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Ophiostoma clavigerum is the mycangial fungus of the Jeffrey pine beetle, *Dendroctonus jeffreyi*

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Abstract: *Dendroctonus jeffreyi* and *D. ponderosae* are sibling species of bark beetles (Coleoptera: Scolytidae) with few morphological and molecular genetic differences. The two species are believed to have diverged relatively recently. *Dendroctonus jeffreyi* colonizes only *Pinus jeffreyi*, while *D. ponderosae* colonizes up to thirteen *Pinus* spp., but not *P. jeffreyi*. Adult beetles of both *D. jeffreyi* and *D. ponderosae* carry symbiotic fungi in mycangia located on the maxillary cardines. *Dendroctonus ponderosae* was known to carry two fungi, *Ophiostoma clavigerum* and *O. montium*, in its mycangia. However, it was not known which fungi might be carried by *D. jeffreyi*. Fungi were isolated from the mycangia of over 900 *D. jeffreyi* collected from a large portion of its geographic range. Using morphology, isozyme phenotypes, and growth rates at different temperatures, all isolates from *D. jeffreyi* mycangia were determined to be *O. clavigerum*; *O. montium* was never isolated from *D. jeffreyi* mycangia.

Key Words: *Ophiostoma montium*, *Dendroctonus ponderosae*, bark beetles, mycangia, isozymes, Coleoptera, Scolytidae

INTRODUCTION

The Jeffrey pine beetle, *Dendroctonus jeffreyi* Hopkins, is a bark beetle exclusively attacking *Pinus jeffreyi* Grev. & Balf (Wood, 1982). Its geographic range is roughly that of its host tree, extending from the southern Oregon/northern California border to northern Baja California (Wood, 1982). The mountain pine beetle, *Dendroctonus ponderosae*, a polyphagous species utilizing up to thirteen *Pinus* spp. but not *P. jeffreyi*, and *D. jeffreyi* are considered sibling species due to their almost-identical morphologies and indistinguishable gallery systems (Higby and

Stock, 1982; Wood, 1982). The beetles are sympatric over portions of their range (Higby and Stock, 1982; Wood, 1982). Genetic evidence suggests that the Jeffrey pine beetle and the mountain pine beetle have diverged relatively recently from a common ancestor (Higby and Stock, 1982). Physiological tolerances of the two beetles to resin chemical constituents of their respective host tree species also support the designation of these two beetles as separate species (Smith, 1963; 1965; Lanier and Wood, 1968).

In both species, adults of both sexes carry symbiotic fungi in mycangia located on the maxillary cardines (Whitney and Farris, 1970; T. D. P., personal observation). Mycangia are structures of the integument specialized for carrying fungi (Batra, 1963; Francke-Grosmann, 1967; Barras and Perry, 1971; Happ et al., 1971). Although bark beetles and the related ambrosia beetles are frequently associated with a range of fungi passively disseminated on the exoskeleton, more specific associations are maintained between mycangia and the fungi associated with them.

The relationship between bark beetles and mycangial fungi has long been assumed to be mutualistic due to the presence of mycangia and the prevalence of these associations in Scolytidae. The fungus clearly benefits through consistent transport to an ephemeral and relatively uncommon resource, the phloem of dying or newly-killed trees. The benefit(s) gained by the beetle are less clear. It has been suggested that mycangial fungi may reduce the impact of antagonistic blue-staining fungi (Barras, 1970; Franklin, 1970; Whitney, 1971; Whitney and Cobb, 1972, but see Ross et al., 1992), assist the beetles in overcoming the defenses of the host tree (Berryman, 1972), alter the chemical or moisture composition of the phloem (Nelson, 1934; Reid, 1961; Webb and Franklin, 1978; Wagner et al. 1979), or provide nutrients or some other factor essential for the insect's development and reproduction (Barras, 1973; Bridges, 1983; Goldammer et al., 1990).

Dendroctonus ponderosae is known to carry two mycangial fungi, *Ophiostoma clavigerum* (Robinson-Jeffrey & Davidson) Harrington and *O. montium* (Rumbold) von Arx (Whitney and Farris, 1970). The mycangial fungus carried by *D. jeffreyi* has not been identified previously, but a morphological similarity

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to *O. clavigerum* has been noted (Paine and Hanlon, 1994). Significant differences in tolerances to various host tree resin components were found between *O. clavigerum* carried by *D. ponderosae* and the morphologically similar fungus carried by *D. jeffreyi* (Paine and Hanlon, 1994). The chemical environments presented by the host trees of the two beetles differ substantially, and the fungi may have been selected for different tolerances. *Pinus jeffreyi*, has n-heptane as its main resin component, while hosts of *D. ponderosae* have monoterpenes and resin acids as major resin components (Mirov, 1929; Smith, 1967; Anderson et al., 1969).

Fungi, in general, are morphologically simple organisms, and as such, morphological differences may be slight to nonexistent between related species even long after an initial speciation event occurs (Brasier, 1986). Although the chemical environments of the mycangial fungi in the host tree differ, the physical environments within the host tree's tissues are similar. Also, both fungi apparently remain dependent upon the beetles for dispersal. Therefore, selection pressure for morphological change may not be strong, and indeed, selection may act to conserve extant structures (e.g., arthropod disseminated propagules) while at the same time acting upon physiological traits that affect survival and exploitation of the chemical environment of the tree.

There were two objectives for this study. First, we wished to identify the fungus or fungi carried in the mycangia of *D. jeffreyi*. Many species of *Dendroctonus* including *D. ponderosae*, are known to carry more than one species of mycangial fungus (Whitney and Farris, 1970; Barras and Perry, 1972; Bridges, 1983; Paine and Birch, 1983). Therefore, although isolations from several beetles from a single population yielded a fungus morphologically similar to *O. clavigerum* (T. D. P., unpublished data), it was not known if this was the only fungus carried by *D. jeffreyi* across its entire geographic range. Second, we wished to compare *O. clavigerum* (carried by *D. ponderosae*) with the morphologically similar fungus carried by *D. jeffreyi* using morphology, isozymes, and temperature tolerances to determine whether these fungal isolates have remained conspecific or have diverged along with their host beetles. Isolates of *O. montium* isolated from *D. ponderosae* mycangia were also included in the study to assess its relatedness to the other two mycangial fungi.

MATERIALS AND METHODS

Collections and isolations.—*Dendroctonus jeffreyi* adults were collected at ten sites, and *D. ponderosae*, at two sites from California. Additional *D. ponderosae* were

collected from bark and from pheromone traps in Sawtooth National Recreation Area, Idaho (Barbara Bentz, USDA Forest Service, Logan, Utah). Brood adults, just prior to emergence, were collected from the bark of infested trees by removing bark from the entire circumference of the trunk of trees from near soil level to a height of ca. 2 m. Beetles were collected from as many broods and trees as possible per site in order to gain a representative sample of mycangial fungi. Only at one site were beetles taken from a single tree (Indiana Summit). Live beetles were held individually in microcentrifuge tubes on ice and transported to the laboratory.

In the laboratory, each beetle was decapitated. The head was dissected to remove the maxillary cardines, which contained the fungus-bearing mycangia. The cardines were surface sterilized for two min in modified White's solution (Barras, 1970) and submerged into malt agar and *Ophiostoma* selective agar (malt agar with 100 ppm cycloheximide and 100 ppm streptomycin) (Harrington, 1981). Once the mycangial fungi began to grow out from the mycangia, a plug of agar containing the fungus was removed from the growing edge and placed onto 2% water agar to facilitate hyphal tipping. Fungal mycelia grow sparsely on water agar allowing hyphal tip cultures to be made with relative ease. After a few days growth on water agar, hyphal tips were taken from each subculture and placed on malt agar.

Additional strains of *O. clavigerum* (TABLE I) were acquired from a vouchered collection (T. C. Harrington, Department of Plant Pathology, Iowa State University, Ames, IA).

Morphological comparisons.—Conidiophores were taken from 2–3-wk old cultures of all mycangial isolates, mounted on glass microscope slides in lactophenol, visually inspected through a compound microscope (200X), and scored for the presence of the large, clavate, septate conidia typical of *O. clavigerum* or the smaller unicellular, globular, truncate conidia of *O. montium*. Ten strains each of *O. clavigerum* (from *D. ponderosae*) and the *D. jeffreyi* mycangial fungus were used for comparisons of morphological characters. For each fungus type, at least one isolate was taken directly from a beetle pupal chamber by removing a small clump of conidiophores with sterile forceps which were then placed onto *Ophiostoma* selective media. Conidiophores from the same pupal chamber were also placed directly on microscope slides, mounted in lactophenol, and used in morphometric comparisons. Nine additional mycangial isolates of each fungus were grown both on malt agar and phloem agar (autoclaved Jeffrey pine phloem chips in 2% agar). All isolates used in morphological

TABLE I. Host tree species, substrate, and location of origin of isolates of *Ophiostoma montium*, *O. clavigerum* from *D. ponderosae*, and the *D. jeffreyi* mycangial fungus used in isozyme electrophoresis and in determining radial growth rates at different temperatures

	Isolate	Host tree	Substrate	Location
<i>Ophiostoma montium</i>	15	<i>Pinus ponderosa</i>	Pupal chamber	Poison Lake, Lassen Nat'l Forest, CA
	49	<i>P. ponderosa</i>	Mycangium	Poison Lake, Lassen Nat'l Forest, CA
	55	<i>P. ponderosa</i>	Mycangium	Poison Lake, Lassen Nat'l Forest, CA
	57	<i>P. lambertiana</i>	Mycangium	Bogard Buttes, Lassen Nat'l Forest, CA
	69	<i>P. lambertiana</i>	Pupal chamber	Bogard Buttes, Lassen Nat'l Forest, CA
	84	<i>P. lambertiana</i>	Mycangium	Bogard Buttes, Lassen Nat'l Forest, CA
	934	<i>P. contorta</i>	Mycangium	Sawtooth Nat'l Recreation Area, ID
	937	<i>P. contorta</i>	Mycangium	Sawtooth Nat'l Recreation Area, ID
	940	<i>P. contorta</i>	Mycangium	Sawtooth Nat'l Recreation Area, ID
	941	<i>P. contorta</i>	Mycangium	Sawtooth Nat'l Recreation Area, ID
<i>O. clavigerum</i>	998	<i>P. contorta</i>	Pupal chamber	Deadman Summit, Toiyabe Nat'l Forest, CA
	999	<i>P. contorta</i>	Mycangium	Deadman Summit, Toiyabe Nat'l Forest, CA
	1049	<i>P. contorta</i>	Mycangium	Truckee, Tahoe Nat'l Forest, CA
	C295 ^a	<i>P. contorta</i>	<i>D. ponderosae</i>	Invermero, Br. Columbia, Canada
	C291	<i>P. contorta</i>	<i>D. ponderosae</i> gallery	West Castle, Br. Columbia, Canada
	C294	<i>P. contorta</i>	From tree	Carbondale, Alberta, Canada
	C293	<i>P. contorta</i>	<i>D. ponderosae</i> gallery	Bally River, Alberta, Canada
	C292	<i>P. contorta</i>	<i>D. ponderosae</i> gallery	Carbondale, Alberta, Canada
	C132	<i>Pinus</i> sp.	Assc'd <i>D. ponderosae</i>	California
	C86	<i>P. contorta</i>	Unknown	Teton Nat'l Forest, WY
<i>D. jeffreyi</i> mycangial fungus	35A	<i>P. jeffreyi</i>	Mycangium	Coon Creek, San Bernardino Mtns, CA
	39A	<i>P. jeffreyi</i>	Mycangium	Fish Creek, San Bernardino Mtns, CA
	71	<i>P. jeffreyi</i>	Pupal chamber	Butte Creek, Lassen Nat'l Forest, CA
	83	<i>P. jeffreyi</i>	Mycangium	Cone Lake, Lassen Nat'l Forest, CA
	840	<i>P. jeffreyi</i>	Mycangium	Smokey Bear Flats, Toiyabe Nat'l Forest, CA
	841	<i>P. jeffreyi</i>	Mycangium	Deadman Summit, Toiyabe Nat'l Forest, CA
	842	<i>P. jeffreyi</i>	Mycangium	Bell Meadows, Stanislaus Nat'l Forest, CA
	844	<i>P. jeffreyi</i>	Mycangium	Monitor Pass, Toiyabe Nat'l Forest, CA
	884	<i>P. jeffreyi</i>	Mycangium	Monitor Pass, Toiyabe Nat'l Forest, CA
	885	<i>P. jeffreyi</i>	Mycangium	Greenhorn Summit, Sequoia Nat'l Forest, CA

^a Isolates beginning with C are from the culture collection of T. C. Harrington. All others are from the collection of D. L. Six.

comparisons were grown in darkness at room temperature (ca. 21 C). The origin of each isolate used in morphological comparisons is listed in TABLE I. Isolates 884 and 35A have been deposited in the American Type Culture Collection (Rockville, MD).

Morphological characters of conidiophores produced on artificial media as well as of those taken from pupal chambers were measured or noted for each fungus. These characteristics were: color of conidiophore, number of septations of conidiophore, width at base and apex of conidiophore, number and width of primary metulae, length of conidiogenous apparatus, and type of conidial proliferation. Conidia

were inspected for color, shape, size, and presence and number of septations.

The same cultures used for morphological analysis, as well as ten cultures of *O. montium*, were also grown on malt agar at 21 C in darkness and observed for differences in cultural appearance, growth rate, and conidiophore production.

Isozyme electrophoresis.—The isolates of *O. montium*, *O. clavigerum*, and the *D. jeffreyi* mycangial fungus used in morphological and/or cultural comparisons were also used in horizontal starch gel electrophoresis to compare isozyme phenotypes of the three fungi.

TABLE II. Enzymes, buffers, and staining procedures used in starch gel electrophoresis of *Ophiostoma montium*, *O. clavigerum*, and the *D. jeffreyi* mycangial fungus

Enzyme (EC number) ^a	Abbreviation ^b	Buffer systems ^c	Stain references ^d
Aconitase (4.2.1.3)	ACO	A ₁	1
Aspartate aminotransferase (2.6.1.1)	AAT	D ₂	1
Glucose-6-phosphate dehydrogenase (1.1.1.49)	G6PD	A	1
Glucosephosphate isomerase (5.3.1.9)	GPI	D	1
Isocitrate dehydrogenase (1.1.1.42)	IDH	E ₃	1
Leucine aminopeptidase (3.4.11.1)	LAP1,2	M ₄	2
Malate dehydrogenase (1.1.1.37)	MDH1,2	D, E	2
Menadione reductase (1.6.99.2)	MNR1,2,3	A	1
Peptidase (3.4.13.1)	PEP	A	3 ^c

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

^b Multiple enzymes designated in order of decreasing anodal migration.

^c A = Discontinuous TRIS citrate/lithium borate system (Marty et al., 1984). D = Continuous morpholine citrate system (Conkle et al., 1982). E = D with pH 8.1 adjusted with morpholine citrate (Conkle unpubl. in Zambino and Harrington, 1989). M = Continuous TRIS borate EDTA system (Micales et al., 1986).

^d 1 = Conkle et al. (1982). 2 = Marty et al. (1984).

^c Filter paper overlay method (Vallejos, 1983).

Mycelium for enzyme extraction was obtained by placing agar plugs taken from growing cultures of each isolate in 30 mL liquid medium (20 mg malt extract, 1 mg yeast extract/mL) in 125 mL Erlenmeyer flasks for 14 d at 21 C (Zambino and Harrington, 1992). Enzymes were extracted by vacuum drying the fungal mats, grinding the mats in liquid nitrogen to a fine powder, and then grinding the powder in extraction buffer (Zambino and Harrington, 1989). The resulting liquid extract was absorbed onto 4 mm #1 Whatman filter paper wicks (Whatman International, Ltd., Kent, England) and loaded onto 10% horizontal starch gels (Sigma starch, Sigma Chemical Co., St. Louis, MO) along with reference wicks at two positions. Reference wicks were made from homogenates of a single isolate of *O. clavigerum* isolated from a mycangium of an individual *D. jeffreyi* collected at Deadman Summit, Toiyabe National Forest, Mono Co., California. Following electrophoresis, gels were sliced horizontally and stained for enzyme activity. Buffer systems and staining procedure references are listed in TABLE II. Nine enzymes exhibiting 13 putative gene loci that resolved well were selected for use (TABLE II). Different electromorphs were assumed to be the result of different alleles.

Growth rates at different temperatures.—Growth rates of the three fungi were assessed using the same isolates used in electrophoresis. Isolates were inoculated centrally in petri dishes containing malt agar using 4 mm plugs of agar colonized by actively growing cultures. One replicate plate of each isolate was placed in the dark at each of five temperatures (5, 16, 21, 27, and 32 C). Colony diameters were measured at 2-, 4-, 6-, and 10-d intervals. All isolates were inocu-

lated onto plates on the same day and then grown and measured over the same 10-d period.

Production of the teleomorph.—While *O. clavigerum* and *O. montium* are known to produce a sexual stage, the teleomorph of the *D. jeffreyi* mycangial fungus has not been observed. Production of the teleomorph was attempted by pairing several sympatric and allopatric isolates from *D. jeffreyi* mycangia on slants of phloem agar. Twenty-six pairs were placed in darkness at room temperature (ca. 21 C) and checked periodically over 3 mo for the production of ascomata.

Statistical analyses.—Morphological characters of the *O. clavigerum* and *D. jeffreyi* anamorphs were compared using t-tests. Growth rates among *O. montium*, *O. clavigerum*, and the *D. jeffreyi* mycangial fungus at five temperatures were compared using one-way analysis of variance (ANOVA). Mean separations were done using Sheffe's F-test. All statistical procedures were conducted using StatView statistical software (Abacus Concepts, 1991).

RESULTS

Morphological comparisons.—Both *O. clavigerum* and the 938 isolates from *D. jeffreyi* mycangial fungus produced two distinct conidial types; small obovate, cylindrical to ellipsoidal, amero-spores when cultures were young, and large septate or aseptate broadly fusiform clavate conidia typical of *O. clavigerum* when cultures were at least 2 wk old, as well as many intermediate forms. *Ophiostoma montium* was never isolated from *D. jeffreyi* mycangia. Conidiophores taken

TABLE III. Morphometric comparisons [N = 50, mean μm (SD)] of *Ophiostoma clavigerum* and the *Dendroctonus jeffreyi* mycangial fungus

Character	<i>O. clavigerum</i>	<i>D. jeffreyi</i> mycangial fungus	Range from descriptions of <i>O. clavigerum</i> ^a	
			A	B
Length of conidiophore incl. conidiogenous apparatus	337.6 (86.8)	620.0 (273.9) ^b	up to 1040	100–300
Width of stipe (base of conidiogenous apparatus)	8.2 (1.6)	7.9 (1.8)	6.0–10.5	
Width of stipe (at site of attachment to stolon)	7.4 (1.6)	7.5 (1.6)	6.0–7.5	5–12
Number of septations of stipe	4.5 (2.1)	7.5 (3.2) ^b	3–5	
Number of primary metulae	2.3 (0.5)	2.1 (0.3)	2–3	
Width of primary metulae	5.6 (1.3)	5.8 (1.1)	4–6	
Width of small conidia	3.0 (0.7)	3.2 (1.0)	up to 3.8	1–2.5
Length of small conidia	7.8 (2.8)	7.8 (3.1)	up to 12.6	2–4
Width of large clavate conidia	3.9 (0.4)	4.4 (0.9) ^b	4.2–5.6	4–6
Length of large clavate conidia	32.5 (12.1)	33.4 (10.9)	35–68	12.5–62.5
Number of septations of large clavate conidia	1.3 (1.5)	0.84 (1.21)		0–7

^a Column A from Robinson-Jeffrey and Davidson (1968); column B from Upadhyay (1981).

^b Size significantly different ($P < 0.05$), *t*-test.

from pupal chambers exhibited both the monone-matous *Leptographium* and synnematus *Graphium* anamorphs, however, *Graphium* anamorphs were rare. Only monone-matous forms were produced in culture. Conidiophores removed from pupal chambers and those from cultures grown on phloem agar were more complex than those produced on malt agar. The use of artificial media and repeated sub-culturing has been found to quickly decrease the complexity of the anamorphs of *O. clavigerum* (Tsuneda and Hiratsuka, 1984). Therefore, isolates grown on phloem agar were used for morphological analyses.

Morphological comparisons of the two fungi are summarized in TABLE III. Conidiophores of both fungi were similar in appearance and in number and size of primary metulae. Both fungi produced brown conidiophores which graded into hyaline metulae and conidiogenous cells and produced conidia by annelidic proliferation. Some conidiophores appeared to produce sympodulate conidia, however, as only light microscopy was used, this could not be determined with certainty. The *t*-tests comparing measurements of conidia and conidiophores of the two fungi showed no significant differences between the fungi for most characteristics; however, length of conidiophore (including the conidiogenous apparatus), number of septations of the stipe, and width of large conidia were significantly different. The significant difference in number of conidiophore septations is due to the greater length of the *D. jeffreyi* fungus' conidiophores. Despite differences found in these comparisons, all measurements, including width of conidia and length of conidiophore for both fungi, agreed closely with formal descriptions of *O. clavi-*

gerum associated with *D. ponderosae* (Robinson-Jeffrey and Davidson, 1968; Upadhyay, 1981) (TABLE III).

Cultural characteristics.—Cultural appearances of *O. clavigerum* and the *D. jeffreyi* mycangial fungus were very similar. Both fungi were at first hyaline but soon (after ca. 4 d) became gray to dark brown and then finally black. When held at 21 C, both fungi grew to cover petri dishes (80 × 15 mm) within 7–9 d. *Ophiostoma montium* cultures were more brownish, slower to darken (after ca. 6 d), and produced copious whitish-gray, sterile, felty, aerial mycelium over dark, closely appressed, or submersed mycelium. *Ophiostoma montium* was also very slow to produce conidiophores in culture (2–4 wk) while *O. clavigerum* and the mycangial fungus of *D. jeffreyi* produced profuse conidiophores within just a few days. Conidial masses on cultures were yellowish to whitish-gray and conspicuous for all three fungi.

Production of the teleomorph.—No paired cultures of the *D. jeffreyi* mycangial fungus placed on phloem agar slants produced ascomata.

Isozyme electrophoresis.—Most of the thirteen loci assayed resolved well and were easily interpreted. Polymorphism was very low in the fungi. Of the twelve loci assayed, only MDH2 and PEP were polymorphic in *O. clavigerum* and the *D. jeffreyi* mycangial fungus, and AAT, IDH, and PEP in *O. montium* (TABLE IV). *Ophiostoma clavigerum* and the mycangial fungus of *D. jeffreyi* shared alleles at all loci assayed and could not be distinguished with the enzyme systems used in this study. *Ophiostoma montium*, however, shared alleles with *O. clavigerum* and the mycangial fungus of *D. jeffreyi* at only six of thirteen loci (TABLE IV).

TABLE IV. Isozyme phenotypes of *Ophiostoma montium*, *O. clavigerum*, and the *D. jeffreyi* mycangial fungus

Locus	<i>O. montium</i>	<i>O. clavigerum</i>	<i>D. jeffreyi</i> mycangial fungus
AAT	B,C,D _a	A	A
ACO	A	B	B
G-6PD	A	A	A
GPI	A	B	B
IDH	B,C	A	A
LAP1	A	A	A
LAP2	A	—	—
MDH1	A	B	B
MDH2	A	B,C	B,C
MNR1	A	A	A
MNR2	A	A	A
MNR3	A	A	A
PEP	A,B,C	A,B,C	A,B,C

^a Electromorphs are designated alphabetically with the most anodally migrating electromorph designated A for each enzyme.

While *O. clavigerum* and the *D. jeffreyi* mycangial fungus exhibited only one band for LAP, *O. montium* exhibited two bands. Because hyphal tip cultures of haploid mycelia of the fungi were used in all preparations, the two bands observed for LAP in *O. montium* could not be due to the presence of heterozygotes. Therefore, the two bands are believed to be products of two loci, with one locus not resolving for *O. clavigerum* and the *D. jeffreyi* mycangial fungus, perhaps due to fixation for a null allele.

All three fungi initially appeared to be monomorphic at the MDH loci. Differences were revealed when different buffer systems were used. With the D buffer system (TABLE II), a cathodally migrating electromorph for MDH2 appeared to be monomorphic and present in all *O. montium* isolates as well as present in three *D. jeffreyi* mycangial fungus isolates. However, when the E buffer system (TABLE II) was used, the enzyme bands of the *D. jeffreyi* mycangial fungus and *O. clavigerum* separated and migrated different distances anodally than did the bands resolving for *O. montium*. With MDH1, a similar phenomenon was observed. Using the E buffer, this locus appeared monomorphic for all three fungi. However, using the D buffer system, two electromorphs were revealed; one for *O. clavigerum* and the *D. jeffreyi* mycangial fungus, and one for *O. montium*.

Growth rates at different temperatures.—Growth rates for the three fungi held at five temperatures are summarized in FIG. 1. Growth rates for *O. clavigerum* and the *D. jeffreyi* mycangial fungus were similar at most temperatures tested over the 6-d period, except on

FIG. 1. Radial growth (mm) of *Ophiostoma clavigerum* associated with *Dendroctonus ponderosae*, *O. clavigerum* associated with *D. jeffreyi*, and *O. montium* at 5, 16, 21, 27, and 32 C. A. After 2 days. B. After 4 days. C. After 6 days. At 45 mm, isolates had filled the plates and no further measurements were possible.

the sixth day, the *D. jeffreyi* mycangial fungus grew significantly faster at 27 C than did *O. clavigerum*. At the lower temperatures tested (5, 16, and 21 C), few differences in growth rate among the three fungi occurred. The most striking differences in growth rate occurred at 32 C where *O. clavigerum* and the *D. jeffreyi* mycangial fungus grew only poorly or not at all, while *O. montium* grew almost as well as at 27 C. *Ophiostoma clavigerum* and the *D. jeffreyi* mycangial fungus growth rates were also significantly depressed below those of *O. montium* at 27 C.

DISCUSSION

Our results indicate that the mycangial fungus of *D. jeffreyi* is *O. clavigerum*. Because the sibling species of

this beetle also carries *O. clavigerum*, this indicates that the fungi did not diverge as the host beetles diverged from a common ancestor and began to exploit different host trees. The differences in resin component tolerances of the two fungi may be due to relatively small changes in the genome (at even a single locus) which allow different physiological responses to host tree chemicals, but that are not detectable using isozymes, which mostly resolve neutral variation in the genome and not variation due to selection. Although Paine and Hanlon (1994) demonstrated that there were different physiological tolerances to host resin constituents between *O. clavigerum* and the mycangial fungus of *D. jeffreyi*, only one isolate of each fungus was used in the trials. It is possible that there is wide variation in tolerances among isolates from many populations that was not captured in that study. Although low levels of genetic diversity were observed among strains in the isozyme assays presented here, the question of variation among strains in physiological response to host characters remains unresolved.

While the fungi appear to be isolated in different host trees by the behavior of their beetle hosts, this may not actually be the case. *Ophiostoma* spp. produce propagules well-adapted for arthropod dispersal (Malloch and Blackwell, 1993). Numerous other insects, including predators, secondary bark beetles, and saprophagous species, also inhabit trees killed by these primary bark beetles. These other subcortical coinhabitants may occasionally transport conidia or ascospores among the host trees of these two beetles where they are sympatric. Indeed, with some mycangial fungi, it has been shown that while beetles carry the asexual form of the fungus, the sexual ascospores may be carried by phoretic mites (Moser et al., 1995). However, it is unknown if ascospores of the mycangial fungi of *D. jeffreyi* and *D. ponderosae* are dispersed in a similar manner.

If the mycangial fungi are occasionally dispersed among host trees of both beetles and are capable of colonizing these hosts, then gene flow may occur either through sexual recombination or by the subsequent dispersal of clones by the beetles. If the fungi have remained sexually compatible, divergence may not occur or may be substantially slowed. Even very low amounts of gene flow can prevent the divergence of semi-isolated populations (Hartl, 1988).

In this study, using a limited number of pairings, we were unable to produce the teleomorph of the *D. jeffreyi* mycangial fungus in culture. We have also never observed the sexual state in beetle-colonized trees. However, we cannot conclude that the sexual state does not exist in this fungus. The sexual states of many fungi are difficult to produce in culture, and

as the ascomata of *Ophiostoma* spp. are small, they may have been overlooked in natural situations.

The fungi in this study all exhibited a high degree of monomorphism. Low levels of polymorphism can indicate that sexual recombination is not common (Zambino and Harrington, 1989; Zambino and Harrington, 1992; Harrington et al., 1996). It is possible that *D. jeffreyi* is associated with an asexual lineage that originated from the isolation of a single mating type with this beetle. Further, it is the asexual state of *O. clavigerum* that is present in the pupal chamber, acquired in the mycangium, and transmitted to new host trees. If a sexual state is produced, it may form distant from the developing brood (e.g., in old adult galleries) or after the beetles have dispersed. Thus, if a sexual state is formed, it may actually be inconsequential in the life cycle of the fungus relative to the primary beetle vector. If this is the case, the asexual cycle would predominate and a high degree of monomorphism would be expected.

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