

Mycelial Incompatibility and Molecular Markers Identify Genetic Variability in Field Populations of *Sclerotinia sclerotiorum*

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ABSTRACT

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Sixty-three sclerotial strains of *Sclerotinia sclerotiorum* were obtained from transects in two fields of canola (*Brassica napus*) in Ontario. Mycelial pairings of the strains in all combinations on agar medium produced either an incompatible reaction in which a reaction line between the two strains developed in the interaction zone, or a compatible reaction in which no reaction line developed. The reaction line was a distinct discontinuity between the two strains, visible as a red line on the colony reverse in pairings made on medium amended with red food coloring. Among the 33 strains from the first field, six mycelial compatibility groups (MCGs) were recognized, the largest group including 19 strains. Among the 30 strains from the second field, many more MCGs were defined. In pairings of 10 monosporous strains from each of six apothecia collected along the transects, all sibling monosporous strains were compatible and no segregation for mycelial compatibility was observed among siblings. Analysis with three molecular markers indicated that each of the MCGs was genetically uniform. With one of these markers, each MCG was uniquely fingerprinted. This fingerprint was produced by a random fragment of nuclear DNA (approximately 4.5 kb) from *S. sclerotiorum*, pLK44.20, which when used as a cloned probe in Southern hybridizations

of DNAs restricted with *Bam*HI detected polymorphisms that corresponded exactly with strain groupings defined by mycelial compatibility. Southern hybridization of high molecular weight DNAs separated by pulsed-field electrophoresis showed that the repetitive element is located on at least five to six chromosomes. Another probe, plasmid pGP637, carrying the mitochondrial 24S rRNA gene from *Neurospora crassa*, in *Hind*III-digested DNA, produced six phenotypes. With the exception of phenotypic heterogeneity within one MCG, which had three phenotypes, only one phenotype was observed in strains from each MCG; each of four of the six phenotypes was shared by a group of MCGs. The third molecular marker was a length polymorphism in a segment of the small mitochondrial rRNA gene amplified by the polymerase chain reaction; of four phenotypes, each of two (fragment size of either 0.6 or 2.0 kb) was shared by a large group of MCGs. The data from this study demonstrate that a field population of *S. sclerotiorum* is genetically heterogeneous, and suggest that the distinct genotypes that compose the population are each conserved, increasing either by clonal asexual or by homothallic sexual reproduction.

Additional keywords: DNA fingerprinting, oilseed rape, *Sclerotinia* stem rot.

Sclerotinia sclerotiorum (Lib.) de Bary, unlike many fungal species, has been taxonomically well-defined by a wide range of criteria, including morphological features (17,18,31), isoenzymes (7,31), and DNA restriction fragment length polymorphisms (RFLP) (20). A necrotrophic pathogen with cosmopolitan distribution and a wide host range (28), *S. sclerotiorum* shows a high level of intraspecific phenotypic variability (21,28). Like other diseases caused by *S. sclerotiorum*, *Sclerotinia* stem rot of canola is an important, yield-reducing disease, but as yet there is no germplasm resistance to this pathogen in canola and pathogen genotype-cultivar relationships are unknown. Whether a local population of *S. sclerotiorum*, for example a field infested by the pathogen, is composed of one or several genetically (and

phenotypically) different individuals has not been determined. Rational, systematic definition of intraspecific groupings based on factors such as pathogenicity, virulence, or geographic distribution has been difficult. In part, this has been because of problems with assay procedures, but also there has been a lack of independent criteria, such as mycelial or vegetative incompatibility or molecular-genetic markers, with which to characterize intraspecific heterogeneity. Once such heterogeneity is identified, groups can be defined and the phenotypes of these groups, such as factors influencing aggressiveness and host specificity, can be described. If reproduction is by clonal, asexual means or by homothallic, inbred, sexual means, then all independent genetic determinants of phenotype including mycelial compatibility would remain associated.

Mycelial incompatibility, the inability of two strains to fuse and form one colony, has been used in studies of fungi such

as *Cryphonectria parasitica* (1) and *Ophiostoma ulmi* (5) as an effective means of identifying intraspecific variation within field populations of a plant pathogen. Mycelial incompatibility is one of several events associated with vegetative incompatibility, the inability of two strains to fuse and form a stable heterokaryon. Vegetative incompatibility appears to be common among Ascomycetes and ascomycetous anamorphs, and has been documented in several species (3,19). Grouping of isolates on the basis of vegetative compatibility has been used to study intraspecific variation, including variation in virulence, within several species, such as *Fusarium oxysporum* (14,16,26,27).

In a recent study (19), we examined mycelial incompatibility in 31 strains of *S. sclerotiorum* isolated from a variety of hosts and geographic areas and found that each of 21 strains were mycelially incompatible with all others, each representing an MCG made up of one strain. Among the 10 remaining strains there were four MCGs, each group contained two to three strains. All self-self pairings were compatible. The data from this study demonstrated that a high level of mycelial incompatibility exists among strains of *S. sclerotiorum*, that genetic heterogeneity exists within the species, and that mycelial compatibility-incompatibility interactions may be an effective method of categorizing this heterogeneity. The genetic consequences of mycelial incompatibility, however, remained unknown. Because nitrogen nonutilizing mutants are not available in this species, the MCGs cannot easily be tested for heterokaryon incompatibility in complementation tests. Alternatively, a variety of molecular criteria for examining genetic diversity within and between MCGs of *S. sclerotiorum* are readily available.

In the present study, we sought to determine whether genetic heterogeneity, based on mycelial incompatibility interactions, was also observed in a local field population of *S. sclerotiorum*. Sclerotia and apothecia were collected in transects within two fields of canola (oilseed rape) heavily infected with *Sclerotinia* stem rot. The first objective was to assign the strains to MCGs by pairing. After determining that many MCGs occurred in the fields, our second objective was to develop molecular criteria that would characterize intraspecific variability to determine whether MCGs identified by pairing studies were genetically distinct and homogeneous (i.e., clonal) entities.

MATERIALS AND METHODS

Two fields of winter canola, *Brassica napus* L., were sampled in Harriston, Ontario, on 28 June 1989. The fields were planted with cultivars Tandem and Ceres, respectively, and were separated by a distance of approximately 3 km. At the time of sampling, the fields were heavily infected; many plants along the transects (approximately $\geq 50\%$) had stem lesions and some showed lodging. The first field was 100 acres, with a cropping history of wheat (1988) and white beans (1987). *S. sclerotiorum* was detected in previous years on white beans and soybeans. The second field was 8 acres, previously planted with oats (1988), winter wheat (1987), and canola (1986). *S. sclerotiorum* was observed on the previous canola crop. Because the crop canopy was extremely dense, scouting paths were used to minimize crop damage. In each field, sampling was conducted in two transects, with sampling at 25-m intervals along one transect and at 50-m intervals along the other transect. At each sampling station, indicated by a number in Table 1, two sclerotia were collected from the soil surface. Two apothecia were also collected where they could be located, at 15 of the 32 stations. A strain was isolated from each surface-sterilized sclerotium, but with the exception of station 32 where because of the absence of sclerotia, strains were isolated from infected stem tissue. Six apothecia from different stations were chosen randomly, and from a mass of spores from each single apothecium, 10 single ascospores were selected at random with the aid of a dissecting microscope (50 \times) and needle. Two single ascospore isolates were prepared from each of the remaining apothecia and stored under liquid nitrogen for future studies. Strains were grown on potato-dextrose agar (Difco Laboratories, Detroit, MI) in the dark at room temperature (20–22 C).

Strains were paired on modified Patterson's medium (MPM) amended with six drops of red food coloring (McCormack Corp., Dallas, TX) per liter (19). The red food coloring was originally used to distinguish MPM from other media used in our laboratory, but the accumulation of this red color in the interaction zone of incompatible pairings, caused by an accumulation of the red coloration in the cytoplasm of hyphal tips in the interaction zone (19), was useful in scoring. Each strain was grown on MPM for 10–14 days before pairing. For the pairing experiments, 2-mm blocks of mycelial inoculum removed from the inner colony (>1 cm from the growing margin) were placed 3.5 cm apart on MPM in a 9-cm petri dish, one pairing per dish, and incubated in the dark at room temperature. Sclerotial strains were confronted in 1,460 of 1,953 combinations. Representatives of each MCG were paired with representatives of each of the other MCGs. From each of six apothecia (see sampling methods), three apothecia from each field, 10 single ascospore-derived strains were paired in all combinations. Two monosporous strains, chosen randomly for each of the six apothecia, were also paired with tester strains representing each MCG of the sclerotial strains. Pairings were examined 7 and 14 days after inoculation.

Mycelia were grown, harvested, and freeze dried for DNA extraction as described by Kohn et al (20). DNA was extracted from 10–15 mg of freeze-dried mycelium by the small-scale method of Zolan and Pukkila (33). When greater amounts of mycelium (e.g., 40 mg) were used, the extracts contained large amounts

TABLE 1. Mycelial compatibility groups (MCGs) among collections of *Sclerotinia sclerotiorum*

Station ^a	First field		Second field		
	Strain ^b	MCG ^c	Station	Strain	MCG
1A	198	2	18A	231	10
1B	199	1	18B	232	11
2A	200	1	19A	233	12
2B	201	1	19B	234	13
3A	202	1	20A	235	14
3B	203	1	20B	236	15
4A	204	1	21A	237	4
4B	205	3	21B	238	4
5A	206	1	22A	239	16
5B	207	3	22B	240	17
6A	208	1	23A	241	5
6B	209	1	23B	242	5
7A	210	2	24A	243	18
7B	211	2	24B	244	2
8A	212	2	25A	245	19
8B	213	7	25B	246	20
9A	214	1	26A	247	21
9B	215	1	26B	248	22
10A	216	1	27A	249	23
10B	217	1	27B	250	24
11A	218	2	28A	251	25
11B	219	1	28B	252	26
12A	220	3	29A	253	27
12B	221	8	29B	254	28
13A	222	2	30A	255	29
13B	223	1	30B	256	30
14	224	1	31A	257	31
15A	225	9	31B	258	32
15B	226	1	32A	259	6
16A	227	2	32B	260	6
16B	228	2			
17A	229	1			
17B	230	1			

^a Station is a numbered point (1–32) on one of four transects at which, with the exception of station 32, two sclerotia (A and B) were collected from the soil. Strains were isolated from each sclerotium from all stations, except for strains from station 32, which were isolated from stem lesions. Each station from 1A to 9B and from 18A to 26B was 25 m apart; each station from 10A to 17B and from 27A to 32B was 50 m apart.

^b Strains are designated with a unique accession number (prefix LMK) in the culture collection of L. M. Kohn.

^c A group of strains that, based on pairing tests, are all compatible with each other.

of viscous carbohydrate and the yield of DNA was reduced. RNA extraction, radiolabeling of DNA probes with ^{32}P by nick translation, and both Southern and northern hybridizations were as described by Anderson et al (2). One heterologous plasmid probe, pGP637, which contains three contiguous *Pst*I fragments of mitochondrial (mt) DNA including the 24S rRNA gene from *Neurospora crassa* (13), was used in Southern hybridizations. Of several clones carrying random fragments of genomic DNA from *S. sclerotiorum*, three were used as probes in Southern hybridizations. All aspects of cloning were exactly as described by Anderson et al (4), except that genomic DNA of strain LMK44 and the vector, pUC9, were digested to completion with *Bam*HI and *Eco*RI before ligation.

The primer pairs used to amplify the internal transcribed spacer (ITS1 and ITS4), and segments of the small and large mitochondrial rRNA genes (MS1 and MS2, ML7 and ML8, respectively) are as described by White et al (30). The oligonucleotide primers were synthesized and supplied by the Regional DNA Synthesis Laboratory, Calgary, Alberta, Canada. The reaction components for the polymerase chain reaction (PCR) were: 0.1–10 ng of genomic DNA, 50 pmol of each primer, 10 mM Tris (pH 8.3 at 25 C), 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100, and 2 units of Taq DNA Polymerase (BioCan, Mississauga, Ontario, Canada). For each reaction, 50 μ l of a 200-fold dilution of DNA from a small-scale extraction was placed in a 0.5-ml polypropylene tube to which 50 μ l of a 2 \times mix of all other components was then added. Three drops of autoclaved mineral oil were then added to prevent evaporation. Each tube was then sealed into the block of a thermal control device (MJ Research Inc., Cambridge, MA) with mineral oil. To monitor temperature, a thermocouple was placed in each of two tubes that contained 150 μ l of mineral oil and located in different parts of the block. The thermal program was as follows: 1) 93 C, 3 min; 2) 40 C, 1 min; 3) 62 C, 10 sec; 4) increase 9 C at rate of 1 C every 5 sec; 5) 71 C, 1 min; 6) 93 C, 1 min; 7) cycle back to step 2, 24 times; 8) 40 C, 1 min; 9) 62 C, 10 sec; 10) increase 9 C, 1 C every 5 sec; 11) 71 C, 5 min; 12) 4 C, hold. PCR products were observed on 1.5% agarose gels in Tris-acetate-EDTA (TAE) buffer.

Protoplasts were prepared for separation of large DNA molecules by electrophoresis on a clamped homogeneous electric field (CHEF) by the following method. For each strain examined, each of four 9-cm petri dishes containing 20 ml of liquid complete yeast medium (CYM) was inoculated with 10, 1-mm mycelial plugs. After 3 days of stationary growth, the four cultures were combined, trapped on 10- μ filters (Gelman), and washed with protoplast buffer (0.8 M MgSO₄, 0.2 M sodium citrate, pH 5.5). Mycelium was digested with 20 ml of NovoZym 234 (Novo Ind., Wilton, CT; 10 mg/ml in protoplast buffer) for 1 hr at room temperature on an Adams Nutator (Becton Dickinson, Parsippany, NJ). Residual mycelium was removed by filtration through glass wool, and 30 ml of KCl (0.6 M) was added to the filtrate. Protoplasts were recovered by centrifugation (setting 3 for 15 min) on a clinical centrifuge. The resulting pellets were washed with 10 ml of ST (sorbitol [1.0 M], EDTA [0.05 M]),

recentrifuged, and suspended in ST to a final concentration of 1×10^8 /ml. Protoplasts (1×10^7) were embedded in low-melting-temperature agarose plugs (LKB) and treated according to Orbach et al (23). A CHEF-DR II system (Bio-Rad Laboratories, Richmond, CA) was used for chromosomal separations. The gel contained 0.8% agarose in 0.5 \times Tris-borate-EDTA (TBE), and was run at 125 V with a switching ramp of 2–8 min for 3 days at 14 C. Chromosomal DNA from *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* (Bio-Rad) were used as standards.

Additional cultures used in this study were: *S. sclerotiorum* (LMK2 and LMK44), *S. minor* (LMK3 and LMK45), *S. trifoliorum* (LMK47 and LMK104), *N. crassa* (Adiopodumé [FGSC no. 430] and 74-OR23-1A [FGSC no. 987] from R. A. Collins), *F. oxysporum* f. sp. *raphani* and *conglutinans* (PHW666 and PHW808, respectively, from W. A. Powell), *O. ulmi* (TOR and two additional strains from M. Hubbes), *C. parasitica* (EP287 from W. A. Powell), *Cenococcum geophilum* (no. 155 from K. Lobuglio), *Aspergillus nidulans* (FGSC nos. 315 and 222 from the Fungal Genetics Stock Center, Kansas City, KS), *Armillaria bulbosa* (427-1 and 434-1 from J. B. Anderson), and *Agaricus brunnescens* (C4 from Canadian Spawn Co., Leamington, Ontario).

RESULTS

Determination of MCGs. Mycelial pairings were scored either as incompatible when a red reaction line on the colony reverse and a discontinuity were observed in the interaction zone between two strains 7 days after inoculation (Fig. 1A and B), or compatible when no reaction line was observed and two strains grew together (Fig. 1C and D). No variation was observed between compatible and incompatible reactions. Both compatible and incompatible reactions were remarkably uniform, although occasionally a pairing failed to produce a red line and was still scored as incompatible on the basis of the discontinuity between the two strains. From pairings of 63 strains, 32 MCGs (within each of which all strains were intercompatible) were distinguished. Of the 32 MCGs, six MCGs, each consisting of two or more strains, were identified with the largest including 19 strains (MCG 1; Table 1) observed only in the first field, and another including nine strains (MCG 2) observed in both fields. The other 26 MCGs were each made up of one strain, compatible only with itself.

For each of six apothecia, the 10 monosporous strains produced compatible interactions when paired in all combinations of siblings. The monosporous strains were paired with tester strains representing each of the MCGs identified among sclerotial isolates. The monosporous strains of three apothecia from the first field belonged to two MCGs, respectively. Strains from the apothecia from stations 8 and 10 were compatible with MCG 7 (containing one sclerotial strain from station 8B), and the strains from an apothecium at station 17 were compatible with MCG 3 (containing three sclerotial strains from stations 4B, 5B, and 12A; Table 1). The monosporous strains of the other three apothecia, from stations 19, 24, and 30, respectively, in the second field, belonged to three MCGs not characterized among the sclerotial strains.

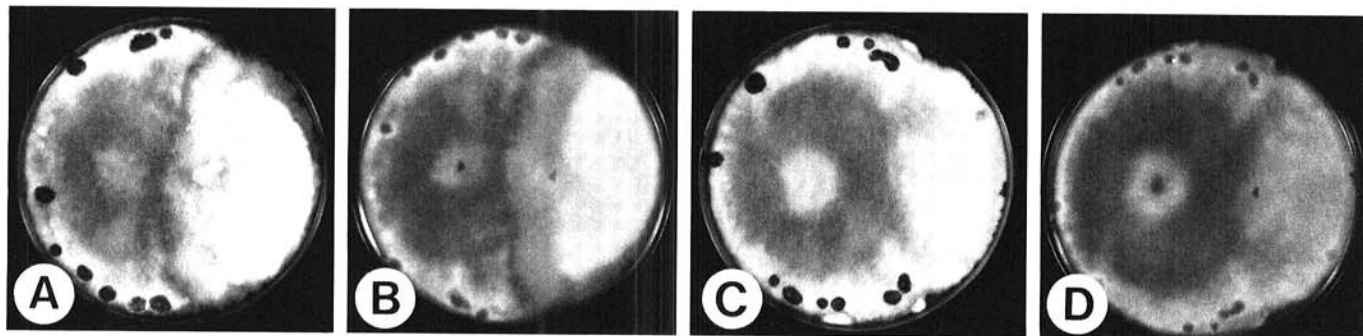


Fig. 1. Pairings of strains of *Sclerotinia sclerotiorum* that demonstrate representative reactions 7 days after inoculation. A and B show an incompatible interaction, with a reaction line, between strains 220 and 213. C and D show a compatible interaction between strains 220 and 205. A and C, colony surface in a 90-mm petri dish; B and D, colony reverse.

Molecular characterization of intraspecific variability. PCR amplification of both the ITS and a portion of the large mt rRNA gene from each of 58 of the 63 strains produced no size polymorphisms (data not shown). Amplification of the small mt rRNA gene, however, yielded four phenotypes (Fig. 2), with each of the two major phenotypes (fragment size of either 0.6 or 2.0 kb) shared by a large group of MCGs. With the exception of strains in MCG 2, with two phenotypes, strains in each MCG had only one length phenotype. The "aberrant" phenotype in MCG 2 (strains 210 and 211) included the 2.0-kb fragment observed in the other strains of the MCG. The origin of the additional fragments was not contamination; when DNA from independent mycelial samples was amplified again, exactly the same phenotype was observed. Controls with no target DNA did not yield any amplified products. A slightly different three-banded phenotype was also observed in both strains (241 and 242) of MCG 5 (Fig. 2B).

Six phenotypes were observed in Southern hybridization of *Hind*III-digested, genomic DNA from 58 of the 63 strains with pGP637 (Fig. 3). With the exception of heterogeneity in MCG 2, with three phenotypes, only one phenotype was observed within an MCG. With the exception of two of the phenotypes of MCG 2 and the phenotype of MCG 11 (consisting of one strain), each of the large mt rDNA phenotypes was shared by a group of MCGs.

A unique phenotype for each MCG was produced in Southern hybridizations of *Bam*HI-digested, genomic DNA from 58 strains with a plasmid probe containing a random fragment (about 4.5 kb) of nuclear DNA from *S. sclerotiorum*, pLK44.20 (Fig. 4).

Only one phenotype was observed for each MCG and no phenotypes were shared between MCGs. The high level of variability, the complex hybridization patterns, and the strength of the signal all suggested that this fragment was repeated several times and was dispersed among several chromosomes. The other two random fragments of homologous DNA that were used as cloned probes produced no polymorphisms in Southern hybridizations with DNA from the 58 strains.

The "fingerprint" probe pLK44.20 was characterized in a variety of ways. To determine whether this fragment is dispersed among chromosomes, large DNA molecules were separated on a CHEF gel. Six to seven chromosome-sized DNAs were resolved (Fig. 5), with five showing a strong signal in Southern hybridizations with pLK44.20. In northern hybridization of total RNA from *S. sclerotiorum* with pLK44.20, no transcript signal was observed after a 24-hr exposure. Very strong hybridization signals, corresponding to 18S, 26S, and 5S/5.8S RNAs, were observed when the same northern blot was hybridized with pAm2, an rDNA clone from *Armillaria* (3) after a 2-hr exposure (data not shown). In Southern hybridizations of *Bam*HI-digested DNA from strains of *Sclerotinia minor*, *S. trifoliorum*, *N. crassa*, *F. oxysporum*, *O. ulmi*, *C. parasitica*, *A. nidulans*, *C. geophilum*, *A. bulbosa*, and *A. brunnescens*, with pLK44.20, complex hybridization patterns, polymorphism between strains, and strong signals were observed only with *S. minor*. One, two, and three weakly hybridizing restriction fragments were observed with *N. crassa* (both strains), *S. trifoliorum* (both strains), and *Fusarium* strain 699, respectively. No hybridization was observed with DNA from the other species (data not shown).

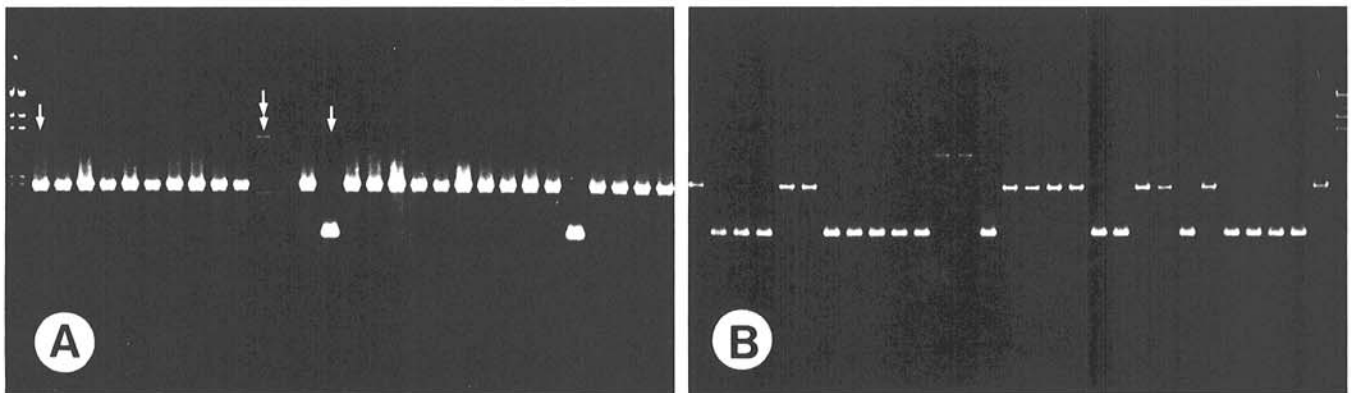


Fig. 2. Amplification of the segment of the small mitochondrial rRNA gene of *Sclerotinia sclerotiorum* defined by primers MS1 and MS2. Samples appear from left to right by order of strain number beginning with 198, as in Table 1, except that strains 203, 206, 217, 250, and 260 are omitted. **A**, Polymerase chain reaction (PCR) products of target DNAs of strains 198–229 from the first field. **B**, PCR products of target DNAs of strains 230 from the first field and 231–259 from the second field. *Hind*III-digested bacteriophage lambda DNAs are in the leftmost lane in **A** and the rightmost lane in **B**. Single arrows designate the two main phenotypes. The double arrow identifies the "aberrant" phenotype of strains 210 and 211 (MCG 2). A similar, but slightly different aberrant phenotype is seen in **B** for strains 241 and 242 (MCG 5).

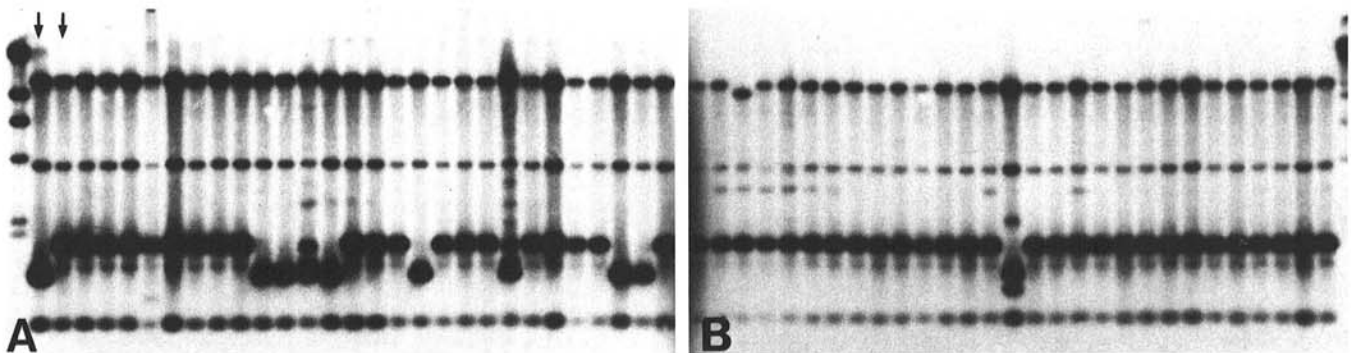


Fig. 3. Southern hybridizations of *Hind*III-digested DNAs of *Sclerotinia sclerotiorum* with pGP637, a plasmid carrying the large mitochondrial rRNA gene of *Neurospora crassa*. DNAs appear from left to right by order of strain number beginning with 198, except that strains 203, 206, 217, 250, and 260 are omitted. **A**, DNAs of strains 198–229 from the first field. **B**, DNAs of strains 230 from the first field and 231–259 from the second field. *Hind*III-digested bacteriophage lambda DNAs are in the leftmost lane in **A** and the rightmost lane in **B**. Arrows in **A** designate the two main DNA fragment phenotypes observed.

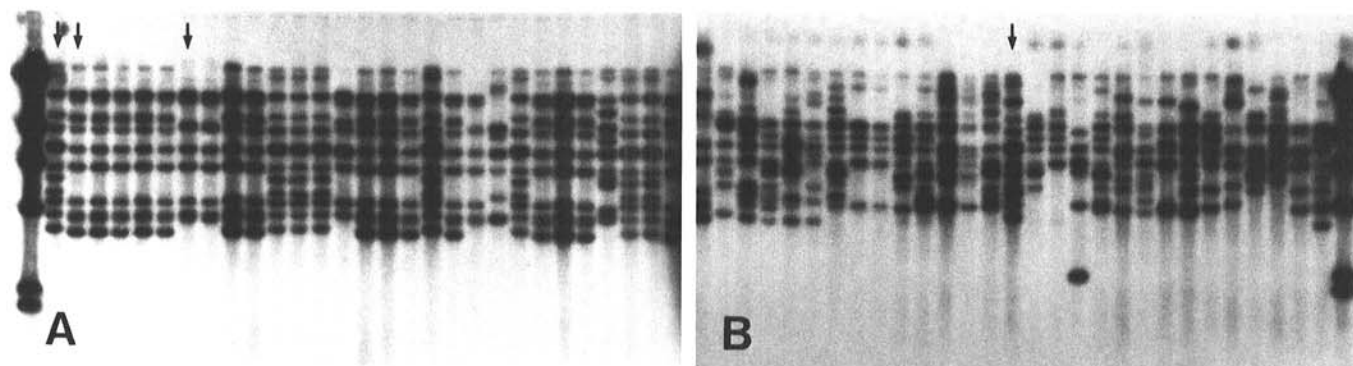


Fig. 4. Southern hybridizations of *Bam*HI-digested DNAs of *Sclerotinia sclerotiorum* with pLK44.20, a plasmid carrying a dispersed repetitive element. DNAs appear from left to right by order of strain number beginning with 198, except that strains 203, 206, 217, 250, and 260 are omitted and strains 225 and 226 are reversed. A, DNAs of strains 198-229 from the first field. B, DNAs of strains 230 from the first field and 231-259 from the second field. *Hind*III-digested bacteriophage lambda DNAs are in the leftmost lane in A and the rightmost lane in B. Arrows in A designate, from left to right, the DNA fingerprints of MCGs 2, 1, and 3, respectively. Arrow in B designates the DNA fingerprint of the single isolate of MCG 2 found in the second field.

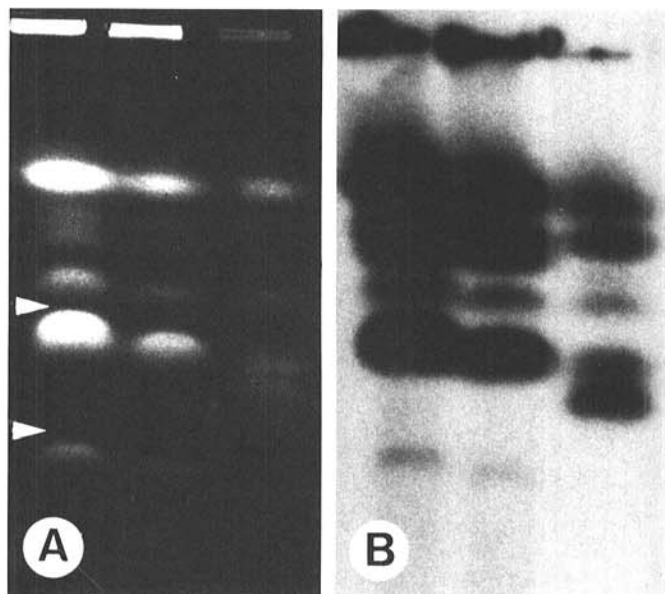


Fig. 5. Chromosome-sized DNAs of *Sclerotinia sclerotiorum*. A, Gel stained with ethidium; from left to right, DNA of strains 199, 224, and 44. B, Southern hybridization with radiolabeled pLK44.20. Arrows represent the positions of the 2.2 and 1.6 million base-pair chromosomal DNAs of *Saccharomyces cerevisiae*.

DISCUSSION

The data from this study demonstrate that mycelial incompatibility in *S. sclerotiorum* occurs within local field populations, as well as among strains from a worldwide sampling (19), and that a field population of *S. sclerotiorum* is composed of more than one MCG. Three molecular criteria, length polymorphisms in the small mt rRNA gene, RFLP phenotypes in Southern hybridizations with a heterologous probe containing the large mt rRNA gene, and the "fingerprint" RFLP phenotypes with the homologous, nuclear DNA probe, all confirm that each MCG in the sample is genotypically unique. No segregation of MCGs was evident among the single ascospore strains derived from six apothecia. With the possible exception of MCG 2, which had only one nuclear DNA "fingerprint" phenotype but more than one phenotype by the mitochondrial DNA criteria, each MCG in the field study appears to be genetically uniform. Taken together, these observations suggest that the MCGs represent genetically distinct "individuals" that have propagated either by clonal, asexual means or by homothallic (8), inbred, sexual means in the two fields sampled. Mycelial compatibility groups, like

vegetative compatibility groups (25), are thought to be determined by the alleles at several loci in the genome. The very large number of MCGs observed is entirely consistent with this model. If the isolates of a given MCG in this sample had originated independently in different crosses, genetic heterogeneity within the MCG, especially with respect to the nuclear "fingerprinting" element, would have been expected. At this point, we do not know whether or not the MCGs containing a single isolate arose through recent outcrossing; certainly the potential for outcrossing exists in *S. sclerotiorum* as it does in other homothallic ascomycetes such as *Sordaria fimicola* (9).

Size polymorphisms in DNA amplified by PCR yield valuable information, especially when comparing a large group of strains. Differences in length of a segment of the small mt rRNA gene in *S. sclerotiorum* suggest the presence of an intron. Introns, while common in the large mt rRNA gene (30), have not to our knowledge been reported in small mt rRNA gene of fungi. The amplification products of a segment of the large mt rRNA gene and the ITS showed no variation in size among the 58 strains examined (data not shown). In other studies, we have sequenced most of the ITS in six strains of *S. sclerotiorum* and have found no variation (J. B. Anderson and L. M. Kohn, unpublished).

Several dispersed, repetitive sequences, much like pLK44.20, have been located in other fungi (12,22) and are beginning to be applied in population and epidemiological studies. Dispersed, repetitive elements useful in genetic fingerprinting are also known in humans (15), birds (6), and plants (24). The exact correlation of complex RFLP phenotypes with mycelial (or vegetative) compatibility groupings reported here is, to the best of our knowledge, novel. The data from the study demonstrate that pLK44.20 is repeated, as shown in the complex hybridization patterns, and dispersed, as shown in Southern hybridization of the CHEF gels. It should be noted that the six to seven fragments resolved in the CHEF gels approach karyotype estimations based on cytological examination of six (10) and eight chromosomes (32). The insert in pLK44.20 does not contain any rRNA genes based, first, on the absence of any signal in a northern hybridization with RNA from *S. sclerotiorum*, and second, on the lack of correspondence in the sizes of restriction fragments hybridizing with pLK44.20 and cloned rDNA (20). Another experiment further indicated that pLK44.20 does not include the 5S rRNA gene, which is dispersed in several locations in the genome of *N. crassa* outside the tandemly repeated rDNA containing the 15S, 5.8S, and 25S genes (11,29). In our experiment, only a single restriction fragment of *N. crassa* DNA hybridized with pLK44.20, while in the study of Selker et al (29), several fragments hybridized with a cloned 5S rRNA gene. The strain of *N. crassa* used here and that used by Selker et al (29) are related to one another by seven generations of backcrossing (R. A. Collins, personal communication). We are currently sequencing this fragment with

the goal of comparing the nucleotide sequence with standard DNA sequence databases, and the sequences of dispersed, highly repetitive elements in other fungi.

The difference in distribution of MCGs between the two fields was notable. The dominance of two large MCGs, each containing relatively many strains, in the first field (with a total of six MCGs) contrasts with the predominance of groups, each containing only one strain, in the second field (with a total of 26 MCGs). Only one MCG was represented in both fields. The results for the first field are consistent with the existence either of a "founder effect," or of an initially diverse population that was subjected to selection. The second field appears to have been infected primarily by inoculum from a large number of sources outside of the field, with no selection for certain MCGs evident. It is important to note that sclerotia collected from the soil surface, while most likely to have fallen from lesions in the current season, could be one to several years old. Apothecia are produced from sclerotia that have carried over in the field from at least one previous season. Also, because the fields were heavily infected at the time of sampling, the inoculum that caused infection was from apothecia produced and probably decayed before sampling. Three of six apothecia tested belonged to two MCGs identified among the soil-surface sclerotia, which may reflect persistence of at least some MCGs from year to year. Studies to determine whether selection for certain MCGs occurs or whether some MCGs are more aggressive pathogens than others, demand a different approach with sampling before and after infection occurs for more than one year. Based on the confirmation of genetic heterogeneity in field populations reported here, such a study is now feasible and worthwhile.

The data from this study demonstrate that field populations of *S. sclerotiorum* are genetically heterogeneous and that the combination of mycelial compatibility grouping and molecular fingerprinting offer powerful tools for understanding this heterogeneity. In future studies combining field sampling, pathogenicity testing, and laboratory characterization, we hope to determine whether features pertinent to pathogenesis, such as aggressiveness, host specificity, and geographic distribution, correlate with clonal genotypes defined by mycelial compatibility grouping and molecular markers. The maintenance of genetic diversity reflected in the high level of mycelial incompatibility in local environs is intriguing and may be due to any of several factors. Through time, selection might result in certain MCGs being amplified or lost. Random drift might tend to reduce the diversity of MCGs, while immigration would tend to increase diversity. Outbreeding would release genetic variability through meiotic recombination and therefore increase the numbers of MCGs. With the availability of molecular-genetic markers, each of these factors is accessible to study.

LITERATURE CITED

1. Anagnostakis, S. L. 1987. Chestnut blight: The classical problem of an introduced pathogen. *Mycologia* 79:23-27.
2. Anderson, J. B., Bailey, S. S., and Pukkila, P. J. 1989. Variation in ribosomal DNA among biological species of *Armillaria*, a genus of root-infecting fungi. *Evolution* 43:1652-1662.
3. Anderson, J. B., Kohn, L. M., and Leslie, J. F. Genetic mechanisms in fungal adaptation. In: *The Fungal Community: Its Organization and Role in the Ecosystem*. 2nd ed. G. C. Carroll and D. T. Wicklow, eds. Marcel Dekker, New York and Basel, Switzerland. In press.
4. Anderson, J. B., Petsche, D. M., and Smith, M. 1987. Restriction fragment polymorphisms in biological species of *Armillaria mellea*. *Mycologia* 79:69-76.
5. Brasier, C. M. 1983. Inter-mycelial recognition systems in *Ceratocystis ulmi*: Their physiological properties and ecological importance. Pages 451-497 in: *The Ecology and Physiology of the Fungal Mycelium*. D. H. Jennings and A. D. M. Rayner, eds. Cambridge University Press, London.
6. Burke, T., and Bruford, M. W. 1987. DNA fingerprinting in birds. *Nature* 327:149-151.
7. Cruikshank, R. H. 1983. Distinction between *Sclerotinia* species by their pectic zymograms. *Trans. Br. Mycol. Soc.* 80:117-119.
8. Drayton, F. L. 1934. The sexual mechanism of *Sclerotinia gladioli*. *Mycologia* 26:46-72 and Pl. 5-7.
9. Fincham, J. R. S., Day, P. R., and Radford, A. 1979. *Fungal Genetics*. 4th ed. University of California Press. Berkeley. 636 pp.
10. Frandsen, K. J. 1946. *Studier over Sclerotinia trifoliorum* Eriksson. Danske Forlag. Copenhagen. 222 pp.
11. Free, S. J., Rice, P. W., and Metzner, R. L. 1979. Arrangement of the genes coding for ribosomal ribonucleic acids in *Neurospora crassa*. *J. Bacteriol.* 137:1219-1226.
12. Hamer, J. E., Farrall, L., Orbach, M. J., Valent, B., and Chumley, F. G. 1989. Host species-specific conservation of a family of repeated DNA sequences in the genome of a fungal plant pathogen. *Proc. Natl. Acad. Sci., USA* 86:9981-9985.
13. Heckman, J. E., and RajBhandary, U. L. 1979. Organization of tRNA and rRNA genes in *N. crassa* mitochondria: Intervening sequence in the large rRNA gene and strand distribution of the rRNA genes. *Cell* 17:583-595.
14. Jacobson, D. J., and Gordon, T. R. 1988. Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f. sp. *melonis*. *Phytopathology* 78:668-672.
15. Jeffreys, A. J., Wilson, V., and Thein, W. L. 1985. Hypervariable "minisatellite" regions in human DNA. *Nature* 314:67-73.
16. Katan, T., and Katan, J. 1988. Vegetative-compatibility grouping of *Fusarium oxysporum* f. sp. *vasinfectum* from tissue and the rhizosphere of cotton plants. *Phytopathology* 78:852-855.
17. Kohn, L. M. 1979. A monographic revision of the genus *Sclerotinia*. *Mycotaxon* 9:365-444.
18. Kohn, L. M. 1979. Delimitation of economically important plant pathogenic *Sclerotinia* species. *Phytopathology* 69:881-886.
19. Kohn, L. M., Carbone, I., and Anderson, J. B. 1990. Mycelial interactions in *Sclerotinia sclerotiorum*. *Exp. Mycol.* 14:255-267.
20. Kohn, L. M., Petsche, D. M., Bailey, S. R., Novak, L. A., and Anderson, J. B. 1988. Restriction fragment length polymorphisms in nuclear and mitochondrial DNA of *Sclerotinia* species. *Phytopathology* 78:1047-1051.
21. Le Tourneau, D. 1979. Morphology, cytology, and physiology of *Sclerotinia* species in culture. *Phytopathology* 69:887-890.
22. McDonald, B. A., and Martinez, J. P. 1990. Restriction fragment length polymorphisms in *Septoria tritici* occur at high frequency. *Curr. Genet.* 17:133-138.
23. Orbach, M. J., Vollrath, D., Davis, R. W., and Yanofsky, C. 1988. An electrophoretic karyotype of *Neurospora crassa*. *Mol. Cell. Biol.* 8:1469-1473.
24. Nybom, H., and Schaal, B. A. 1990. DNA "fingerprints" applied to paternity analysis in apples. *Theor. Appl. Genet.* 79:763-768.
25. Perkins, D. D. 1988. Main features of vegetative incompatibility in *Neurospora*. *Fungal Genet. Newsl.* 35:44-46.
26. Ploetz, R. C., and Correll, J. C. 1988. Vegetative compatibility among races of *Fusarium oxysporum* f. sp. *cubense*. *Plant Dis.* 2:325-328.
27. Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative incompatibility. *Can. J. Bot.* 63:179-183.
28. Purdy, L. H. 1979. *Sclerotinia sclerotiorum*: History, diseases, symptomatology, host range, geographic distribution, and impact. *Phytopathology* 69:875-880.
29. Selker, E. U., Yanofsky, C., Driftmier, K., Metzner, R. L., Alzner-DeWeerd, B., and RajBhandary, U. L. 1981. Dispersed 5S RNA genes in *N. crassa*: Structure, expression and evolution. *Cell* 24:819-828.
30. White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal DNA for phylogenetics. Pages 315-322 in: *PCR Protocols: A Guide to Methods and Applications*. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, Inc. San Diego, CA. 482 pp.
31. Willetts, H. J., and Wong, J. A-L. 1980. The biology of *Sclerotinia sclerotiorum*, *S. trifoliorum*, and *S. minor* with emphasis on specific nomenclature. *Bot. Rev.* 46:101-165.
32. Wong, J. A-L., and Willetts, H. J. 1979. Cytology of *Sclerotinia sclerotiorum* and related species. *J. Gen. Microbiol.* 112:29-34.
33. Zolan, M. E., and Pukkila, P. J. 1986. Inheritance of DNA methylation in *Coprinus cinereus*. *Mol. Cell. Biol.* 6:195-200.