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ISOZYME VARIATION WITHIN AND AMONG HOST-SPECIALIZED VARIETIES OF *LEPTOGRAPHIUM WAGENERI*

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ABSTRACT

Isozyme variation of 76 isolates representing the three taxonomic varieties of *Leptographium wageneri* was studied using starch gel electrophoresis. Of 21 enzymes tested, 10 were polymorphic, having from two to six electromorphs. Only 14 combinations (electrophoretic types) of the 29 electromorphs of the polymorphic enzymes were found, each restricted to a single variety. Within each variety, one electrophoretic type was abundant and broadly distributed, and populations of additional types were geographically isolated or restricted. Ordination of genetic distances (D) among the electrophoretic types revealed three non-overlapping, well separated clusters that corresponded to the three varieties. Gene diversity (H) in each variety was low (0.017 to 0.040), but genetic differentiation between varieties was high, with a coefficient of gene differentiation (G_{ST}) for the species of 0.860. These results suggest that the designation of the three host-specialized, morphologic variants as varieties is justified and suggest the rarity or lack of genetic recombination in nature.

Key Words: gene diversity, *Ophiostoma wageneri*, black-stain root disease, population genetics, electrophoretic types.

Leptographium wageneri (Kendrick) Wingfield is a dematiaceous hyphomycete that causes a unique and serious wilt-type disease of conifers in western North America, black-stain root disease (Cobb, 1988). Three physiologically and morphologically distinct variants of the fungus are specialized to different host species (Harrington and Cobb, 1984). These host-specialized variants have recently been recognized as distinct taxonomic varieties (Harrington and Cobb, 1986, 1987). *Leptographium wageneri* var. *wageneri* is pathogenic to pinyons; variety *ponderosum* (Harrington et Cobb) Harrington et Cobb is primarily specialized to western hard pines and rarely attacks white pines and hemlocks; the variety *pseudotsugae* Harrington et Cobb causes black stain in Douglas-fir and has also been isolated from western hemlock (Harrington and Cobb, 1984).

Cultures of the three varieties grown on agar medium differ slightly in growth rates at 25 C, in pigmentation, in the relative production of conidiophores, and in width of conidiophore apices (Harrington and Cobb, 1987). There has been only a single report of a teleomorph in this species: *Ophiostoma wageneri* (Goheen et Cobb) Harrington was described from perithecia in beetle galleries in roots of a diseased ponderosa pine (Goheen and Cobb, 1978).

Although taxonomy is based on observable differences in morphology, genetic and biochemical techniques have become increasingly important as an adjunct to traditional morphologic studies. Electrophoresis of soluble enzymes is an indirect method of determining genetic differences at enzyme loci and has recently been used to delineate taxa that are morphologically similar or variable (e.g., Bonde et al., 1984; Cruikshank and Pitt, 1987; Micales and Stipes, 1987). Isozyme frequency data have also been useful for identifying factors that affect genetic variation in populations or species. For example, the lack of sexual reproduction (Leung and Williams, 1986; Tooley et al., 1985), environmental homogeneity (Spieth, 1975), and founder effects (Spieth, 1975) have all been correlated with low electrophoretic variability.

In a recent study, Orosina and Cobb (1987) used starch gel electrophoresis of ten enzymes to study isozyme variation among 26 isolates representing the three varieties of *L. wageneri*. Their data suggested low genetic variation in the species, with seven of the ten enzymes having only one electromorphic form. The data from the three polymorphic enzymes supported the concept of three taxonomic varieties.

Genetic variation in *Leptographium wageneri* was further examined in the current study, but

TABLE I

ISOLATES OF *LEPTOGRAPHIUM WAGENERI* ARRANGED BY VARIETY, ELECTROPHORETIC TYPE, AND HOST OF ORIGIN

Variety	Type	Host	Isolates ^a
var. <i>wageneri</i>	A	<i>Pinus monophylla</i>	CAS4 (ATCC 64194), CAS5, CAS7, CAS9
	B	<i>Pinus edulis</i>	COE1 (ATCC 58576), COE2, COE6, COEN, IDE1, NME1 (ATCC 58579), UTE1
		<i>Pinus monophylla</i>	CAS1 (ATCC 64193), CAS2, CAS3, CAS15 (ATCC 64195), NES1 (ATCC 64192), NES2, NES3, NES4
var. <i>pseudotsugae</i>	C	<i>Pseudotsuga menziesii</i>	IDD2, MOD22
	D	<i>Pseudotsuga menziesii</i>	MOD1 (ATCC 58578)
	E	<i>Pseudotsuga menziesii</i>	NMD1
	F	<i>Pseudotsuga menziesii</i>	NMD2
	G	<i>Tsuga heterophylla</i>	BCH1 (ATCC 42953)
	H	<i>Pseudotsuga menziesii</i>	BCD1 (ATCC 58574), BCD11, BCDJ, CAD1, CAD2, CAD5, CAD6, CAD18 (ATCC 64196), CAD19, CAD22, CAD27, CAD30, CAD31, CAD32, CAD40, CAD55, CAD56, CADF, CADX, IDD1, ORD1, ORD2, ORD3, ORD4, ORD5, ORDP
	I	<i>Pseudotsuga menziesii</i>	ORDQ, WADU
	J	<i>Pseudotsuga menziesii</i>	COD2 (ATCC 64191)
var. <i>ponderosum</i>	K	<i>Pinus contorta</i>	BCL1 (ATCC 42954), BCL2, BCL4
		<i>Pinus monticola</i>	BCW1
		<i>Pinus strobus</i>	MOW2
	L	<i>Pinus contorta</i>	BCL3
	M	<i>Pinus jeffreyi</i>	CAJ1, CAJ3
		<i>Pinus ponderosa</i>	CAP3, CAP19 (ATCC 58575), CAP36, CAPC, CAPD, CAPH, CAPI, CAPW, IDP1 (ATCC 58577)
	N	<i>Tsuga mertensiana</i>	ORMS (ATCC 58581)
<i>Pinus contorta</i>		ORL1	
<i>Pinus ponderosa</i>		CAPY, ORP1	
	<i>Tsuga heterophylla</i>	ORH1 (ATCC 58580)	

^a Culture numbers are those used in the collection of T. C. Harrington. The first two letters designate the state or province of origin. Numbers in parentheses are those of the American Type Culture Collection.

ing each electromorph of *L. wageneri* and examples of other fungi in *Leptographium*, *Ophiostoma*, and *Ceratocystis* were included as references. Wicks were removed after 10–15 minutes of electrophoresis at the voltages specified in TABLE II.

After electrophoresis, up to five slices per gel were stained for enzyme activity. A cathodal slice was included along with an anodal slice in stains for enzymes that were determined to have electromorphs with cathodal migration in initial tests (e.g., MDH2).

Several staining procedures had the potential to detect activity of more than one enzyme. In each case, the identification of electrophoretic bands was determined by staining other slices of the same gel using staining procedures specific for activity of the other enzymes that may have been detected. Sets of bands on subsequent slices having the same patterns of electrophoretic mo-

tility were assumed to represent activity of the same enzyme system. Bands on gel slices stained for the two MDH systems were compared in this manner. Slices stained for DIA and MNR were compared with each other and with slices stained for glutathione reductase (EC 1.6.4.2) for the presence of shared banding patterns.

Enzymes were selected that had well-resolved, well-stained bands and an equal number of bands in all isolates (FIG. 2, TABLE II). Electromorphs were determined for each isolate after a comparison of its banding pattern in one or more gels of each of the buffer systems used for the enzyme.

Genetic distances (standard genetic distance D; Nei, 1972, 1983) were calculated between all pairs of isolates regardless of variety and arranged in matrix form. Because of minimal loss of information and the ability to use data without *a priori* classification (Clifford and Stephenson, 1975), ordination of the matrix data by principal

TABLE II

ENZYMES USED IN STARCH GEL ELECTROPHORESIS STUDIES OF *LEPTOGRAPHIUM WAGENERI*, THE NUMBER OF ELECTROMORPHS DETERMINED PER ENZYME, AND BUFFERS AND STAINING PROCEDURES FAVORING RESOLUTION

Enzyme name (EC number) ^a	Enzyme abbreviation ^b	Electro- morphs determined	Buffer system ^c	Stain reference ^d
Aconitase (4.2.1.3)	ACO	2	A, HC7	2
Aspartate aminotransferase (2.6.1.1)	AAT	3	B2, D	2
Catalase (1.11.1.6)	CAT	1	B2	1
Diaphorase (1.8.1.4)	DIA1	1	A	2
	DIA2	5	A, D	2
Esterase (3.1.1.1)	EST	3	D	3
Fumarase (4.2.1.2)	FUM	1	A	2
Glucose-6-phosphate dehydrogenase (1.1.1.49)	G6PD	1	A, B2	2
Glucosephosphate isomerase (5.3.1.9)	GPI	2	A, B	1
β -Glucosidase (3.2.1.21)	β -GLU	6	B, D	2
Glutamate dehydrogenase (1.4.1.3) NADP cofactor	GDH	1	B, B2	2
Isocitrate dehydrogenase (1.1.1.42)	IDH	2	E	2
Leucine aminopeptidase (3.4.11.1)	LAP	1	M	1
Malate dehydrogenase (1.1.1.37) NAD cofactor	MDH1	1	D, E	2
(1.1.1.40) NADP cofactor	MDH2	1	D, E	2
Menadione reductase (1.6.99.2)	MNR1	1	A	1
	MNR2	1	A	1
Peptidase (3.4.13)	PEP	1	A	2
Phosphoglucomutase (5.4.2.2)	PGM	2	B, D	2
Superoxide dismutase (1.15.1.1)	SOD	2	HC7	4
Triose-phosphate isomerase (5.3.1.1)	TPI	2	A	2

^a Nomenclature Committee of the International Union of Biochemistry (1984).

^b Multiple enzyme forms are designated in order of decreasing anodal migration.

^c Buffer systems, electrical requirements, and references: A = pH 8.5/8.1 discontinuous Tris citrate/lithium borate system (RW) using 50 mA constant current until wave front reaches 8 cm, Marty *et al.* (1984); B = pH 5.7 continuous histidine citrate system using 250 V constant voltage for 4.5 h, Shields *et al.* (1983); B2 = pH 8.8/8.0 discontinuous Tris citrate/sodium borate system (B) using 50 mA constant current until wave front reaches 8 cm, Conkle *et al.* (1982); D = pH 6.1 continuous morpholine citrate system using 250 V constant voltage for 5.0 h, Conkle *et al.* (1982); E = buffer D with pH adjusted to 8.1 using morpholine citrate, with same voltage and run time as D, Conkle, unpubl.; HC7 = pH 7.0/7.0 histidine/citrate system (HC) using 250 V constant voltage for 5.0 h, Marty *et al.* (1984); M = pH 8.9 continuous Tris borate EDTA system using 275 V constant voltage for 4.5 h, Micales *et al.* (1986).

^d 1 = Conkle *et al.* (1982). 2 = Marty *et al.* (1984). 3 = Fluorescent esterase stain of Marty *et al.* (1984). 4 = destained SOD bands on blue background; 6-phosphogluconic dehydrogenase stain of Marty *et al.* (1984).

coordinate analysis was used to show genetic distance relationships among all isolates, among isolates of var. *pseudotsugae*, and among isolates of var. *ponderosum* but was not used to show

relationships among isolates of the less variable var. *wageneri*. For each analysis, a scatter plot was constructed using the first three principal coordinates.

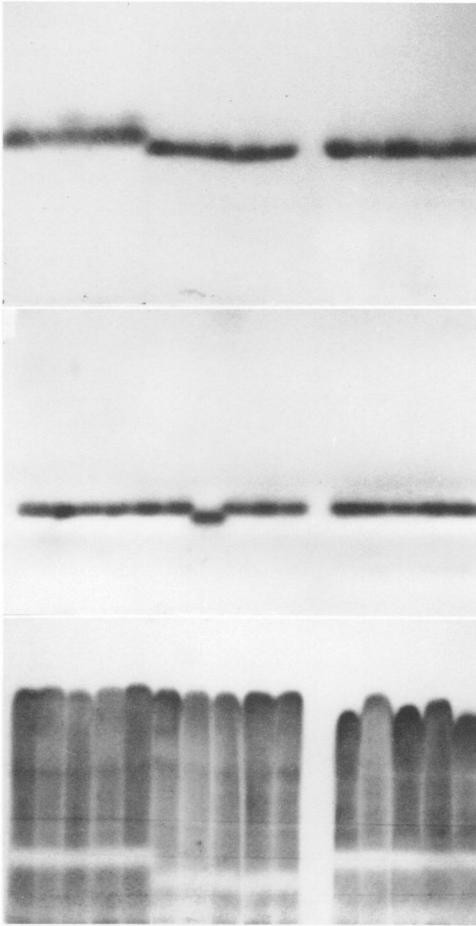


FIG. 2. Electromorphs of *L. wageneri* for aconitase (top), glucosephosphate isomerase (middle), and superoxide dismutase (destained bands, bottom). From left to right, the lanes of each gel are as follows: lanes 1–5 are isolates CAS1, CAS3, CAS4, CAS5, and CAS7 of var. *wageneri*; lanes 6–10 are isolates BCL1, BCL3, BCL4, BCW1 and CAJ1 of var. *ponderosum*; lanes 11–15 are isolates BCD1, BCD11, BCH1, CAD18, and CAD31 of var. *pseudotsugae*.

Genetic distances were also calculated between each pair of varieties, and gene diversity (H ; Nei, 1973) within and between varieties and gene differentiation (G_{ST} ; Nei, 1973; R_{ST} ; Nei and Roychoudhury, 1972) within *L. wageneri* at the level of variety were determined.

Attempts were made to induce the sexual state of *L. wageneri* in order to investigate the genetic inheritance of isozyme variation in this fungus. Isolates representing each of 14 electrophoretic types (*i.e.*, groups of isolates with detectable elec-

trophoretic differences, TABLE III) were paired in all combinations on water agar containing sterile sections of twigs of *Pinus resinosa* Ait., 1.0 ppm thiamine hydrochloride, 0.75 ppm pyridoxine hydrochloride, and 0.05 ppm biotin. Plates were incubated at 18 C and examined for the production of perithecia and protoperithecia at various intervals.

RESULTS

Each of the enzymes from TABLE II yielded dark, well-resolved patterns and showed electrophoretic variation between the isolates of *L. wageneri* and distantly related fungi. In most of the enzymes, only one major enzyme form was indicated, but there were two electrophoretic forms of DIA, MDH and MNR, giving a total of 21 scorable enzymes. Eleven of these had only one detectable electromorph in all isolates of *L. wageneri*. The number of electromorphs in the ten polymorphic enzymes ranged from two to six (TABLES II, III).

Although variation in most enzymes was easy to read (FIG. 2), results from four enzymes needed particular care in interpretation and determination of electromorphs. The enzyme GDH was apparently monomorphic, but had a great deal of variability in activity and darkness of staining between isolates. No GDH activity was detected for isolates CAS9 and CAP19 despite repeated attempts. Although the lack of staining could be interpreted as representing one or possibly two null alleles, we have treated it as missing data. Because of this, allele frequency data from this enzyme could not be used in genetic distance and gene diversity calculation.

A triple banding pattern was found in the enzyme G6PD. The band with the least anodal migration was broader and less resolved than the other two bands in all isolates, but in certain isolates the band was particularly diffuse and slower in migration. These observed differences between isolates in resolution and migration of the third band were eliminated when isolates were grown on a medium with a high glucose content (unpubl. data). We have therefore considered the triple-banded patterns to indicate the product of one monomorphic locus. In contrast, the triple banding pattern has been interpreted by Otrosina and Cobb (1987) to indicate one monomorphic and one polymorphic locus.

The enzyme β -GLU had five electromorphs

TABLE III

ELECTROMORPHS OF THE 14 ELECTROPHORETIC TYPES FOUND FOR 76 ISOLATES OF *LEPTOGRAPHIUM WAGENERI*. ENZYME ELECTROMORPHS^a ARE DISPLAYED IN ROWS, ELECTROPHORETIC TYPES IN COLUMNS ARRANGED ACCORDING TO VARIETY

	Electrophoretic types ^b													
	var. <i>wagneri</i>			var. <i>pseudotsugae</i>							var. <i>ponderosum</i>			
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
ACO	a ^c	a	b	b	b	b	b	b	b	b	b	b	b	b
AAT	a	a	a	a	a	a	b	b	b	b	c	c	c	c
DIA2	d	d	e	c	e	c	e	e	e	e	a	a	b	b
EST	a	a	b	b	b	b	c	b	b	b	b	b	b	b
GPI	a	a	a	a	a	a	a	a	a	a	a	b	a	a
β -GLU	c	c	a	a	a	d	a	a	e	a	b	b	b	n ^d
IDH	b	b	a	a	a	a	a	a	a	a	a	a	a	a
PGM	a	b	a	a	b	b	a	a	a	b	b	b	b	b
SOD	b	b	a	a	a	a	a	a	a	a	b	b	b	b
TPI	b	b	b	b	b	b	b	b	b	a	a	a	a	a

^a All isolates were monomorphic for an additional eleven enzymes: CAT, DIA1, FUM, G6PD, GDH, LAP, MDH1, MDH2, MNR1, MNR2 and PEP.

^b For each electrophoretic type, the number of isolates and their hosts are shown in TABLE I.

^c Electromorphs were designated alphabetically in order of decreasing anodal migration.

^d Electromorph "n" of B-GLU is a non-resolving, low activity electromorph.

that resolved into sharply defined fluorescent bands. However, four isolates of var. *ponderosum* had minimal activity of this enzyme, detectable only as a diffuse background fluorescence (TABLE III). Since the four isolates were from the same geographic area (FIG. 1, top) and were identical for each of the other enzymes, the minimal staining was attributed to a unique electromorph with low activity, giving a total of six electromorphs for β -GLU.

The enzyme AAT, reported by Orosina and Cobb (1987) as having two electromorphs, had three electromorphs that were only revealed when stained slices of two different gel types were compared. On B2 gels, electromorph "b" of AAT migrated at the same rate as electromorph "a." On D gels, however, electromorph "b" migrated slower than electromorph "a" but at the same rate as electromorph "c."

Four additional enzymes resolved after electrophoresis and staining but could not be used in the study. Activity of uridine diphosphoglucose pyrophosphorylase (EC 2.7.7.9) was minimal and could not be detected in many isolates used in our study. Our incomplete data suggest that the enzyme is monomorphic in *L. wagneri*, as previously reported by Orosina and Cobb (1987). The banding pattern of acid phosphatase (EC 3.1.3.2) was also faint and apparently monomorphic. Conversely, the enzymes glutathione

reductase (EC 1.6.4.2) and alcohol dehydrogenase (EC 1.1.1.1) stained well for most isolates but could not be used in this study due to extreme electrophoretic variability, with variation in the number of bands from isolate to isolate.

Successful crosses were not obtained in any of the pairings among *L. wagneri* isolates, so the actual genetic basis of electrophoretic variation in enzymes of *L. wagneri* could not be determined. Since genetic distances, heterozygosity, and gene diversity are based on frequencies of alleles, we have had to assume that each of the 21 enzymes listed in TABLE II are coded for by a different genetic locus, with different electromorphs representing the products of different alleles. This interpretation of the data conforms to results from studies of isozyme variation in unrelated fungi where the genetic basis has been determined (Burdon *et al.*, 1986; Gessner *et al.*, 1987; May and Royse, 1982; Royse *et al.*, 1983; Shattock *et al.*, 1986; Spear *et al.*, 1983) and our own unpublished studies of isozyme variation in the related fungus *Ophiostoma nigrocarpum* (Davids.) deHoog.

Fourteen combinations of electromorphs (electrophoretic types) were detected among the 76 isolates tested (TABLE III). Each type was found in only one variety of *L. wagneri*. Principal coordinate analysis of genetic distances among the 14 electrophoretic types yielded three principal

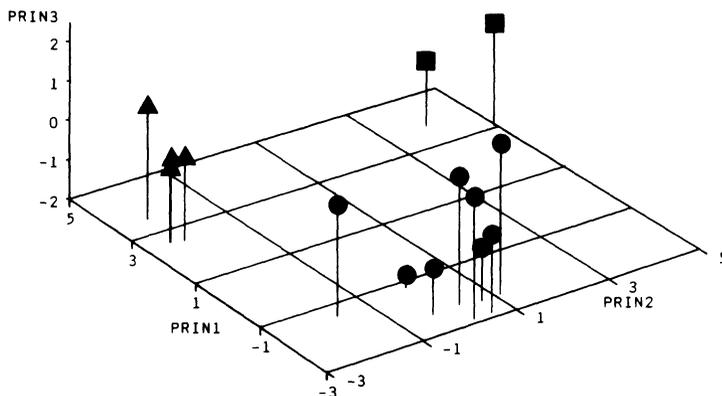


FIG. 3. Ordination in three dimensions of genetic distance relationships among electrophoretic types of the varieties of *L. wagneri*. Axes are the first three principal coordinates of the distance matrix transformed by principal coordinate analysis. Electrophoretic types of var. *wagneri* are represented by squares; var. *pseudotsugae* by circles; and var. *ponderosum* by triangles.

coordinates that contained 96.8 percent of the information of the original 14-variable distance matrix. The three principal coordinates were used to construct a three dimensional scatter plot to depict genetic distance relationships within *L. wagneri*. The scatter plot (FIG. 3) revealed three distinct clusters of electrophoretic types within the species. The clusters did not overlap and no intermediate types were found. The three clusters corresponded to the taxonomic varieties of Harrington and Cobb (1986, 1987).

As shown in FIG. 1, each variety appeared to consist of one common electrophoretic type that was broadly distributed geographically and one or more types that were either geographically isolated from the common type or occurred in a local area within its range. Of the eight types found in variety *pseudotsugae*, H represented 74% of the isolates and had the broadest geographic occurrence. Four types (C, D, G, and I) were of local occurrence and three (E, F, and J) occurred at the edges of the range of the variety. Variety *ponderosum* had four electrophoretic types, with K and L geographically isolated from M and N by the Bitterroot Range of the Rocky Mountains. In variety *wagneri*, electrophoretic type A was only found on a single plateau and differed from the more common type B only at the electromorph for PGM.

The correspondence or lack of correspondence between genetic distances among the electrophoretic types and their geographic distributions can be seen by comparing FIG. 1 with FIGS. 3 and 4.

FIGURE 1 shows considerable overlap between the distributions of the electrophoretic types of var. *pseudotsugae* and those of the other two varieties. Despite this geographic overlap, there are much greater genetic distances among the electrophoretic types of different varieties than within varieties (FIG. 3). Within varieties, however, there is some correspondence between genetic distances and geographic distributions of different electrophoretic types. The first three principal coordinates from principal coordinate analysis of subset genetic distance matrices for vars. *pseudotsugae* and *ponderosum* contained 94.2 and 100 percent of the information of the respective original genetic distance matrices and were used to construct the three dimensional scatter plots shown in FIG. 4. In var. *pseudotsugae*, geographic separation (FIG. 1, middle) and genetic distance (FIG. 4, top) were both small between types I and G, and large between type J and most other types. In other cases the genetic distances were greater (e.g., between E and F) or smaller (e.g., between C and E) than would be expected on the basis of geographic separation alone. In var. *ponderosum*, there were only four electrophoretic types and the variety was more restricted in geographic distribution (FIG. 1, top). There were also fewer isozyme differences (TABLE III) and smaller genetic distances (FIG. 4, bottom) among the types of var. *ponderosum*.

Gene diversity (H) was low within each of the three varieties, ranging from 0.017 to 0.040 (FIG. 5). In contrast, there was a high degree of genetic

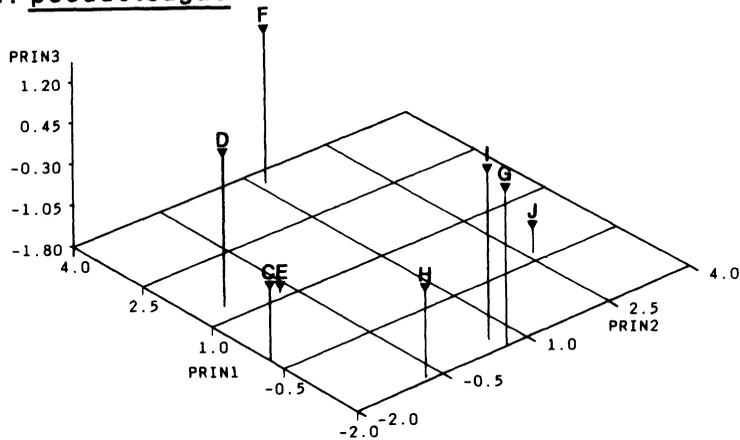
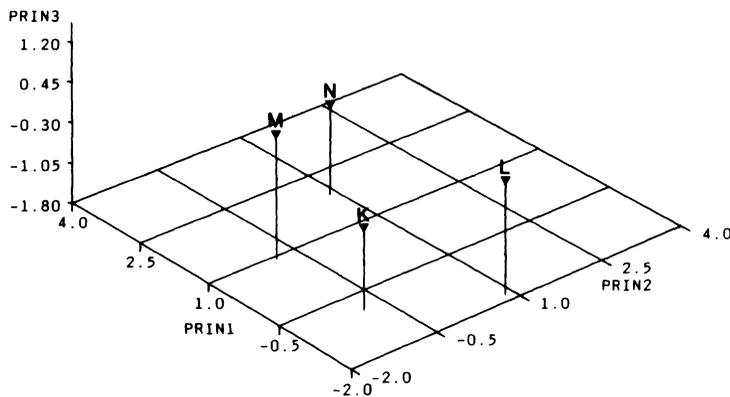
var. pseudotsugaevar. ponderosum

FIG. 4. Ordination in three dimensions of genetic distance relationships within varieties of *L. wagneri*. Top = var. *pseudotsugae*, bottom = var. *ponderosum*.

differentiation among varieties as indicated by large genetic distances (D) between varieties and high coefficients of gene differentiation (R_{ST} and G_{ST}) for the species. The smallest genetic distance (D) calculated between varieties of *L. wagneri* was between varieties *ponderosum* and *pseudotsugae*. This value of 0.257 indicates that an average of nearly 26 electrophoretically detectable substitutions would be found per hundred loci. The interpopulational (inter-variety) gene diversity (R_{ST}) was 9.38, indicating a nine- to tenfold greater amount of gene diversity occurring strictly among varieties versus that occurring within varieties. Similarly, the coefficient of gene differentiation (G_{ST}) was 0.860, indicating that

86% of the total gene diversity in the species was due to differences among varieties.

DISCUSSION

Two types of analysis of the electrophoretic data (*i.e.*, analysis of electrophoretic types separately and analysis of combined data from the types of each variety) strongly supported the division of the species into three taxa. The ordination of genetic distance relationships among the electrophoretic types eliminated bias in assigning isolates to taxa prior to analysis and confirmed the lack of significant genetic intermediates between the varieties. Further analysis of pooled data at the varietal level gave estimates

that may be assumed to be affected by the presence or absence of recombination in the fungus. The magnitude of genetic distances between pairs of closely related species has been suggested to depend in part on the speed with which reproductive isolation mechanisms have developed and their efficacy during the process of speciation (Ayala, 1975). If this is true, the large genetic distances among varieties of *L. wagneri* may indicate the early and effective operation of reproductive isolation. If one considers reproduction by strictly asexual means to be an extreme form of genetic isolation, the data may be interpreted as supporting the idea that the teleomorph is rare in nature.

The low number of electrophoretic types and the low gene diversity within varieties of *L. wagneri* also suggest a rarity or lack of sexual reproduction. Similarly, Roelfs and Groth (1980) found that asexual populations of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn. have far fewer virulence phenotypes (combinations of virulence traits) than do sexual populations, and the distribution of such phenotypes was extremely non-random. Genetic diversity has also been reported to be lower in asexual populations of *Phytophthora infestans* (Mont.) de Bary (Tooley *et al.*, 1985) and *Magnaportha grisea* (Hebert) Barr (Leung and Williams, 1986) than in populations of the same species in which sexual reproduction occurs.

Finally, the widespread occurrence of some of the electrophoretic types of *L. wagneri* can also be interpreted as evidence for the lack of sexual recombination in this fungus. Although selection acts on the level of the allele or allelic combination in an organism with sexual recombination, the genome may be the unit of selection in strictly asexual organisms, resulting in competition among clones (Crow and Kimura, 1965). Widespread occurrence of a very few electrophoretic types and geographic isolation of others, as was found in the varieties of *L. wagneri*, has also been found in the asexual pathogen *Alternaria mali* Roberts (Hwang *et al.*, 1987) and in asexual populations of *Magnaportha grisea* (Leung and Williams, 1986).

It has been suggested that the development of a strictly asexual mode of reproduction may not be detrimental to a population or species if progeny are: 1) consistently dispersed into an environment similar to that of the parent; 2) if there is intense selection pressure; and 3) if popula-

tions are very small (Maynard Smith, 1971). An examination of the life cycle of *L. wagneri* reveals that this species may meet all three of these criteria.

Leptographium wagneri occurs in nature in discrete infection centers. Initial infections are established as root-feeding bark beetles transport the fungus to new areas (Cobb, 1988; Harrington *et al.*, 1985; Witcosky *et al.*, 1986). The pathogen/vector relationship is not strong, however, making the establishment of a new infection center a rare event—especially establishment of infection centers in distant areas. Spread within an infection center is primarily by limited growth of mycelium through soil between infected and uninfected roots, or more rarely, by direct root contact (Cobb, 1988). Aside from brief periods of movement between roots or as spores carried on the exoskeletons of insect vectors, *L. wagneri* exists only in the xylem of living or recently killed trees. Thus, the hosts of *L. wagneri* may provide a relatively uniform and protected environment for the fungus from generation to generation, meeting Maynard Smith's first criterion. Secondly, the host specialization of the three varieties suggests that host-pathogen interactions exert strong selective pressure on the fungus. Since genetic differences are immediately expressed in organisms with a predominant haploid stage in the life cycle (*e.g.*, Deuteromycotina and most Ascomycotina), selection pressure could be expected to be particularly intense. Thirdly, the dependence of the fungus on an uncertain vector provides that population size is at least intermittently low. Although *L. wagneri* infection centers covering several hectares may develop through root-to-root spread without the aid of vectors (Cobb *et al.*, 1982), the founding of each new infection center could be seen as a genetic bottleneck and a great reduction in effective population size.

In spite of the limited variation found in *L. wagneri*, it is possible that the fungus is a sexually competent, heterothallic species with an uneven distribution of mating types. Because of the low probability of establishing infection centers, there may be a high probability of losing one mating type during establishment of any infection center. Mating types could become geographically isolated in this way, with rare sexual recombination limited to regions of contact between infection centers carrying the complementary mating types. However, the lack of identi-

fiable regions of high variability and the lack of ability of paired isolates from different locations and/or different electrophoretic types to form perithecia in culture argue against the possibility. If the suggested scenario of predominantly asexual reproduction is accurate, it would be more likely that an organism would lose the ability to express loci for unused sexual capabilities over time through either random fixation of mutations or through selection of asexual strains without the "cost of sex" (Maynard Smith, 1971) associated with maintaining active alleles at those loci.

In conclusion, the results of isozyme study in *Leptographium wageneri* have supported the division of the species into three taxonomic varieties and suggest that sexual recombination is rare or lacking in this fungus.

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