Techniques

Restriction Fragment Length Polymorphisms in Nuclear and Mitochondrial DNA of *Sclerotinia* Species

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ABSTRACT

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Restriction fragment length polymorphisms were observed in nuclear, ribosomal DNA of *S. sclerotiorum*, *S. minor*, *S. trifoliorum*, *S. asari*, *S. ficariae*, *Sclerotinia* n. sp., and *Sclerotiorum cepivorum*. Whole-cell DNAs of 42 isolates were digested separately with *Eco*RI, *Bam*HI, and *Hind*III and probed with cloned rDNA from *Neurospora* in Southern hybridizations. Cloned rDNAs from *Schizophyllum* and *Armillaria* hybridized to the same restriction fragments as did cloned rDNA from *Neurospora*. Most of the polymorphism in rDNA restriction fragment lengths was between rather than within species, with application as taxonomic characters in comparing species. Specifically, the rDNA restriction fragment phenotypes indicated that the newly discovered species *S. asari* and *Sclerotinia* n. sp. are genetically distinct entities, and that the synonymy of *S. ficariae* under *S. sclerotiorum* is justified. Three isolates of *Sclerotiorum cepivorum* had an identical set of rDNA restriction fragment sizes. The rDNA restriction fragment phenotype of this form-species was unique, with no obvious relationship with any of the *Sclerotinia* species tested. To examine variation in mitochondrial DNA, plasmid pGP637, which carries the mitochondrial 24S rRNA gene from *Neurospora crassa*, was used as a probe in Southern hybridizations with *Hind*III-digested DNAs of *S. sclerotiorum*, *S. minor*, and *S. trifoliorum*. There was extensive variation in the restriction fragment sizes of mitochondrial DNA between species, with only one fragment shared by all isolates. Each species had between one and four fragments that were unique to, and constant within, that species. There was considerable variation in the sizes of several other fragments within each of the three species examined.

Although over 250 species names have been accommodated in *Sclerotinia* over the years, Kohn (11) reduced the genus to three species, *Sclerotinia sclerotiorum* (Lib.) de Bary, *S. minor* Jagger, and *S. trifoliorum* Erikss. Purdy (20) synonymized *S. minor* and *S. trifoliorum* under *S. sclerotiorum*, but most workers, using a wide variety of morphological, cytological, biochemical, cultural, and epidemiological characters (4,12,19,23,24,28,32), recognize the three species as distinct though closely related.

The ability to distinguish these species from one another has not entirely stabilized the taxonomy of *Sclerotinia*. When comparing novel strains with the recognized species, the full range of taxonomic criteria may not be at hand, especially those criteria pertaining to the sexual state. Three facets of this problem have emerged in *Sclerotinia*. First, two putatively new species of *Sclerotinia* are now known from Asia, *S. asari* Wu and C. R. Wang in China and an undescribed species currently being investigated by I. Saito in Japan. Using morphological characteristics of apothecia and sclerotia, it is difficult to distinguish these taxa from the pre-existing three species. On the basis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or isoelectric focusing of soluble proteins, the new taxa are distinct from the old (J. Wong, pers. commun.; I. Saito, pers. commun.). A single taxonomic characteristic is not, however, an adequate criterion for recognizing a new species. Second, using morphological characteristics of the dried type specimen, Kohn (11) synonymized *S. ficariae*, a pathogen of a woodland plant, *Ranunculus ficaria*, under *S. sclerotiorum*. Although merging the two species epithets has been questioned by some European workers, no other criteria for comparing *S. ficariae* with *S. sclerotiorum* have been applied to the problem. Third, the affinities of "orphan" sclerotial fungi, which do not produce a sexual state, such as *Sclerotiorum cepivorum*, have been elusive, although on the basis of sclerotial anatomy and histochemistry, this form-species appears close to *Sclerotinia*, *Botryotinia*, and *Ciborinia* (13) rather than to *Stromatinia* as proposed by Whetzel (31). Techniques for comparing and characterizing somatic isolates of *S. sclerotiorum*, *S. minor*, and *S. trifoliorum* that will yield new pools of taxonomic characters are needed to demonstrate the relatedness of somatic isolates in the absence of a sexual state, as well as to detect intra- and interspecific variants in the genus *Sclerotinia*.

Presumably, the phenotypic characteristics that either

differentiate these three species or unite them phylogenetically as a genus are genetically determined and are correlated with differences or similarities in genomic DNA. In the studies reported here, independent molecular characters, restriction fragment length polymorphisms (RFLPs) in nuclear, ribosomal DNA (rDNA) and in mitochondrial DNA (mtDNA) were used to compare isolates of a group of species with affinities in *Sclerotinia*.

RFLPs have been studied in many organisms including humans (3), crop plants (9,21), and fungi (1,17,26,35). These polymorphisms can be generated by loss or gain of restriction sites resulting from point mutations, or from rearrangement of DNA sequences. One of the problems in applying RFLPs to taxonomic problems is in selecting DNA segments that "resolve" at the appropriate taxonomic level, showing neither excessive and uninterpretable variation, nor homogeneity at the taxonomic rank of interest (16). In fungi, rDNAs and mtDNAs are abundant and easily accessible segments that have been shown to resolve at the species or the strain level (1,5,6,10,22,29).

In the studies reported here, RFLPs were observed in rDNA and mtDNA of *S. sclerotiorum*, *S. minor*, *S. trifoliorum*, *S. asari*, *S. ficariae*, *Sclerotinia* n. sp., and *Sclerotiorum cepivorum*. Most of the polymorphisms in restriction fragment length were between rather than within species, with application as taxonomic characters in comparing species.

MATERIALS AND METHODS

The host and geographic origins of the isolates used in this study are listed in Table 1. Isolates came from the following sources: W. R. Jarvis, Agric. Canada Research Station, Harrow, Ontario, Canada N0R 1G0; American Type Culture Collection (ATCC); J. R. Steadman, University of Nebraska, Lincoln 68583; R. G. Gilbert, USDA ARS/IAREC, P.O. Box 30, Prosser, WA 99350; D. J. Jardine, Kansas State University, Manhattan 66506; J. A.-L. Wong, Agric. Tasmania, G.P.O. Box 192B Hobarg, Tasmania 7001; P. B. Adams, USDA ARC-West, Plant Protection Institute, Beltsville, MD 20705; E. L. Stromberg, VPI and SU, Blacksburg, VA 24061; T. Schumacher, University of Oslo, P.O. Box 1045, Blindern, 0316 Oslo 3, Norway; and I. Saito Kitami Agric. Research Station, Kunneppu-cho, Tokoro-gun, Hokkaido, Japan 099-14.

Mycelium for DNA isolation was grown in standing-liquid culture in potato dextrose broth (27) in 9-cm petri dishes. Liquid cultures were inoculated with 4–5 1-mm^3 plugs of agar and mycelium and incubated 7–10 days in the dark at ambient room temperature. Mycelium was harvested on cheesecloth, rinsed with glass-distilled H_2O, frozen in liquid nitrogen, and lyophilized. Mycelium, once completely freeze-dried, could be stored indefinitely, without degradation of DNA, over silica gel in a sealed container at room temperature. DNA was extracted from 30–50 mg of lyophilized mycelium by the method of Zolan and Pukkila (36) or from 200 mg of mycelium by the method of Murray and Thompson (18) with purification in ethidium bromide-CsCl density gradients (15). The latter method yielded 20–100 μg of DNA per 200 mg of mycelium.

Restriction enzymes were purchased from Pharmacia (Canada) Ltd. (Dorval, Québec), and reactions were done according to the manufacturer's recommendations. Generally, 1–2 μg of DNA was incubated with 5–10 units of enzyme for 2–6 hr at 37 C. Electrophoresis of DNAs was in 20 × 24 × 0.4 cm, 0.75% agarose gels in Tris-borate or Tris-acetate buffer (15) at 1.5 v/cm for 16 hr. The molecular weight standard was bacteriophage lambda DNA digested with *Hind*III. Under these conditions the mobility of a fragment between 0.5 and 10 kb was inversely proportional to the $\log_n$ of molecular size, and the standard deviation for any given fragment within this size range was less than 0.1 kb in independent experiments. Capillary transfer of DNAs from gel to nylon hybridization membrane (Genescreen Plus, NEN Research Products, Boston, MA) was done according to the manufacturer's recommendations.

Several plasmid probes were used: pMF2 contains the portion of the rDNA repeat with the 18S, 5.8S, and 26S ribosomal RNA

TABLE 1. List of *Sclerotinia* and *Sclerotium* strains

LMK number	host	Location	Collector/ source
S. sclerotiorum			
1	lettuce	LaSalle, Ontario	Jarvis (128)
34	cabbage	New South Wales, Australia	Wong (S11) ATCC 34325
44	lettuce	River Canard, Ontario	Jarvis (150)
77	soybean	Greeley, Colorado	Steadman (143)
80	lettuce	Yuma, Arizona	Steadman (144)
81	soybean	Australia	Steadman (147)
82	bean cull	Mitchell,, Nebraska	Steadman (152b)
83	snapbean	New York	Steadman (155)
84	snapbean	Hancock, Wisconsin	Steadman (156)
85	lima bean	Westley, California	Steadman (160)
86	Canada thistle	Montana	Steadman (170b)
87	Canada thistle	Montana	Steadman (176)
88	navy bean	Harrow, Ontario	Steadman (182)
89	dry bean	Alberta	Steadman (184)
90	Charlevoix bean	Michigan	Steadman (194)
91	alfalfa	Touchet, Washington	Gilbert
92	soybean	Manhattan, Kansas	Jardine
93	lettuce	Bradford Marsh, Ontario	Kohn/Grenville
121	rapeseed	Guangxi, China	Wong/ Wu Yusan
122	soybean	Heilongjiang, China	Wong/ Wu Yusan
123	sunflower	Heilongjiang, China	Wong/ Wu Yusan
S. minor			
3	lettuce	LaSalle, Ontario	Jarvis (129)
35	potato	New South Wales, Australia	ATCC 34324[a] Wong (S3)
45	lettuce	River Canard, Ontario	Jarvis (151)
115	peanut (soil)	Cortland, Virginia	Adams (Ss42)
116	peanut (soil)	SW of Cortland, Virginia	Adams (Ss50)
118	lettuce (soil)	Salinas, California	Adams (Ss70)
S. trifoliorum			
36	white clover	Tasmania	ATCC 34327[a] Wong (S33)
47	alfalfa	Rockbridge Co., Virginia	Stromberg (LAL1)
55	alfalfa	Wythe Co., Virginia	Stromberg (C AL1)
104	red clover	Japan	ATCC 52596[b] Fujii (R 31)
105	red clover	Japan	ATCC 52597[b] Fujii (R 38-3)
106	lucerne	New South Wales, Australia	ATCC 34326[a] Wong (ST-L2)
S. ficariae			
57	*Ranunculus ficaria*	Norway	Schumacher
58	*Ranunculus ficaria*	Norway	Schumacher
S. asari			
124	*Asarum heterotropoides*	China	Wong/ Wu Yusan
Sclerotinia n. sp.			
99	burdock	Japan	Saito (SI-BA-1)
100	carrot	Japan	Saito (SI-CM-1)
101	*Angelica acutiloba*	Japan	Saito (SI-ANG-T)
Sclerotium cepivorum			
1	onion	Wageningen, Netherlands	Gams (83-11)
71	onion (soil)	Elmer, New Jersey	Adams (Sc-103)
73	soil	Brazil	Adams (Sc-W-10)

[a] Vouchers from Wong and Willets (32,33).
[b] Vouchers from Uhm and Fujii (29).

cistrons and lacks most of the nontranscribed spacer of *Neurospora crassa* (7); pR1 contains the entire rDNA repeat, including the 18S, 5.8S, 26S, and 5S genes, as well as the nontranscribed regions of *Schizophyllum commune* (B. Buckner, pers. commun.); pAM1 and pAM2 contain the entire rDNA repeat from *Armillaria mellea* (J. B. Anderson, unpublished), and pGP637 contains three contiguous *Pst*I fragments of mitochondrial DNA containing the 24S rRNA gene from *Neurospora crassa* (8).

Plasmid probes were labeled with [α-^{32}P]dCTP (3,000 Ci/mmol.; NEN Research Products) to a specific activity of about 1×10^8 cmp/μg DNA by nick translation with a kit from Bethesda Research Laboratories (Gaithersburg, MD). Usually 10 ng of bacteriophage lambda DNA was added to 500 ng of plasmid DNA for nick translation to visualize the lambda marker DNAs at a signal intensity roughly comparable to fragments in fungal DNAs in Southern hybridization. Concentration of labeled probe in hybridizations was between 10 and 20 ng/ml. Prehybridization was 6× saline sodium citrate (SSC), 5× Denhardt's solution, 1% SDS for 2–4 hr at 65 C, after which the prehybridization solution was removed. The hybridization was in 6× SSC, 5× Denhardt's, 1% SDS, 10% dextran sulfate, 150 μg/ml of sheared salmon sperm DNA, and probe DNA at 65 C for 16–24 hr. Salmon DNA and probe DNA were brought to 1.0 ml with gd H_2O and denatured by boiling for 10 min just before hybridization. Blots were washed twice for 5 min in 2× SSC at room temperature, twice for 30 min in 2× SSC, 1% SDS at 65 C, and twice for 15 min in 0.1× SSC at room temperature. Autoradiography was with Kodak X-Omat AR film with a Dupont Cronex Lightning-Plus intensifying screen at −70 C for 1–4 hr or with no screen at room temperature for 4–24 hr.

RESULTS

Whole-cell DNAs of 42 isolates were digested separately with *Eco*RI, *Bam*HI, and *Hind*III and probed with pMF2 in Southern hybridizations. The sizes of all rDNA fragments detected are given in Table 2. Autoradiograms for Southern hybridizations of digested DNAs of six isolates of *S. minor* and *S. trifoliorum*, and 18 isolates of *S. sclerotiorum* with pMF2 are shown in Figure 1. Each of the seven species examined had a characteristic pattern of rDNA fragments and, with the exception of *S. ficariae* and the majority of *S. sclerotiorum* isolates, which shared an identical rDNA fragment phenotype, all species were clearly different from one another. The only intra-specific variability was found in *S. sclerotiorum* and *S. trifoliorum*. Of 21 isolates of *S. sclerotiorum*, 20 were identical in rDNA fragment phenotype; only one isolate had a slightly different phenotype, with a 7.1-kb *Eco*RI fragment instead of the 4.8 and 2.2 kb fragments in DNAs of the other

TABLE 2. Sizes in kilobases of DNA fragments hybridizing to pMF2

Species	Phenotype[a]	Number of isolates	Restriction enzyme		
			*Eco*RI	*Bam*HI	*Hind*III
S. sclerotiorum	a	20	4.8, 3.9, 2.2	≈11	7.2
	b	1	7.1, 3.9	≈11	7.2
S. trifoliorum	c	4	7.2, 4.1	>30	6.9, 1.1
	d	1	7.2, 4.1	>30	6.5, 1.4
	e	1	7.6, 3.7	>30	7.5
S. minor	f	6	6.4, 3.5	>30	6.6
Sclerotinia n. sp.	g	3	7.2, 3.5	>30	6.9
S. ficariae	a	2	4.8, 3.9, 2.2	≈11	7.2
S. asari	h	1	4.0, 3.2, 1.7	9.5	6.8, 2.3
Sclerotium cepivorum	i	3	5.0, 3.2	>30	6.2, 2.0

[a]Phenotype a includes LMK 2, 34, 44, 77, 80–93, 121, and 122 of *S. sclerotiorum* and 57 and 58 of *S. ficariae*; b: 123; c: 36, 55, 105, and 106; d: 104; e: 47; f: 3, 35, 45, 115, 116, and 118; g: 99, 100, and 101; a: 57 and 58; h: 124; i: 1, 71, and 73.

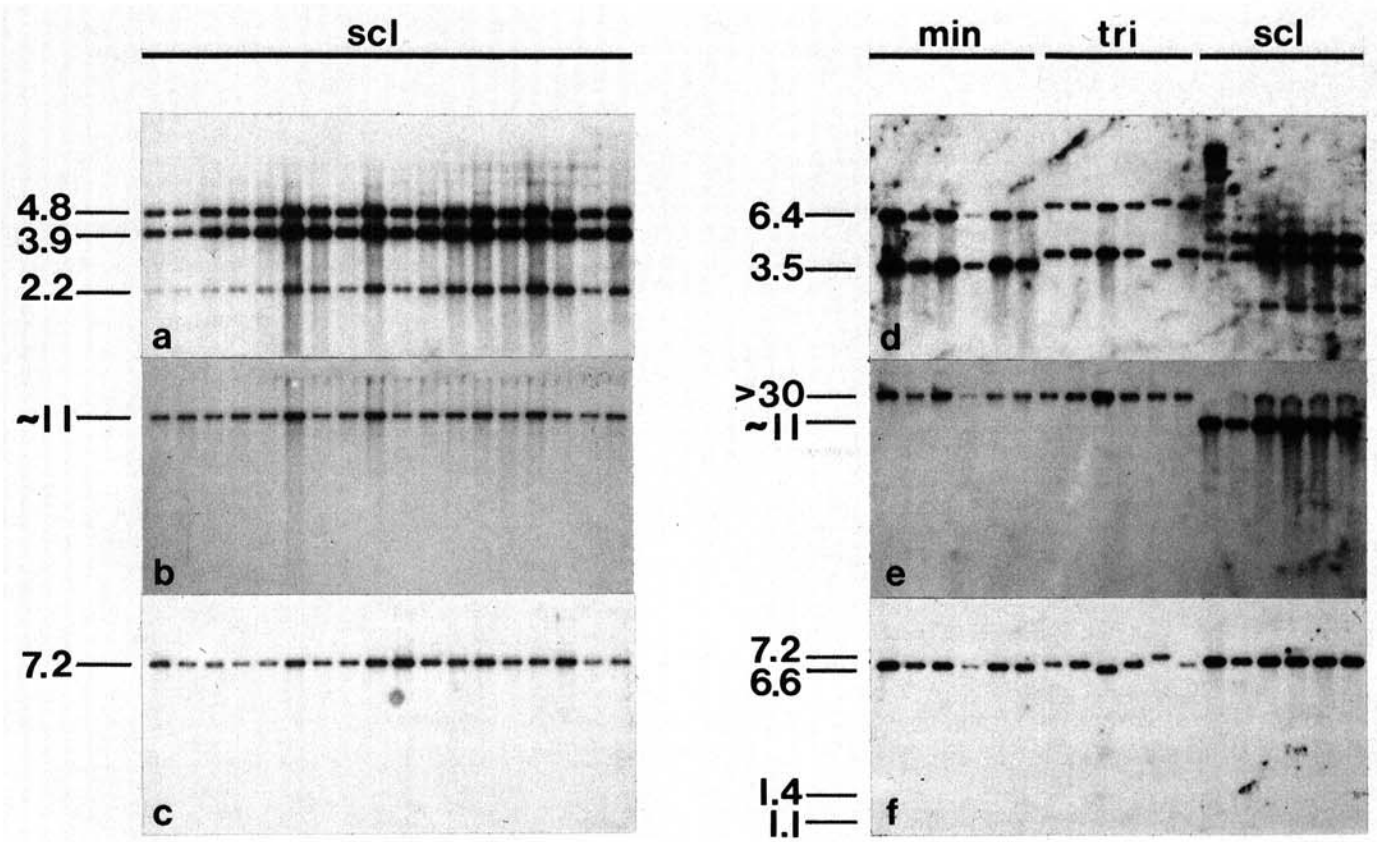

Fig. 1. Southern hybridizations of digested *Sclerotinia* DNAs with radiolabeled, rDNA-containing plasmid, pMF2. Target DNAs were digested with: **a** and **d**, *Eco*RI; **b** and **e**, *Bam*HI; **c** and **f**, *Hind*III. From left to right DNAs were from **a, b,** and **c,** *Sclerotinia sclerotiorum* (scl) isolates LMK 93, 80, 81, 77, 34, 44, 85, 2, 82, 91, 87, 92, 90, 89, 83, 86, 88, and 84; **d, e,** and **f,** *S. minor* (min) LMK 118, 116, 115, 45, 35, and 3, *S. trifoliorum* (tri) LMK 106, 105, 104, 55, 47, and 36, and *S. sclerotiorum* (scl) LMK 85, 83, 81, 44, 34, and 2.

isolates. This polymorphism may be due to the loss of a single *Eco*RI site adjoining the 4.8 and 2.2 kb fragments. Three slightly different phenotypes were observed for *S. trifoliorum*. Five of six isolates of *S. trifoliorum* had two *Hind*III fragments that hybridized to pMF2. Although the larger fragment and the smaller fragment varied slightly in size, the sum for the two fragments was constant at about 8 kb. One isolate of *S. trifoliorum* had only one *Hind*III fragment of 7.5 kb. In one isolate of *S. trifoliorum* the two *Eco*RI fragments of rDNA were different in size from those in the other five isolates. The sum of the sizes of the *Eco*RI fragments, however, was constant for all six isolates of *S. trifoliorum*.

Polymorphisms among the strains of *S. trifoliorum* could not be correlated with differences in host or geographical distribution. A much larger sample would be needed to determine if such correlations exist. In *S. sclerotiorum*, where a much larger sample of strains with broad host and geographical distribution was examined, the rarity of polymorphism was notable. One isolate from sunflower in China showed an alternative rDNA fragment phenotype, although two other Chinese isolates from rapeseed and soybean showed the same phenotype as the majority of isolates from North America and Australia.

The size of the rDNA repeat estimated from *Eco*RI digests (Table 2) is 10.9 kb for *S. sclerotiorum*, 9.9 kb for *S. minor*, 11.3 kb for *S. trifoliorum*, 10.7 kb for *Sclerotinia* n. sp., 10.9 kb for *S. ficariae*, 8.9 kb for *S. asari*, and 8.2 for *Sclerotium cepivorum*. The rDNA repeats of *S. sclerotiorum*, *S. ficariae*, and *S. asari* each apparently had only one *Bam*HI fragment similar in size to the sum of the sizes of the *Eco*RI fragments for each of these species, respectively. The rDNA repeats of *S. minor*, *S. trifoliorum*, *Sclerotinia* n. sp. and *Sclerotium cepivorum* apparently had no *Bam*HI sites, giving a very large fragment whose size could not be measured accurately. For *S. asari* and *Sclerotium cepivorum*, the total of the sizes of *Hind*III fragments equaled the total for *Eco*RI fragments. For each of *S. sclerotiorum*, *S. minor*, *S. trifoliorum*, *Sclerotinia* n. sp., and *S. ficariae*, the total of the sizes of the *Hind*III fragment(s) was less than that for the respective *Eco*RI fragments. Possibly, certain *Hind*III fragments in rDNAs of these species are located entirely within the nontranscribed spacer and do not hybridize to pMF2, which contains little of the nontranscribed spacer.

To determine whether cloned rDNAs from other sources would hybridize to the same or to different fragments as those hybridizing to pMF2, we used pR1, which contains the entire rDNA repeat from *Schizophyllum commune* and pAM1 and pAM2, which together represent the entire rDNA repeat from *Armillaria mellea*. Plasmic pR1 was used as a probe in Southern hybridizations with *Eco*RI- and *Bam*HI-digested DNAs of isolates LMK 2, 34, and 44 of *S. sclerotiorum*, LMK 3, 35, and 45 of *S. minor*, and LMK 36 and 47 of *S. trifoliorum*. Clones pAM1 and pAM2 were used simultaneously as a probe in Southern hybridizations with the same *Hind*III-digested DNAs of *S. minor*, *S. trifoliorum*, and *S. sclerotiorum* as in Figure 1f. In all cases the *Schizophyllum* and *Armillaria* rDNA probes hybridized to exactly the same fragments as pMF2.

Plasmid pGP637, which carries the mitochondrial 24S rRNA gene from *Neurospora crassa*, revealed considerable polymorphism in fragment size in Southern hybridizations with *Hind*III-digested DNAs of *S. sclerotiorum*, *S. minor*, and *S. trifoliorum* (Fig. 2). There was extensive variation between species, with only one band shared by all isolates. Each species had between one and four fragments that were unique to, and constant within, that species. There was considerable variation in the sizes of other fragments within each of the three species examined.

DISCUSSION

The restriction fragment length polymorphisms revealed with both nuclear rDNA and mtDNA probes in this sample of isolates occur mostly between species. Those species for which multiple isolates were available appeared to have a characteristic restriction fragment phenotype or "fingerprint." A reasonable hypothesis is that the RFLPs observed here are representative of the divergence that has occurred in the genome as a whole. Furthermore, the data strongly suggest that the best characterized taxonomic species in this study, *S. minor*, *S. trifoliorum*, and *S. sclerotiorum*, are also reproductively isolated "biological species." Were this not the case, we might expect to observe as many polymorphisms within species as between species. That the rDNA and mtDNA fragment patterns correlated so decisively with these three species of *Sclerotinia* suggests their usefulness as characters in the taxonomy of this group of sclerotial fungi.

This study provides an example of how RFLPs can be used as taxonomic characters, in combination with morphological, epidemiological, and other criteria. First, rDNA fragment phenotypes support the recognition of *S. asari* and *Sclerotinia* n. sp. as species distinct from *S. sclerotiorum*, *S. minor*, and *S. trifoliorum*. Second, the observation of identical rDNA fragment phenotypes supports the synonymy of *S. ficariae* under *S. sclerotiorum*, as proposed earlier by Kohn (11). Third, RFLPs were used to confirm two identifications and to verify records. Isolate LMK 92 was the first record of *S. sclerotiorum* in Kansas (D. J. Jardine, pers. commun.), and data on LMK 91 confirmed an identification of *S. sclerotiorum* on alfalfa, an unusual host for this species in Washington (R. G. Gilbert, pers. commun.).

The problem of connecting "orphan" form-species with no known sexual state, such as *Sclerotiorum cepivorum*, to genera such as *Sclerotinia* was not solved in these studies. Because the reference rDNAs were polymorphic at species level, we have only demonstrated that *Sclerotium cepivorum* is distinct from the other species examined, notably *Sclerotinia sclerotiorum*, *S. minor*, and *S. trifoliorum*. Other DNA polymorphisms, with resolution at generic rank, are needed to associate with this form-species with a genus in the Sclerotiniaceae. Alternatively, comparisons with other species in the Sclerotiniaceae can continue to be made, but only an identical RFLP phenotype will be sufficient to suggest synonymy.

Within the present sample there was more viability in restriction fragment lengths of mtDNA than of rDNA. This is not surprising, because length mutations are reported to be extremely common in mtDNA of closely related isolates (29). For this reason, when comparing isolates representing more than one species by measurement of restriction fragment sizes, nuclear rDNA would appear to be preferable to mtDNA. Another advantage of rDNA is that this segment is tandemly repeated on the order of 100-fold compared with single-copy nuclear DNAs. Because of the high copy number, rDNA restriction fragments may sometimes be seen on ethidium-stained gels and are easy to assay in Southern hybridizations. Cloned rDNAs are available from a wide variety of fungi; all seem to cross-hybridize due to the highly conserved nature of certain regions of the rRNA genes. In this study rDNA

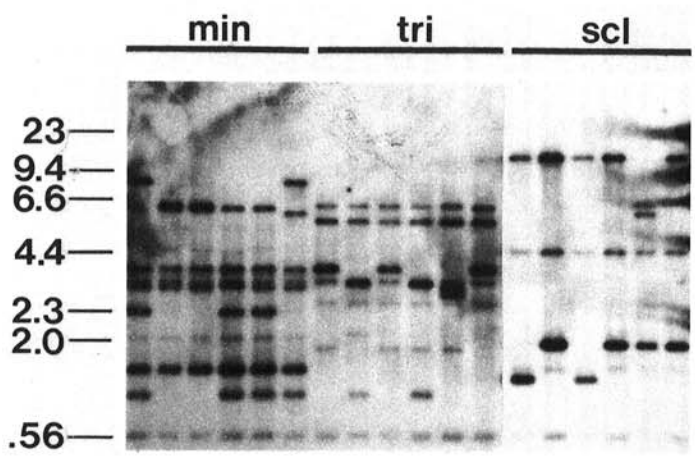

Fig. 2. Southern hybridizations of *Hind*III-digested *Sclerotinia* DNAs with radiolabeled, mitochondrial DNA-containing plasmid, pGP637. DNAs were from the same isolates of *S. minor*, *S. trifoliorum*, and *S. sclerotiorum* as in Figures 1d, e, and f.

clones from *Schizophyllum* and *Armillaria*, both Holobasidiomycetes, gave results identical to those for an rDNA clone from *Neurospora*. This was not necessarily expected, because the *Schizophyllum* rDNA repeat, represented by pR1, is known to contain the 5S gene in addition to the 18, 5.8, and 26S genes (B. Buckner, pers. commun.), whereas the *Neurospora* rDNA repeat, represented by pMF2, has only the 18, 5.8, and 26S genes. The 5S genes in *Neurospora* (25) and in *Aspergillus* (2,14) are located outside of the tandemly repeated rDNA at other chromosomal locations. Although the locations of the 5S genes in genomic DNA of *Sclerotinia* spp. are not known, we did not detect any hybridization between the 5S genes of *Sclerotinia* and the 5S genes of *Schizophyllum*.

The total size of the rDNAs varied from 8.2 to 11.3 kb in our sample. The best estimates of the size were obtained by summing the *Eco*RI fragments, each of which was of a size to be accurately measured. In some species total sizes for *Hind*III were lower than for the respective *Eco*RI fragments. We suspect that certain *Hind*III fragment(s) may be located completely within the nontranscribed spacer region and were therefore not homologous to pMF2, which does not contain most of the nontranscribed spacer. We also suspect that these same *Hind*III fragments were not homologous to the *Schizophyllum* or *Armillaria* clones because of evolutionary divergence in nontranscribed spacer regions.

This study shows that RFLPs exist in nuclear and mitochondrial DNAs of this sample of isolates with affinities in *Sclerotinia*. All species for which there were multiple isolates appeared to be distinct at the molecular level. Specifically, the recognition of *S. asari* and *Sclerotinia* n. sp. as distinct species, and the synonymy of *S. ficariae* under *S. sclerotiorum*, are supported by our data. Although our data strongly suggest that the form-species *Sclerotiorum cepivorum* is a distinct, taxonomic entity, we cannot as yet infer any relationship between *Sclerotiorum cepivorum* and any of the other species, nor can we make conclusions about phylogenetic relationships among the other species studied. Restriction mapping of rDNAs followed by alignment and comparison of maps, however, may well clarify phylogenetic relationships in this group.

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