6.1.1	GOT	B
Malate dehydrogenase	1.1.1.37	MDH	E
Phosphoglucoisomerase	5.3.1.9	PGI	A
Glutamate dehydrogenase	1.4.1.2	GDH	B
Aconitase	4.2.1.3	ACO	E
Alpha esterase	3.1.1.2	AEST	A
Leucine aminopeptidase	3.4.11.1	LAP	A

[a] Gel buffer systems prepared following Conkle et al (12). A = Tris-borate; B = Tris-citrate; E = morpholine citrate.

et al (12). The three-gel buffer systems employed in this study, designated A, B, and E, respectively, were Tris-borate (pH 8.3), Tris-citrate (pH 8.8), and morpholine-citrate (pH 8.1) (Table 2). Wicks containing the mycelial extracts were loaded onto the gels and were placed in a refrigerator at 3 C. Bags containing cold water were set on top of the gels to dissipate heat. Running voltage and current for the A, B, and E gels were 250–320 V at 75 mA, 200–300 V at 70 mA, and 150–200 V at 60 mA, respectively. Electrophoresis was terminated after 4–5 h or after buffer fronts had migrated 8 cm. The gels were then sliced and stained for enzyme systems (12,19). Eight enzyme systems with 10 putative isozyme loci were employed in this study (Table 2).

Data interpretation and analysis. Gels were scored by using methods previously reported (28), except putative allelic variants within a locus were assigned alphabetic characters. Scoring of allelic variants was also based on the assumption that isolates derived from infected root tissue or from the context tissue of *H. annosum* basidiocarps were dikaryotic. For example, the alphabetic designator AA was given usually for the most common band, which was a single-banded allozyme phenotype presumed to have a homoallelic genotype; BB was designated for the next most common, etc. Two- and three-banded allozyme phenotypes judged to be heteroallelic were consistent with previous studies indicating segregation of alleles in corresponding homokaryons (W. J. Otrosina, *unpublished data*), with published subunit structures of enzyme systems (13), and with interpretations in other fungi (24,29). Heteroallelic bands were designated by the letter given to the two presumed alleles involved (e.g., AB, BC, etc). If two loci appeared to be coding for an enzyme system, the locus coding for the most mobile enzyme molecules was given a "2" after the enzyme abbreviation (e.g., MDH-2), whereas the more slowly migrating enzyme was given a "1". Enzymes were considered to be coded by two loci when their respective bands varied independently from one another with respect to their migration. Null alleles were defined as the absence of banding for certain allozyme loci relative to our gel systems, staining protocols employed, and electrophoretic conditions. The fungal isolates used in this study were run at least twice, and isolates having allozyme loci defined as null were consistent between runs. Other enzyme systems were fully active in situations in which putative null alleles were present for a locus.

Percentage of polymorphic loci, putative allele frequencies, and expected heterozygosity were calculated for isolates in both ISGs. Nei's unbiased genetic distance (23) and other genetic parameters were calculated from allele frequency data by using the computer program BIOSYS-1 (33). Binomial confidence limits based on the allele frequencies and on sample sizes were obtained from the statistical tables of Miller and Freund (20).

RESULTS

Intersterility. The majority of basidiocarps sampled yielded collections of viable single basidiospore cultures. Basidiospore

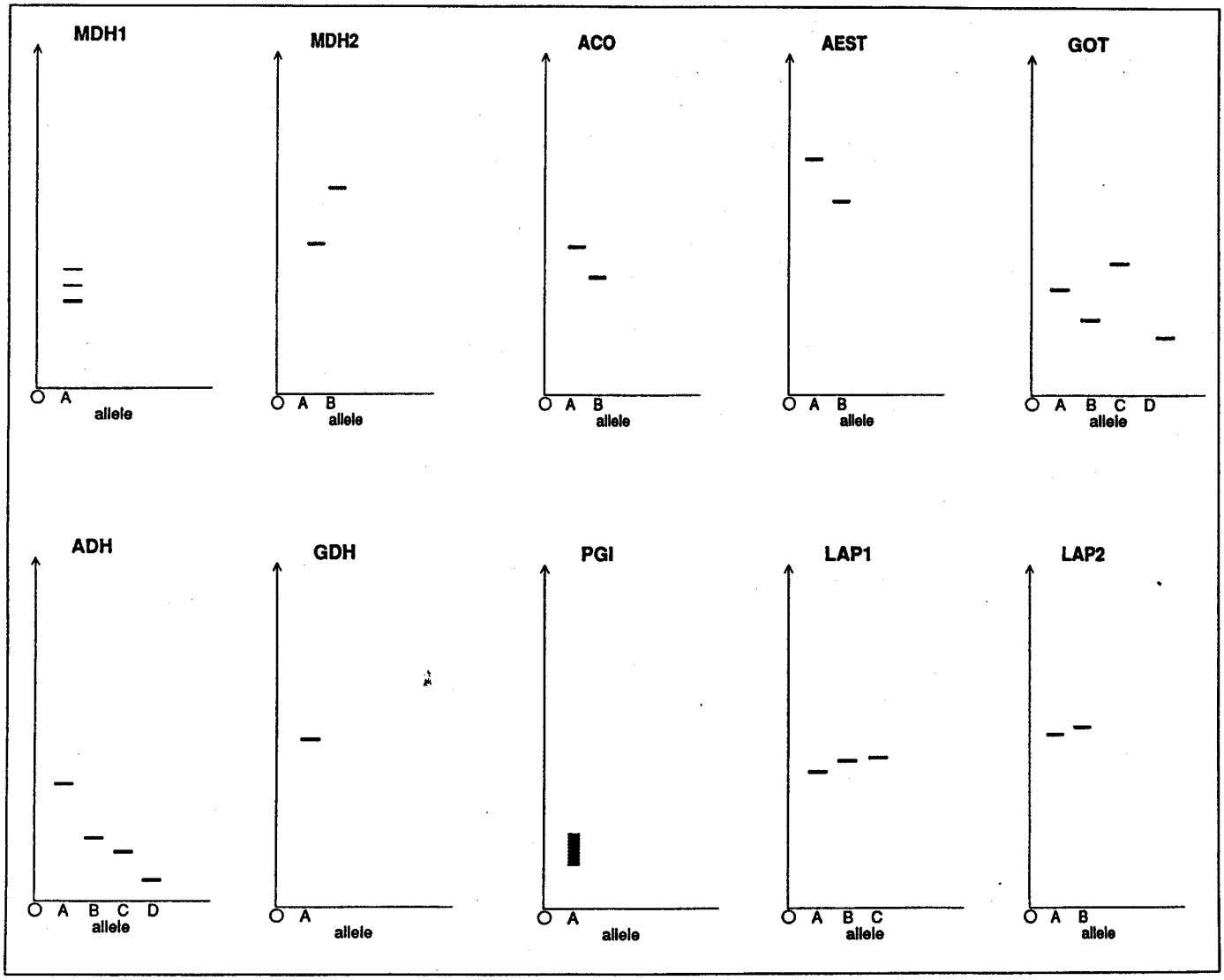

Fig. 1. Relative mobilities of putative alleles in 10 allozyme loci analyzed from *Heterobasidion annosum* isolates with known intersterility genotypes. Arrow indicates anodal direction of migration of bands from origin (O).

germination was nearly 100% in all collections. In mating experiments with homokaryons, tester strains from Finland clearly delineated S and P ISGs in our collections. One isolate, however, yielded only clamped single basidiospore cultures. Presence of clamp connections precluded intersterility testing, and it is possible this isolate represents a homothallic form of *H. annosum*. Approximately one-third of the isolates from pine stumps were in the S group. All isolates from true fir stumps and from fallen *S. gigantea* stems were in the S group (Table 1).

Allozyme analyses. No cathodal migration was observed for any enzyme system assayed. Systems included herein are those that gave clearly interpretable bands without regard for degree of polymorphism within an allozyme locus.

Most enzyme systems displayed single-banded phenotypes for homoallelic allozyme loci. Relative mobilities of allozymes associated with specific putative alleles for enzyme systems are diagrammed in Figure 1. Malate dehydrogenase (MDH), however, displayed a multiple banding pattern that was associated with the MDH-1 locus, a locus that was monomorphic for both ISGs (Fig. 2). Homokaryotic isolates and F1 progeny from compatible crosses yielded banding patterns identical to dikaryons at this locus. Secondary bands can be produced by dissociation of protein subunits or by post-translational modification of the enzyme. Because of the consistency of the banding pattern in this isozyme, its interpretation was presented as a single locus.

Triple bands observed for other loci such as ADH and GOT were interpreted as representing heteroallelic dikaryons and were consistent with the dimeric subunit structure of the enzyme. Homokaryotic isolates were derived from basidiocarps from which dikaryotic cultures heteroallelic for these allozymes were obtained. Isozyme experiments utilizing homokaryotic isolates showed 1:1 segregation ratios for fast and slow alleles in these enzyme systems (*unpublished data*).

Allele frequencies for allozymes from the S and the P ISGs are presented in Table 3. Frequencies for most loci are within 99% confidence limits for binomial proportions (20).

The MDH system developed readable bands within 15–20 min of incubation. We used the MDH-2 system as a quasidiagnostic locus for the S and P groups for this reason, and because MDH-2 A and MDH-2 B were associated with the S and P ISGs, respectively. The seven other enzyme systems also had allozyme patterns associated with the ISGs (Table 3).

Allozyme patterns of isolates from pine stumps not assayed for intersterility genotype (Table 4) also reflect a high proportion of allele A for the MDH-2 locus (presumed to be S group isolates). Isolates obtained from symptomatic pine hosts as opposed to stumps yielded only allele B (P group) for the MDH-2 locus as did isolates from symptomatic manzanita, incense cedar, and juniper. Isolates from white fir stumps with evidence of infection before felling, roots of symptomatic white fir seedlings, or fallen logs or stumps of giant sequoia had only the A allele in the MDH-2 locus. In one case, an isolate from a white fir stump yielded MDH-2 allele C coding for an allozyme with greater mobility than either A or B. Banding patterns of the other allozyme loci for this isolate were consistent with those of S group isolates, however.

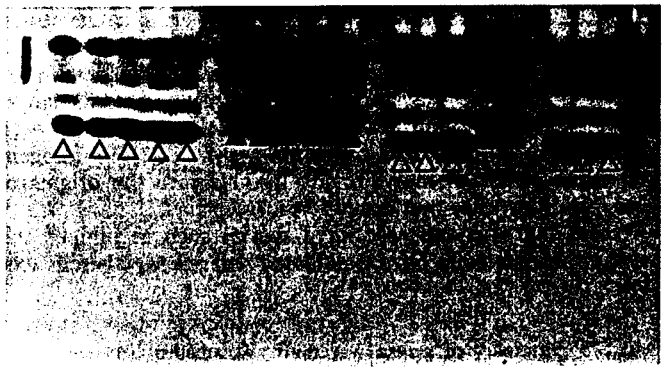

Fig. 2. Portion of gel showing banding patterns of malate dehydrogenase (MDH) and their relationships to intersterility groups of *Heterobasidion annosum* isolates. The upper bands, delimited by the dark vertical bar at the left side of photograph, represent the MDH-1 locus. Multiple bands described in text are observed at this locus. MDH-1 is invariant for all western U.S. isolates surveyed. Direction of migration is toward bottom of photograph (anode). The more mobile bands beyond the vertical bar (toward anode) are defined as the MDH-2 locus. White triangles highlight the more mobile MDH-2 B allele corresponding to the P intersterility group, whereas the dark triangles highlight the MDH-2 A allele corresponding to the S intersterility group for isolates of *H. annosum*.

As defined by our experimental conditions, the PGI and GDH loci had alternative null alleles for the P and S group isolates, respectively. The LAP-2 locus also showed no activity for the P group isolates. These null alleles were treated as active alleles for the purposes of genetic analyses based on allele frequencies. The loci MDH-2, LAP-1, ACO, AEST, GOT, and ADH were fixed or nearly fixed for alternative alleles between ISGs. Also, ADH and GOT were the most polymorphic, with each locus having four alleles between the two ISG. MDH-1 was monomorphic for all isolates.

In general, the S group isolates appeared to be slightly more variable than the P group isolates as measured by number of alleles per locus and percentage of loci that were polymorphic, although there is some degree of overlap (Table 5). The expected heterozygosities, which are a measure of allozyme variability present if alleles combine according to the Hardy-Weinberg law, were nearly five times greater in S group isolates than in the P group isolates.

DISCUSSION

These results demonstrated that a high degree of genetic divergence exists between ISGs of western U.S. isolates of *H. annosum*.

TABLE 3. Allele frequencies for 10 allozyme loci in S and P intersterility groups of *Heterobasidion annosum*

Locus (allele)	S group isolates ($N = 42$)	P group isolates ($N = 22$)
ADH		
A	0.04 (0.01–0.14)[a]	1.00 (0.76–1.00)
B	0.27 (0.12–0.47)	0 (0.00–0.24)
C	0.51 (0.30–0.72)	0 (0.00–0.24)
D	0.18 (0.07–0.38)	0 (0.00–0.24)
GOT		
A	0.90 (0.71–0.98)	0 (0.00–0.24)
B	0 (0.00–0.13)	0.94 (0.66–1.00)
C	0 (0.00–0.13)	0.03 (0.00–0.27)
D	0.10 (0.03–0.27)	0.03 (0.00–0.27)
MDH-1		
A	1.00 (0.86–1.00)	1.00 (0.76–1.00)
MDH-2		
A	1.00 (0.86–1.00)	0 (0.00–0.24)
B	0 (0.00–0.13)	1.00 (0.76–1.00)
PGI		
A	1.00 (0.86–1.00)	Null[b]
GDH		
A	Null	1.00 (0.76–1.00)
ACO		
A	0.99 (0.85–1.00)	0 (0.00–0.24)
B	0.01 (0.00–0.14)	1.00 (0.76–1.00)
AEST		
A	1.00 (0.86–1.00)	0 (0.00–0.24)
B	0 (0.00–0.13)	1.00 (0.76–1.00)
LAP-1		
A	1.00 (0.86–1.00)	0 (0.00–0.24)
B	0 (0.00–0.13)	0.93 (0.65–0.99)
C	0 (0.00–0.13)	0.07 (0.01–0.35)
LAP-2		
A	0.89 (0.69–0.97)	Null
B	0.11 (0.03–0.30)	Null

[a]Numbers in parentheses are 99% binomial confidence limits (20).
[b]Null alleles have no activity or banding for the particular locus represented.

We assume that the allozyme loci analyzed are neutral markers relative to selection influences and represent a sample of the genome with respect to allozyme variation. The 99% confidence level was chosen to provide a conservative interpretation of allele frequencies between the two ISGs (Table 3). It is immediately obvious that there is considerable allozyme differentiation between the ISGs. Preliminary studies have also indicated a high degree of allozyme differentiation between S and P ISGs of *H. annosum* (26,27). The large genetic distances (23) observed between the ISGs in this study (0.926) also indicate very few alleles are shared between groups.

In the laboratory, pairings between homokaryotic isolates of S and P ISGs from the western United States demonstrated 18% interfertility between groups (7). Our allozyme data indicate little or no gene flow occurs in nature between the S and P ISGs in the western United States. We calculated the quantity *F*(st), the ratio of actual variance of the two *H. annosum* ISGs to the maximum possible variance expected if all alleles were completely fixed (36), by using step FSTAT in the computer program BIOSYS-1 (33). The 0.872 value obtained for our allozyme data indicates a high degree of fixation for alternative alleles in most loci. Lack of gene flow between sympatric populations is a necessary condition for the formation of biological species (31), although development of complete reproductive isolation may not necessitate large changes in the genome (2). With time, considerable allozyme differences may accumulate, and estimates about the relative time since divergence of reproductively isolated populations can be examined by using allele frequencies (5). The western U.S. populations of *H. annosum* ISGs differ so completely that they appear to have either been divergent for a long time or to have evolved their genetic differences at high rates.

There are relatively few studies dealing with the relationships between genetic divergence and intersterility barriers in fungi. DNA hybridization studies among ISGs in *Collybia dryophila* and anastomosis groups in *Rhizoctonia solani* indicate that apparent rapid genome divergence is associated with development of genetic barriers to gene flow (34). In *Drosophila* sp., a group on which much information has been gathered relating allozyme data to various levels of speciation, genetic distance of the magnitude reported for *H. annosum* in the present study represented that of different species (2). Recently, studies on pectic isozyme profiles of *H. annosum* have revealed differences between S and P ISGs of this fungus from Europe and North America (16). Allozyme studies and DNA analyses comparing European populations of *H. annosum* S and P ISGs may shed more light on the phylogeny of this pathogen.

Host-parasite interactions and ecological factors may represent some of the forces driving differentiation of the two ISGs. There is experimental evidence that host preference in the fungus (11,15, 35) is related to ISG, which implies that a host selection component is related to the genetic divergence of these two groups. In the portion of our collection for which we performed allozyme analyses on isolates with undetermined ISG, all isolates obtained from symptomatic pine, incense cedar, juniper, and manzanita had the MDH-2 B allele (P group), whereas isolates from symptomatic tissue from true fir had the MDH-2 A allele (S group) (Table 4). All other polymorphic loci assayed for these isolates also had banding patterns consistent with host and S or P group patterns. Other studies have related the S ISG to infected true fir, western hemlock, and western red cedar, and other nonpine hosts, whereas the P group was always associated with infected pine, juniper, and incense cedar hosts (9,15). Allozyme analyses that we conducted on P group homokaryotic isolates of *H. annosum* from red pine stands in Vermont (6) yielded allozyme genotypes equivalent to the western collections of P group isolates, including the MDH-2 B phenotype. Because of the geographic area sampled and generally sympatric ranges of pine and true fir hosts associated with these areas in California and Oregon, our data support the hypothesis of a strong association among ISG, allozyme genotype, and specific host.

A parallel example relating host tree species, allozyme differentiation, and reproductive isolation can be found in the membracid insect species *Enchenopa binotata* (14). Insect reproductive and allozyme data relating sympatric associations of tree host species and insects indicated that *E. binotata* is a complex of reproductively isolated biological species that are differentiated along host lines. In the fruit fly genus *Rhagoletis*, there is considerable evidence for evolution of certain sympatric sibling species along host plant lines (4). Zhu et al (37) presented evidence for the involvement of host selection in allozyme differentiation in the ectomycorrhizal fungus *Suillus tomentosus*.

Paleoecological factors may be important in the genetic differentiation of the two *H. annosum* groups. Apparently a more species-rich conifer flora occupied the Sierra-Cascade range region in California and southeastern Oregon during the middle of the Miocene, when more moist and uniform climatic conditions existed (1). With a climate change to more xeric and colder conditions, less diverse and more uniform species associations now exist. As a speculation, *H. annosum* intersterility barriers in the western United States may have origins in climate-induced changes in forest species composition. Study of relationships between paleobotanical evidence and coevolution of forest tree pathogens such as *H. annosum* may provide insights into the origins of other Basidiomycetes that decay forest tree roots.

Present-day pine and associated species such as juniper and manzanita historically have dominated drier sites, whereas true

TABLE 4. Malate dehydrogenase-2 (MDH-2) genotypes of *Heterobasidion annosum* isolates from stump and symptomatic tree collections that were not mated to determine their intersterility groups

Host	Number of isolates	Locations of collections[a]	MDH-2 genotype[b]
Symptomatic hosts			
Pinus monophylla	1	SBNF	B
P. ponderosa	15	SBNF, YNP	B
P. jeffreyi	3	SBNF	B
P. coulteri	1	SBNF	B
Pseudotsuga menziesii	1	ENF	B
Juniperus occidentalis	1	MNF	B
Arctostaphylos sp.	2	SBNF, ENF	B
Libocedrus decurrens	2	YNP	B
Abies concolor	9	SBNF	A
Stumps			
P. ponderosa	6	FNF, MNF, SBNF	B
P. ponderosa	4	SBNF	A
P. jeffreyi	5	SBNF	B
P. jeffreyi	6	SBNF	A
A. concolor	6	SBNF, SNP	A,C[c]
A. concolor	1	FNF	B
A. magnifica	1	SNP	A
Sequoiadendron giganteum	2	SNP	A

[a]Location designators: FNF = Fremont National Forest, OR; MNF = Modoc National Forest, CA; SBNF = San Bernardino National Forest, CA; ENF = Eldorado National Forest, CA; SNP = Sequoia National Park, CA; YNP = Yosemite National Park, CA.
[b]MDH-2 locus with allele A and C corresponding to the S intersterility group and allele B corresponding to the P intersterility group.
[c]One isolate from SBNF had allele C, as in the MDH-2 locus, that had greater mobility than either allele A or B. All other allozyme loci for this isolate conformed to the S intersterility group pattern.

TABLE 5. Summary of genetic variability parameters between S and P intersterility groups of *Heterobasidion annosum*

Isolate	Sample size	Mean number of alleles per locus	Percentage of polymorphic loci	Mean heterozygosity
S group	42	1.6 (0.3)[a]	40.0[b]	0.10 (0.06)[c]
P group	22	1.3 (0.2)	20.0	0.02 (0.01)

[a]Numbers in parentheses are standard errors.
[b]A locus was considered polymorphic if more than one allele was found.
[c]Expected heterozygosity by Hardy-Weinberg law (unbiased estimate) (23).

fir, western hemlock, and western red cedar predominate on more mesic sites, although the true fir component on pine sites is increasing as a result of fire exclusion and silvicultural manipulations of forest stands (30).

Stumps of pine and true fir are nonselective for ISGs of *H. annosum*. We often obtained S group isolates from basidiocarps found in pine stumps and a P group isolate from a true fir stump. Such nonselectivity of stump tissue has been observed by us and reported by others (15), and it has important implications for disease management. Knowledge of the distribution of S and P groups of the fungus in older stumps and decaying root systems that serve as inoculum in stands of both pine and fir may enable the land manager to make decisions as to which species to encourage silviculturally. Also, such information is essential for the development of predictive models describing the impact and dynamics of *H. annosum* root disease centers in susceptible forest stands.

Allozyme markers such as MDH-2 can be employed to rapidly diagnose the ISG of a given isolate of *H. annosum*. Because the MDH-2 locus develops rapidly and alleles consistently mark S and P groups, a large number of isolates could be electrophoretically analyzed in a short time. The null alleles of PGI and GDH, which alternatively mark S and P groups, are being used in the process of developing a rapid, nonelectrophoretic assay for identifying intersterility groups.

LITERATURE CITED

1. Axelrod, D. I. 1977. Outline history of California vegetation. Pages 139-193 in: Terrestrial Vegetation of California. M. G. Barbour and J. Major, eds. John Wiley & Sons, New York.
2. Ayala, F. J., Tracey, M. L., Hedgecock, D., and Richmond, R. C. 1974. Genetic differentiation during the speciation process in *Drosophila*. Evolution 28:576-592.
3. Bresinsky, A., Fischer, M., Meixner, B., and Paulus, W. 1987. Speciation in *Pleurotus*. Mycologia 79:234-245.
4. Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). Evolution 23:237-251.
5. Carson, H. L. 1976. Genetic differences between newly formed species. BioScience 76:700-701.
6. Chase, T. E. 1985. Genetics of sexuality and speciation in the fungal pathogen *Heterobasidion annosum*. Ph.D. thesis. University of Vermont, Burlington. 244 pp.
7. Chase, T. E., Otrosina, W. J., and Cobb, F. W., Jr. 1989. Interfertility of 'S' and 'P' groups of *Heterobasidion annosum* in North America. (Abstr.) Phytopathology 79:1164.
8. Chase, T. E., and Ullrich, R. C. 1983. Sexuality, distribution, and dispersal of *Heterobasidion annosum* in pine plantations of Vermont. Mycologia 75:825-831.
9. Chase, T. E., and Ullrich, R. C. 1990. Genetic basis of biological species in *Heterobasidion annosum*: Mendelian determinants. Mycologia 82:67-72.
10. Chase, T. E., Ullrich, R. C., Otrosina, W. J., Cobb, F. W., Jr., and Taylor, J. W. 1989. Genetics of intersterility in *Heterobasidion annosum*. Pages 11-17 in: Proc. IUFRO Conf. Root and Butt Rots of Forest Trees. 7th. D. J. Morrison, ed. Pacific Forest Center, Victoria, British Columbia, Canada.
11. Cobb, F., Jr., Chase, T., Otrosina, W., Ratcliff, A., and Popenuck, T. 1989. Comparative virulence of *Heterobasidion annosum* isolates. (Abstr.) Phytopathology 79:1164.
12. Conkle, M. T., Hodgskiss, P. D., Nunnally, L. B., and Hunter, S. C. 1982. Starch gel electrophoresis of conifer seeds: A laboratory manual. USDA For. Serv. Gen. Tech. Rep. PSW-64. 18 pp.
13. Darnall, D. W., and Klotz, I. M. 1972. Protein subunits: A table. Rev. ed. Arch. Biochem. Biophys. 149:1-14.
14. Guttman, S. I., Wood, T. K., and Karlin, A. A. 1981. Genetic differentiation along host plant lines in the sympatric *Enchenopa binotata* Say complex. Evolution 35:205-217.
15. Harrington, T. C., Worrall, J. J., and Rizzo, D. M. 1989. Compatibility among host-specialized isolates of *Heterobasidion annosum* from western North America. Phytopathology 79:290-296.
16. Karlsson, J.-O., and Stenlid, J. 1991. Pectic isozyme profiles of intersterility groups in *Heterobasidion annosum*. Mycol. Res. 95:531-536.
17. Korhonen, K. 1978. Intersterility groups of *Heterobasidion annosum*. Commun. Inst. For. Fenn. 94:1-25.
18. May, B., and Royse, D. J. 1988. Interspecific allozyme variation within the fungal genus *Pleurotus*. Trans. Br. Mycol. Soc. 90:29-36.
19. Micales, J. A., Bonde, M. R., and Peterson, G. L. 1986. The use of isozyme analysis in fungal taxonomy. Mycotaxon 27:405-449.
20. Miller, I., and Freund, J. E. 1965. Probability and statistics for engineers. Prentice-Hall, Inc., Englewood, NJ. 432 pp.
21. Morrison, D. J., and Johnson, A. L. S. 1978. Stump colonization and spread of *Fomes annosus* five years after thinning. Can. J. For. Res. 8:177-180.
22. Morrison, D. J., Thompson, A. J., Chu, D., Peet, F. G., Sahota, T. S., and Rink, U. 1984. Characterization of *Armillaria* intersterility groups by isozyme patterns. Pages 2-11 in: Proc. IUFRO Conf. Root and Butt Rots of Forest Trees. 6th. G. A. Kile, ed. CSIRO, Melbourne, Australia.
23. Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.
24. Old, K. M., Moran, G. F., and Bell, J. C. 1984. Isozyme variability among isolates of *Phytophthora cinnamomi* from Australia and Papua New Guinea. Can. J. Bot. 62:2016-2022.
25. Otrosina, W. J. 1985. Influence of age of culture and two extraction methods on yield of enzymes for starch gel electrophoresis of *Fomes annosus* isozymes. (Abstr.) Phytopathology 75:1368.
26. Otrosina, W. J. 1986. Allozyme relationships between isolates of *Fomes annosus* from pine and true fir in California. (Abstr.) Phytopathology 76:1112.
27. Otrosina, W. J., Chase, T. E., Cobb, F. W., Jr., and Taylor, J. W. 1989. Isozyme structure of *Heterobasidion annosum* isolates relating to intersterility genotype. Pages 406-416 in: Proc. IUFRO Conf. Root and Butt Rots of Forest Trees. 7th. D. J. Morrison, ed. Pacific Forest Center, Victoria, British Columbia, Canada.
28. Otrosina, W. J., and Cobb, F. W., Jr. 1987. Analysis of allozymes of three distinct variants of *Verticicladiella wageneri* isolated from conifers in western North America. Phytopathology 77:1360-1363.
29. Royse, D. J., and May, B. 1982. Use of isozyme variation to identify genotypic classes of *Agaricus brunescens*. Mycologia 74:93-102.
30. Rundel, P. W., Gordon, D. T., and Parsons, D. J. 1977. Montane and subalpine vegetation of the Sierra Nevada and Cascade ranges. Pages 560-599 in: Terrestrial Vegetation of California. M. G. Barbour and J. Major, eds. John Wiley & Sons, New York.
31. Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787-792.
32. Smith, R. S. 1984. Root disease caused losses in the commercial coniferous forests of the western United States. USDA For. Serv., Fort Collins, CO. For. Pest Manag. Appl. Grp. Rep. 85-5.
33. Swofford, D. L., and Selander, R. B. 1981. A computer program for the analysis of allelic variation in genetics. Dep. Genet. Dev., Univ. Ill., Urbana.
34. Vilgalys, R. J., and Johnson, J. L. 1987. Extensive genetic divergence associated with speciation in filamentous fungi. Proc. Natl. Acad. Sci. USA 84:2355-2358.
35. Worrall, J. J., Parmeter, J. R., and Cobb, F. W., Jr. 1983. Host specialization of *Heterobasidion annosum*. Phytopathology 73:304-307.
36. Wright, S. 1969. Evolution and Genetics of Populations. Vol. 2. The Theory of Gene Frequencies. University of Chicago Press, Chicago. 511 pp.
37. Zhu, H., Higginbotham, K. O., Dancik, B. P., and Navatril, S. 1988. Intraspecific genetic variability of isozymes in the ectomycorrhizal fungus *Suillus tomentosus*. Can. J. Bot. 66:588-594.