Local and Trans-Canadian Clonal Distribution of *Sclerotinia sclerotiorum* on Canola

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ABSTRACT

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Clonal variability within and among field populations of *Sclerotinia sclerotiorum* isolated from canola petals in western Canada was determined by analysis of two independent criteria, mycelial compatibility and DNA fingerprinting. Strains were considered to belong to the same clone if they were mycelially compatible and also had identical DNA fingerprints. Thirty-nine clones were identified among 66 strains from seven locations in Alberta, Saskatchewan, and Manitoba. The most widely distributed clone, accounting for 18% of the isolates, was found in all three provinces. Seven other clones were found in two provinces. In 33 out of 36 mycelial compatibility groups (MCGs), each MCG had a unique DNA fingerprint; each of the remaining three MCGs included strains with one of two fingerprints and was interpreted as two clones. A comparison of strains from western Canada with those from a previous study of two fields in Ontario showed that the one clone identified in both Ontario fields was also present in Manitoba and Saskatchewan. This study demonstrates that clones of *S. sclerotiorum* are distributed over long distances geographically and confirms the results of the Ontario study in demonstrating that field populations of *S. sclerotiorum* on canola are composed of more than one clone. Analysis of monosporous siblings from homothallic sexual reproduction in each of two clones showed no meiotic segregation for determinants of either mycelial compatibility or DNA fingerprints. Therefore, intact clonal genotypes can potentially be dispersed as ascospores.

Additional keyword: Sclerotinia stem rot.

Under permissive environmental conditions, Sclerotinia stem rot of canola (oilseed rape, *Brassica campestris* L. or *B. napus* L.), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, can result in serious losses in yield due to lodging and premature shattering of seedpods. The infection process begins when airborne ascospores infest canola blossoms. Infection is initiated when infested petals dehisce, fall, and then adhere to the stem; ascospores germinate, penetrate the stem, and form lesions in which

sclerotia eventually develop. Sclerotia fall out of these lesions into the soil and, after physiological conditioning, including overwintering, germinate to form apothecia (7,18).

In a recent study (14) in Ontario each of two field populations of *S. sclerotiorum* on canola was shown to be genetically heterogeneous, i.e., composed of several clones, each clone capable of increasing through asexual or homothallic sexual reproduction. These clones were identified by two independent approaches, determination of mycelial compatibility groups (MCGs) and assay of molecular markers. All three molecular markers demonstrating intraspecific variability showed most MCGs to be genetically uniform. One of the molecular markers was a dispersed repetitive DNA sequence, pLK44.20, which when used as a cloned probe in Southern hybridizations produced a unique, complex hybridization pattern, a DNA fingerprint, for each MCG. Monosporous isolates of field-collected apothecia showed no evidence of recombination among the determinants of mycelial compatibility or DNA fingerprint. This homogeneity suggests that clones reproduce by inbred homothallic fruiting, in addition to asexual means.

The Ontario study (14) determined that mycelial compatibility and DNA fingerprinting with pLK44.20 were effective tools for identifying clones of *S. sclerotiorum*. That study also indicated that the two fields in Ontario were each a mosaic of different clones. Among the questions raised by these findings was whether some clones are distributed over long distances geographically. Because *S. sclerotiorum* occurs worldwide, infects at least 225 genera of host plants, including weeds (20), and is capable of perennation as resistant sclerotia in soil, knowledge of the distribution of clones could be a significant factor in the management of *Sclerotinia* diseases. If some clones of *S. sclerotiorum* are widely distributed, it should then be determined whether they are more aggressive or more pathogenically specialized than clones with more limited distribution. If so, then knowledge of clonal distributions and phenotypes would be important in screening breeding lines for resistance, as well as in other disease management approaches, such as forecasting.

Other recent studies have used molecular markers to identify clones of fungal plant pathogens. In a survey of a field population of *Septoria tritici* on wheat, McDonald and Martinez examined low-copy DNAs to compare multilocus genotypes (16) and DNA fingerprints (17) of isolates. Most of the *S. tritici* clones were recovered multiple times, most of them were each highly clustered at certain sampling sites, and six of seven sampling sites harbored more than one clone. In studies on the causal agent of rice blast, *Magnaporthe grisea*, Hamer et al (8) identified a dispersed repetitive DNA element that fingerprints clones and distinguishes those pathogenic to rice from those that are nonpathogenic. In a subsequent study (15), although most clonal lineages (isolates that have highly similar but not necessarily identical fingerprints) corresponded to single pathotypes (races), there were clear examples of two types of noncorrespondence. In the first, a clonal lineage was characterized by two pathotypes. In the second, a pathotype consisted of more than one unrelated clonal lineage, suggesting convergent origin of the pathotype. In addition to defining the relationship between clonal lineages and pathotypes, Levy et al (15) reported that several lineages were recovered multiple times and from different regions of the United States.

The objective of the studies reported here was twofold. First, using strains of *S. sclerotiorum* collected from the canola-growing regions of western Canada, we sought to determine whether each of the sampled field populations was genetically heterogeneous, i.e., composed of more than one clone, and whether some clones were distributed over long distances geographically. Clones were identified by both mycelial compatibility and DNA fingerprinting; the association of these two independent characters in clonal genotypes was tested in a large sample of strains. Second, we sought to determine whether segregation for determinants of mycelial compatibility or for the DNA fingerprint would be observed after meiosis in a controlled homothallic sexual fruiting where the MCG and DNA fingerprint of the parent strain were known.

MATERIALS AND METHODS

Two hundred ninety strains were isolated from ascospore-infested canola petals from Alberta, Saskatchewan, and Manitoba in 1990. The sampling was conducted in the final phase of development of a grower-based stem rot forecasting system, under the supervision of R. A. A. Morrall. Strains were isolated from *B. napus* (cultivars Alto, Global, Legend, Tribute, Vanguard, and Westar) and *B. campestris* (cultivars Horizon, Parkland, and Tobin). Field sampling of canola inflorescences and plating of petals on nutrient agar were as described by Turkington et al (24). Colonies of *S. sclerotiorum* were transferred and retained for this study. From these, we selected 66 strains from seven locations, representing one to three farms at each location, up to three fields at each farm, and four sites, at least 50 m apart, in each field (Table 1). Our objective in selecting from the 290 strains was to maximize the number of strains per locality in a hierarchical sampling of locality, farm, field, and site, while also retaining broad geographical sampling across the three provinces. Also important was the elimination of contaminants. Strains were maintained on potato-dextrose agar (Difco Laboratories, Detroit, MI), at room temperature (20–22 C).

Mycelial compatibility testing was performed as previously described (13). For efficiency, the 66 strains were arbitrarily divided into two equal sets, and strains in each set were then paired in all combinations. Tester strains representing MCGs identified from each of the two sets were paired, and all strains were classified in MCGs. Pairings were evaluated 7 days after inoculation. Pairings were scored as incompatible when a discontinuity in the interaction zone between the two strains was observed on the colony surface. This discontinuity was a strip of thin, sparse mycelium, a strip of dense aerial mycelium, and/or a red line in the interaction zone, most easily observed on the colony reverse (13). Pairings were scored as compatible when the two strains grew together to form one colony and no red reaction line was observed. All self-self pairings were compatible. Strains belonging to the same MCG were compatible; strains belonging to different MCGs were incompatible. Assignments to MCGs are shown in Table 1. Each MCG from this study and previous studies (14) in our laboratory is identified by a unique number.

For the DNA fingerprinting analysis, strains were grown on liquid complete yeast medium, containing 0.46 g of KH_2PO_4, 1.00 g of $K_2HPO_4 \cdot 3H_2O$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 20.0 g of D-glucose, 2.0 g of yeast extract (Difco), and 2.0 g of Bacto-peptone (Difco) per liter. Each strain was grown for 2 days before harvesting. Whole-cell genomic DNA was extracted from lyophilized samples by the method of Zolan and Pukkila (25) with modifications (14). DNAs were digested with *Bam*HI. The cloned probe used in these studies, pLK44.20, contains a 4.5-kb repeated dispersed element of nuclear DNA from *S. sclerotiorum* (14). The repeated element is confined to a 1.4-kb restriction fragment within the 4.5-kb insert of pLK44.20 (*unpublished data*). Procedures for radiolabeling and Southern hybridizations were as previously described (4) with the exception of electrophoretic conditions. DNAs of all strains were electrophoresed in 0.8% agarose gels at 1.5 V/cm for 16 h (short run), as in previous studies (4,14). For analysis of individual fragments within fingerprints (Table 2), DNAs of strains representing each unique fingerprint were electrophoresed in 0.8% agarose gels at 3.0 V/cm for 22 h (long run). Long runs, while confirming short-run data, provided better separation of hybridizing fragments for analysis.

A single isolate, LMK 211 (representing MCG 2), whose DNA fingerprint is shown in Figure 1, was used as a size standard against which each fragment in each of the fingerprints was compared. After the presence or absence of each fragment distinguishable by size in the western Canadian sample had been recorded (Table 2) and data from the Ontario study (14) had been included (fragment data are available from the authors upon request), the method of Jeffreys et al (11) was used to calculate the mean probability that all of the fragments present in the fingerprint of one clone would be present in another, independent

TABLE 1. Mycelial compatibility groups (MCGs) among strains of *Sclerotinia sclerotiorum* isolated from canola petals in western Canada

Locality	Farm	Field[a]	Site[a]	Canola cultivar	Strain[b]	MCG[c]
Camrose, Alberta	1	1	1	Alto	RM 1	42
					RM 2	67
			2	Alto	RM 3	33
					RM 4	38
			3	Alto	RM 5	43
			4	Alto	RM 6	39
Manning, Alberta	1	1	1	Parkland	RM 17	33
					RM 18	45
					RM 19	33
			2	Parkland	RM 20	35
			3	Parkland	RM 21	35
					RM 22	35
	2	1	4	Tobin	RM 23	34
			3	Tobin	RM 24	34
			2	Tobin	RM 25	34
					RM 26	38
			1	Tobin	RM 27	34
					RM 28	46
	3	1	4	3 Cultivars[d]	RM 214	34
			1	3 Cultivars[e]	RM 215	33
					RM 216	33
			2	3 Cultivars[e]	RM 217	33
					RM 218	33
Killarney, Manitoba	1	3	4	Westar	RM 166	57
		2	4	Westar	RM 167	2
		3	1	Westar	RM 168	58
			2	Westar	RM 170	59
	2	1	3	Westar	RM 186	61
					RM 187	40
			2	Vanguard	RM 189	62
					RM 190	63
		3	2	Westar	RM 247	40
					RM 248	36
			4	Westar	RM 249	33
					RM 250	38
			3	Westar	RM 251	37
		2	4	Westar	RM 252	36
Blaine Lake, Saskatchewan	1	1	2	Vanguard	RM 7	44
					RM 12	35
Outlook, Saskatchewan	1	1	1	Global	RM 100	47
					RM 101	48
					RM 102	33
					RM 103	39
					RM 104	33
		3	1	Global	RM 107	49
		4	1	...[f]	RM 108	50
					RM 109	51
					RM 110	52
					RM 111	53
Melfort, Saskatchewan	1	3	4	Westar	RM 144	67
			1	Westar	RM 145	54
		2	3	Tribute	RM 146	36
		1	4	Westar	RM 148	55
			1	Westar	RM 149	56
	2	1	...[g]	Legend	RM 285	64
					RM 286	65
					RM 287	33
					RM 288	66
					RM 289	41
					RM 290	41
Carrot River, Saskatchewan	1	1	4	Westar	RM 172	2
			1	Westar	RM 173	37
			3	Westar	RM 174	60
			1	Westar	RM 175	36
			4	Westar	RM 176	33
			2	Westar	RM 177	37

[a] At each farm, the fields sampled were designated 1, 2, 3, and 4. Within each field, each numbered site was at least 50 m from the next nearest site.
[b] Each strain is designated with a unique accession number (prefix RM). Strains were isolated from colonies growing out from petals placed on potato-dextrose agar amended with ampicillin (25 ppm) and streptomycin sulfate (25 ppm).
[c] A group of strains that are all compatible with each other, as determined by pairing tests.
[d] The field is a test plot in which three cultivars of *Brassica napus* (Westar, Alto, and Legend) were sampled.
[e] The field is a test plot in which three cultivars of *B. campestris* (Tobin, Parkland, and Horizon) were sampled.
[f] Strains from field 4 at Outlook farm 1 were isolated from *Sinapis alba*.
[g] Site not designated by collector.

clone.

Fingerprints of each strain in this study from western Canada were then compared with those of strains in the Ontario study (14); shared fingerprints were confirmed by an additional Southern hybridization. In comparisons of DNAs from Ontario strains with fingerprints identical to those of western Canadian strains, mycelial compatibility testing was also performed in order to determine whether strains that had the same fingerprint also belonged to the same MCG.

In studies of homothallic sexual reproduction, sclerotia were removed from 4-wk-old cultures of each of two Ontario strains (14), LMK 200 (MCG 1) and LMK 211 (MCG 2). The sclerotia were conditioned for apothecial development by immersion in aerated distilled water for 3 wk (10) and were then incubated on moist autoclaved sand (12). Three apothecia were harvested from each strain. From a mass of germinating ascospores shot from each apothecium, 20 single ascospores were selected at random, with the aid of a dissecting microscope (50× magnification) and needle. Germination was evaluated after 12 h. Ten germinated ascospores from each apothecium were chosen at random, isolated, incubated as mycelial strains, and prepared for DNA extraction and Southern hybridization with pLK44.20 as previously described. For mycelial compatibility testing, five

TABLE 2. DNA fingerprints of mycelial compatibility groups (MCGs) of *Sclerotinia sclerotiorum* isolated from canola in western Canada[a]

	Fragment number[b]												
MCG	1	2	3	4	5	6	7	8	9	10	11	12	13[c]
2	10	10000	100000	1000	10000	1000	1000	1000	100	1000	1000	1000	0
33	10	10000	100000	1000	10000	1000	1000	0001	100	0001	1000	1000	0
34i	10	10000	000100	1001	10000	1000	0000	1001	100	1000	1100	1001	0
34ii	10	10000	000100	1001	10000	1000	1000	1001	100	1100	1100	1001	1
35	00	00000	000100	0000	10100	1000	1000	0000	000	0000	0000	0001	1
36	00	00000	000000	1001	10000	1000	1000	0000	000	1001	0100	0001	1
37	00	00000	000010	0001	00010	1000	1000	0000	010	0000	0000	0001	1
38i	00	00000	000000	0000	10100	1000	1000	0000	000	0000	0000	0001	1
38ii	10	00100	000010	0000	10010	1100	1000	0000	010	0000	0000	0001	0
39i	10	00100	010000	1000	10000	1000	1000	0000	000	1000	1000	0001	1
39ii	10	00100	010000	1000	10000	1000	1000	0000	000	0000	0000	0001	1
40	00	00000	000000	0000	10000	1010	1000	0000	000	1000	0000	0001	0
41	10	00100	000010	0000	10001	1000	1000	0000	010	0000	0000	0001	0
42	00	00000	000000	0100	00000	1000	1000	0000	000	0000	0000	0001	1
43	00	00000	000000	0000	10001	1000	1000	0000	000	1000	1000	0001	1
44	10	00000	000100	0000	10001	1000	1000	0000	000	0000	1000	0001	0
45	10	00100	010000	0100	10000	1000	1000	0000	000	0000	0000	0001	1
46	00	00000	000100	0000	10000	1000	1000	0000	100	0000	0100	0001	1
47	00	00100	100000	1000	10000	1000	0000	0000	000	1000	1000	1000	0
48	00	00000	110000	0000	10000	1000	1000	0000	010	1000	1000	0001	1
49	00	10000	000000	0000	10000	1000	1000	0000	000	0000	0000	0101	1
50	00	00000	000100	0000	00000	1000	1010	0100	000	0000	0000	0001	0
51	00	00000	000000	0000	00000	0000	0000	0001	000	0001	0001	0001	0
52	00	00001	100100	0001	10000	1010	1000	0000	000	0000	0000	0001	0
53	00	00000	000000	0000	00000	1100	1000	0001	000	0000	0100	0001	0
54	00	00000	000000	0100	10011	1000	1000	0010	000	0001	0000	0101	0
55	00	00000	100000	1000	10100	1001	1010	0000	000	0000	0000	0001	1
56	10	00000	000000	0000	10000	1000	1000	0000	100	0001	1000	0001	0
57	00	00001	010000	0101	10001	1000	1000	0000	000	1000	1000	0010	0
58	00	00000	100000	0000	10000	1100	1001	1000	100	0000	0000	0001	1
59	10	00001	000100	0000	10000	0100	1010	0001	000	0000	0000	0001	0
60	00	00000	000000	0000	00000	1000	1000	0000	010	0000	1000	0001	1
61	00	00000	000000	0000	10000	1000	1000	0001	000	1000	0100	0001	0
62	00	00000	000000	0001	10000	1010	1000	0000	000	0000	1000	0001	0
63	00	00000	000000	0100	10000	1000	1000	0000	000	0001	0100	0001	0
64	00	00000	000010	0100	10001	1000	1000	0000	010	1000	1000	0001	0
65	10	00000	000100	0001	10001	1000	1000	0000	010	0000	1000	0001	1
66	00	00000	000000	0000	00011	1000	1000	0000	100	0001	1000	0101	0
67	11	00010	100010	0000	10001	1000	1010	0000	000	0000	1000	0001	0

[a] The sample from Ontario described by Kohn et al (14) included MCGs 1–32.
[b] Each fragment size class is listed by column, in which presence is indicated by 1 and absence by 0. Fragments 1–12 correspond to those shown in Figure 1; the size of fragment 13 is 0.8 kb. Fragments between numbered columns are of intermediate size. Fragments listed only as 0 were present in one or more strains from the Ontario study (14).
[c] The sizes of the three fragments intermediate in size between fragments 12 and 13 are 3.2, 2.7, and 2.2 kb.

strains were selected from the set of 10 siblings from each apothecium and paired in all combinations. These isolates were also paired with the parental strains and with testers from two other MCGs, strains LMK 207 and LMK 225 (MCGs 3 and 9, respectively). Procedures for testing and scoring were as described above.

RESULTS

Mycelial compatibility testing. A total of 36 MCGs were identified among the 66 western Canadian strains (Table 1). Twenty-five strains were incompatible with all others, each constituting an MCG of one strain. Among the remaining 41 strains, 11 MCGs were recognized. MCG 33 was the largest, consisting of 12 strains, followed by MCG 34, with five strains; MCGs 35 and 36, with four strains each; MCGs 37 and 38, with three strains each; and MCGs 2, 39, 40, 41, and 67, with two strains each. Scoring for compatibility versus incompatibility was consistent through repeated pairings, with only one exception. Strain RM 288 demonstrated "promiscuous" behavior; i.e., it produced apparently compatible reactions with a group of strains that were incompatible in pairings with one another. Furthermore, inconsistent reactions were observed in repeated pairings with this strain. We observed that the colony reverse of this strain, unlike that of typical isolates of *S. sclerotiorum*, was mottled with dark pigment. After several weeks, green *Trichoderma* sectors appeared on the colony surface. We then prepared an axenic isolate of this strain with which consistent compatibility reactions were obtained.

DNA fingerprinting. On the basis of phenotypes produced in Southern hybridizations of *Bam*HI-digested genomic DNA with the ^{32}P-labeled plasmid probe pLK44.20, the 66 strains were classified in 39 phenotypes. From the frequencies of all fragments in clones from Ontario (14) and western Canada (Fig. 1 and Table 2), the mean probability that all of the fragments present in one clone would be present in another, independent clone was calculated to be 8×10^{-8}. If fragments greater in size than 18 kb (fragment 3 in Fig. 1) are excluded, being difficult to measure accurately, this probability becomes 7×10^{-7}.

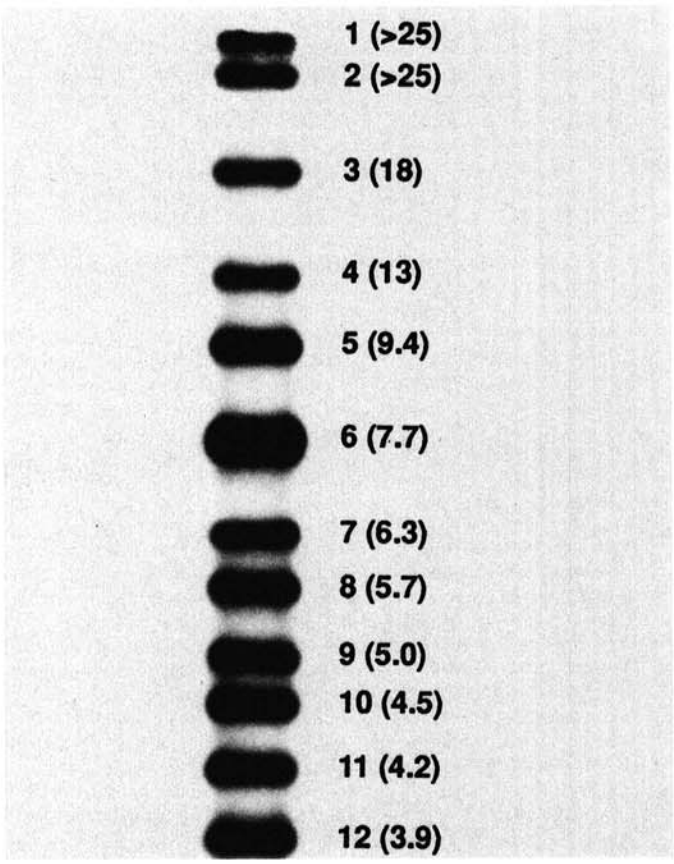

Fig. 1. Southern hybridization of *Bam*H1-digested genomic DNA of strain LMK 211 (mycelial compatibility group 2) of *Sclerotinia sclerotiorum* probed with pLK44.20, a plasmid carrying a dispersed repetitive element. DNA of this strain was used as a reference for all DNA fingerprints in this study. The fragments are numbered according to size, with their sizes in kilobases given in parentheses.

Identification of clones. By pooling the results obtained from mycelial compatibility testing and DNA fingerprinting, we grouped the 66 strains into 39 clones. Each clone is composed of a group of strains that share the same fingerprint and are compatible with all other strains within the group; 33 of the 36 MCGs were exactly equivalent to unique fingerprints (Table 2). In each of the remaining three MCGs, however, one strain had a different DNA fingerprint from the strains with which it was mycelially compatible. In MCG 34, strain RM 27 had three additional fragments not present in strains RM 23, 24, 25, and 214. In MCG 38, strain RM 250 had five hybridizing fragments that were not present in strains RM 4 and 26, which had one low-molecular-weight fragment absent from RM 250. In MCG 39, strain RM 103 lacked two low-molecular-weight fragments that were present in RM 6. By the strictest definition of a clone, in which both mycelial compatibility and DNA fingerprint are used as criteria, MCGs 34, 38, and 39 each consist of two clones.

Some clones were distributed over wide geographical areas. MCG 33, comprising 12 strains, was found in all three provinces. MCG 34 comprised five strains from two farms at Manning, Alberta. MCGs 2, 35, 36, 37, 38, 39, and 67 each consisted of two, three, or four strains and were represented in at least two of the three provinces where samples were obtained. MCGs 40 and 41 each consisted of two strains from one farm (in Killarney, Manitoba, and Melfort, Saskatchewan, respectively).

Comparison of strains from western and central Canada. By comparing the DNA fingerprints of the 63 strains previously isolated in the Ontario field study (14) with the 66 strains from western Canada, we determined that one DNA fingerprint occurred in strains from both areas. Side-by-side Southern hybridization confirmed that Ontario strain LMK 244 (MCG 2) had the same fingerprint as western Canadian strains RM 167 and RM 172. In mycelial compatibility tests, RM 167 and RM 172 were compatible with LMK 244, and they were all found to belong to MCG 2.

Homothallic sexual reproduction. Conditioned sclerotia of strains LMK 211 and LMK 200 produced apothecia after about 60 days of incubation. Ascospore germinability was almost 100% for LMK 211 (almost all transferred ascospores germinated and continued to grow) and 30% for LMK 200. All sibling monosporous isolates showed compatible interactions in pairings, all were compatible in pairings with parental strains, and all showed incompatible reactions (identical to those of parental isolates) in pairings with tester strains representing other MCGs. DNAs of all sibling monosporous isolates had identical restriction fragment length polymorphism phenotypes in Southern hybridizations with pLK44.20, and these phenotypes were identical to those of the respective parental strains (Fig. 2).

DISCUSSION

Data from this study demonstrate that some clones are distributed over long distances geographically (Fig. 3) and that each canola field population examined was infested by more than one clone of *S. sclerotiorum*, confirming observations from a previous field study in Ontario (14). The extent of observed correlation between two independent criteria, mycelial compatibility testing and DNA fingerprinting, among 66 strains further supports the interpretation that MCGs of *S. sclerotiorum* are clones. The identification of 25 clones (of 39) consisting of one strain may be due to the inability of our sample to detect several representatives of a clone. For example, there are several clones comprising two or three strains; sampling could easily result in the detection of only one strain (or no strains) of a clone. Given the number of clones with more than two strains detected in both this study and in the Ontario field study (14), as well as

the wide geographical distribution of some clones, we feel that the evidence for clonality is strong.

It should be noted, however, that MCG or vegetative compatibility group (VCG) systems in fungi are not necessarily clonal. The genetic determinants of mycelial compatibility are unknown in *S. sclerotiorum*, but the large number of MCGs that have been identified in field populations is consistent with the existence of several loci at which there are at least two alleles in the population. If mycelial compatibility, like vegetative compatibility, is determined by alleles at several loci (3,19), then the convergent origin of an MCG as the result of independent crossing events among different strains is unlikely. If the number of determinant loci is small, however, then the convergent origin of an MCG becomes more likely. Therefore, the use of MCGs or VCGs alone might not be sufficient to identify clones unambiguously. In Southern hybridizations with a dispersed repetitive DNA element such as pLK44.20, however, the probability that all strains of a convergently derived MCG would share the same complex fingerprint is estimated to be less than 1 in 10^6 (see Results). This calculation assumes that 1) the fragments of equal electrophoretic mobility represent the same "allele" at the same genetic locus and 2) fragments are distributed randomly among clones. These two conditions might not always obtain. For example, among groups of individuals that are closely related by descent, the distribution of fragments might differ from that in the population at large, and the probability of finding the same set of fragments in two independent clones would be elevated. Given these uncertainties, the inclusion of a second, independent criterion, mycelial compatibility, can only increase our ability to distinguish clones.

If identical clonal genotypes are unlikely to arise independently, how do they move from place to place? This study showed that all determinants for mycelial compatibility and the DNA fingerprint remained associated through meiosis in homothallic sexual reproduction. This means that intact clonal genotypes might be dispersed by ascospores, as well as by the movement of the mitotically produced sclerotia.

In this study of 36 MCGs among 66 strains sampled from several fields, we observed some departures from the correspondence of a unique DNA fingerprint for each MCG, which were not observed in the previous study of 32 MCGs from 63 strains sampled from two fields (14). Although no cases of two different MCGs having the same fingerprint were observed in the present study, there were three cases of an MCG with more than one fingerprint. Among the 36 western Canadian MCGs were three (MCGs 34, 38, and 39) in which one strain had a DNA fingerprint different from that of the majority. In such cases we consider the most plausible explanation to be a mutational or recombinational event in or near the sequence homologous to the fingerprint element, but not at loci governing mycelial compatibility. Whatever the mechanism of change in the fingerprint might be, the data are most consistent with the conclusion that mycelially compatible strains with slightly different fingerprints belong to the same lineage.

The clonal heterogeneity in field populations of *S. sclerotiorum* and the long-distance distribution of some clones present some interesting questions concerning the maintenance of genetic diversity in this species. The coexistence of several clones in field populations of plants and animals (21,22), as well as fungi (16,17), has been documented. *S. sclerotiorum* propagates both sexually and asexually, like many other clonal organisms (22). Homothallic sexual reproduction in *S. sclerotiorum* reduces the opportunity for genetic recombination, although outbreeding may still be possible, as in other homothallic ascomycetes, such as *Sordaria fimicola* and *Aspergillus nidulans* (6). We are currently conducting experiments to determine whether interclonal crosses are possible in *S. sclerotiorum*.

How do clones of *S. sclerotiorum* colonize a field? Sclerotinia stem rot of canola is mainly initiated by airborne ascospores. These ascospores originate from apothecia on soilborne sclerotia in or around the field. The sclerotia are produced asexually in stem lesions and generally overwinter at least once before they are physiologically conditioned to produce apothecia (7). Presumably a field in which a host of *S. sclerotiorum* has been grown within the approximately 4- to 5-yr life span of a sclerotium (2) would be colonized by at least one *S. sclerotiorum* clone. Even during crop rotation to nonhost plants, sclerotia in the field may remain dormant or, under suitable environmental conditions, produce apothecia. Ascospores from these apothecia could infect cultivated hosts or weeds in the field or in neighboring areas (1,5), expanding the range of the clone. Planting a host, such as canola, provides the opportunity for clones to colonize plants competitively, form lesions, and ultimately increase the population of soilborne sclerotia. Clones of *S. sclerotiorum* probably increase rapidly, randomly (depending on ascospore dispersal), and noncompetitively, leaving gaps of uninfected plants or sections

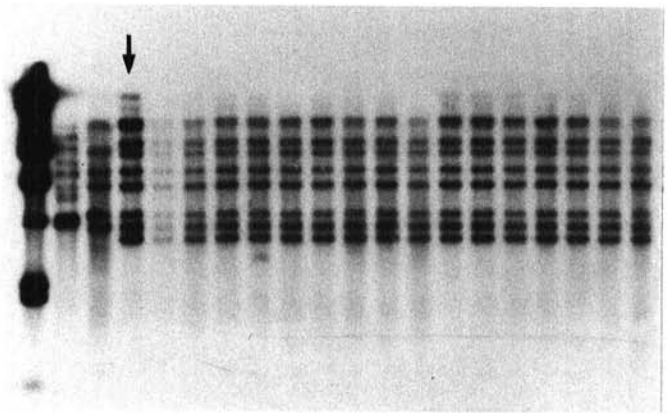

Fig. 2. Southern hybridizations of *Bam*H1-digested genomic DNAs of sibling monosporous isolates of *Sclerotinia sclerotiorum*, obtained from homothallic sexual reproduction of the parent strain, LMK 200, in mycelial compatibility group (MCG) 1, probed with pLK44.20. *Hin*dIII-digested DNA of bacteriophage λ is in the leftmost lane. From the left, lanes 2 and 3 are DNAs of strains LMK 225 (MCG 9) and LMK 207 (MCG 3), respectively. Lane 4 (arrow) is DNA of the parent strain, LMK 200. Lanes 5 through 20 are DNAs from single monosporous sibling strains.

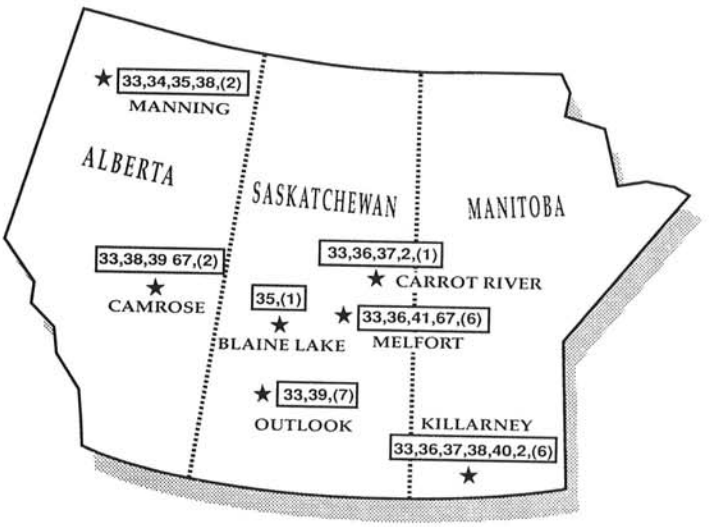

Fig. 3. Distribution of *Sclerotinia sclerotiorum* clones on canola at seven locations in western Canada. Mycelial compatibility groups (MCGs) are each designated by a unique identification number. Numbers without parentheses are the identification numbers of MCGs consisting of two or more strains. Numbers in parentheses indicate the number of MCGs each consisting of one strain identified at the locality; the identification numbers of these single-strain MCGs are given in Table 1.

of soil with few or no sclerotia, overlapping with other clones, and potentially replacing or being replaced by other clones. This can be described as a "guerrilla" growth form, similar to that of plants with nodal rooting, such as strawberries (9), and probably most clonal fungal pathogens. In contrast, organisms with slow, dense, competitive colonization by clones in a "phalanx" growth form are represented by animals such as corals (9) and fungi such as *Armillaria bulbosa* (23). In *A. bulbosa*, each clone is genetically unique and exclusively occupies a discrete territory.

How is a clonal mosaic maintained? Although we have established in this paper that immigration over long distances can occur, we do not necessarily believe that immigration is the sole factor controlling clonal diversity in populations. For example, in the two fields sampled in Ontario (14), one field was represented by six clones, some with many strains, while the other field, only 3 km away, was represented by 27 clones, most with only one strain. This suggested the effect of immigration or outbreeding in increasing the number of clones in the second field and the effect of other factors, such as selection, drift, or a founder effect in reducing the number of clones in the first field. In a field population of *S. sclerotiorum*, clonal diversity could be maintained by factors such as selective neutrality, transient selection, and diversifying selection (22), as well as the more obvious mechanisms of immigration, outbreeding, and mutation. Diversifying selection is an especially pertinent theory which suggests that different genotypes are favored in different environments. In populations subject to changing conditions, the coexistence of several clones would be favored over clonal dominance or homogeneity due to selection. Changing and patchy conditions characterize the environment of *S. sclerotiorum* in the field. Macro- and microclimate, cropping history, host cultivars, and agronomic practices all offer diverse environmental opportunities to a clonal population of *S. sclerotiorum*. A preliminary greenhouse study of six Ontario clones strongly suggests that clones differ in their aggressiveness on three cultivars of *B. napus* (L. M. Kohn, *unpublished data*). Such factors, resulting in a highly disturbed and variable environment, would all tend to support a clonal mosaic in field populations. Now that markers are available for the identification of clones, *S. sclerotiorum* is extremely amenable to the study of factors determining clonal distribution in fungal plant pathogens. We are currently investigating whether the clonal mosaic changes during the disease cycle, i.e., whether clones that predominate as inoculum also predominate in sclerotium-producing lesions. We are also evaluating whether different hosts of *S. sclerotiorum* grown in the same area act as bait for certain clones, which would suggest that the availability of the host exerts selection pressure on clonal distribution.

How are clones of *S. sclerotiorum* distributed over long distances? Dispersal of infested seed by human activity must be considered, although most canola growers use seed that has been treated with a fungicide. No seed treatment is 100% effective, and it has not been determined whether mycelium of *S. sclerotiorum* in the testa or sclerotia mixed with the seed survive this treatment. Also, *S. sclerotiorum* is usually found at a very low level when canola seed is plated on agar (R. K. Gugel, *personal communication*). Seed is cleaned on a spiral cleaner, presumably removing most, but not necessarily all, sclerotia. However, dispersal in seed of canola or other hosts cannot be ruled out, and this should be investigated. It is also possible that some clones have been widely dispersed for some time and that clonal diversity in populations where they occur is maintained by the mechanisms discussed previously. The wide host range and distribution of the species tends to support this theory. Investigation of the biogeography of clones of *S. sclerotiorum*—for example, to determine whether clonal distributions are limited to regions of North America or whether North American, South American, and Australasian clones overlap—would be of interest. Future studies of clonal distribution should include the identification of clones from various hosts in addition to canola.

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