Chromosome Size Polymorphism in *Leptosphaeria maculans*

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ABSTRACT

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Chromosome size polymorphism among nine isolates of *Leptosphaeria maculans* was studied by hybridization of homologous and heterologous DNA fragments to chromosomes separated by contour-clamped homogeneous electric field (CHEF) electrophoresis. Four of the fungal isolates were highly virulent and three were weakly virulent in *Brassica* plants; the remaining two originated from the cruciferous weed *Thlaspi arvense*. There was a high degree of chromosome size polymorphism, and no two isolates had the same karyotype. However, three general patterns could be distinguished by differences in size range, distribution of bands within the size ranges, and hybridization to probes. These three banding patterns corresponded to the three pathogenicity groups. The four highly virulent isolates had 12–14 chromosomal bands with estimated sizes ranging from 0.7 to 3.7 Mb. The three weakly virulent isolates had 11 or 12 bands with sizes between 0.8 and 2.7 Mb, and the two *Thlaspi* isolates had 14 and 16 bands with sizes between 1.0 and 3.2 Mb. Some of the bands were brighter than average and may represent more than one chromosome. The weakly virulent and *Thlaspi* isolates had a higher proportion of small chromosomes, while those of the highly virulent isolates were more evenly distributed throughout the size range. Most of the DNA fragments used as probes hybridized to a single chromosome in each isolate and to chromosomes of similar sizes (± 0.5 Mb) within a pathogenicity group, but often to a chromosome with a very different size in isolates from the other pathogenicity groups. Hybridization to many chromosomes by DNA fragments cloned from one of the highly virulent isolates indicated the presence of repetitive sequences specific for highly virulent isolates. The results indicate that these pathogenicity groups are very different from each other and perhaps different species.

Additional keywords: blackleg, electrophoretic karyotype, phytopathogen, *Phoma lingam*.

The ascomycete *Leptosphaeria maculans* (Desm.) Ces. & de Not. (anamorph: *Phoma lingam* (Tode ex Fr.) Desm.) causes disease in many cruciferous plants (17). In rapeseed (*Brassica napus* and *B. campestris*), the fungus infects the leaf tissue early in the season and produces spots. The fungus spreads down the petiole and the stem through the xylem vessels and eventually invades and kills cells of the stem cortex causing a canker. This stem canker is the symptom from which the name of the disease, blackleg, originates (15). This is not, however, the only manifestation of the disease. In addition to leaf spots and stem cankers, the fungus can cause damping-off of seedlings, root rot, seed infection, and postharvest damage to leaf and root crops, such as cabbage and rutabaga (11).

L. maculans isolates from *Brassica* have been traditionally classified into two groups according to their pathogenicity: highly virulent or aggressive and weakly virulent or nonaggressive (17,31). The weakly virulent isolates cause only mild disease symptoms and cause minor damage to crops. The highly virulent isolates, in contrast, are considered a serious threat to oilseed rape production (13). The number of known differential characteristics between highly virulent and weakly virulent isolates has increased and strongly indicates that these two groups of isolates belong to different species. Some of these differential characteristics are pigment production, growth rate on laboratory media, malate dehydrogenase isozyme patterns (17,18), level of production of certain polysaccharide-degrading enzymes (16), production of the toxin sirodesmin (23,35), chromosomal restriction fragment length polymorphisms (16,21,24), and electrophoretic karyotypes (46,47).

L. maculans also occurs on cruciferous weeds such as *Thlaspi arvense* (stinkweed), *Sisymbrium loeselii, Descurainia richardsonii,* and *Lepidium* sp. (36). Most of these weed isolates closely resemble the *Brassica* weakly virulent group in morphology and pathogenicity to rapeseed, although they are usually more virulent to their weed host (36,38).

Pulsed-field gel electrophoresis (PFGE) was first described by Schwartz and Cantor (41). Since that time a variety of systems have been developed that allow separation of chromosomes up to 12 Mb in size (for reviews see 33,42). Karyotypes obtained by PFGE show the common occurrence of chromosome size polymorphisms in many fungi, including yeasts (1,20), filamentous ascomycetes (4), basidiomycetes (2,19,30), and fungi imperfecti (28,45). Furthermore, some chromosomes are supernumerary and dispensable (B chromosomes) (32).

Taylor et al (46,47) found a large variation in chromosome sizes among isolates of *L. maculans* after separation by transverse alternating field electrophoresis (TAFE). There were, however, two general banding patterns that corresponded to the two pathogenicity groups. Since information about variability in pathogenicity and probability of genetic exchange among the different groups of *L. maculans* is important for the planning of breeding programs for blackleg resistance, we decided to use a PFGE system that yielded chromosome separations suitable for hybridization studies. We report here the separation of chromosomes of *L. maculans* by contour-clamped homogeneous electric fields (CHEF) electrophoresis (3) in a larger range of sizes than previously reported (46) and the assignment of several cloned genes and homologous probes to specific chromosomes.

MATERIALS AND METHODS

Fungal isolates and plasmids. The list of *L. maculans* isolates and their characteristics appears in Table 1. The plasmids used as probes are listed in Table 2.

Culture conditions. Isolates were grown on V8 juice agar plates (36) under fluorescent lights at room temperature with a 12-h photoperiod or, to promote sporulation, under black lights

(fluorescent tube G070, F40/BLB16, Sylvania Lighting Equipment, Danvers, MA) with a 12-h photoperiod and temperatures of 24 C during light and 17 C during darkness. Pycnidiospores were collected from approximately 2-wk-old cultures and stored in 50% glycerol at −20 C. Frozen pycnidiospores were warmed to 4 C for at least 2 h; they were then used at a higher temperature.

Sample preparation for PFGE. Pycnidiospores were diluted in protoplasting solution to approximately 1×10^{10} spores per milliliter and digested for 4 h at 28 C and shaken at 60 rpm. The protoplasting solution was a modification of the one used by Farman and Oliver (6) as follows: Driselase 10 mg/ml (Sigma Chemical Company, St. Louis, MO), lysing enzyme from *Trichoderma harzianum* 100 mg/ml (Sigma), chitinase 0.25 mg/ml (Boehringer GmbH, Mannheim, Germany), β-glucuronidase (type B1) 32 mg/ml (Sigma), and bovine serum albumin 13 mg/ml (Sigma) in 0.8 M NaCl. The digested spore suspension was overlaid on 5 ml of STEN buffer (31.5% sucrose, 10 mM Tris HCl, pH 7.5, 0.1 mM EDTA, 20 mM NaCl) and centrifuged at 3,000 *g* for 15 min at 4 C. The spores that did not pellet but remained in the STEN buffer were collected by repeating the centrifugation after dilution with one volume of 0.8 M NaCl. The treated spores were gently resuspended in STEN buffer to approximately 10^9 spores per milliliter (usually 1–3 ml volume) and mixed with one volume of hot 1.6% low melting point agarose in STEN buffer. The spore mixture was quickly dispensed into a block former (Bio-Rad, Richmond, CA) and allowed to solidify. The blocks were then taken out and digested with E buffer (7.5 mg/ml pronase [self-digested for 1 h at 37 C], 0.5 M EDTA, 10 mM Tris-HCl, pH 8.0, 1% [w/v] *N*-lauroyl sarcosine) (46).

PFGE conditions. The electrophoretic separation of chromosomes was carried out on a Bio-Rad CHEF DRII system using 0.5× TBE (27) at 14 C in 0.8% agarose gels (DNA grade, GIBCO BRL, Gaithersburg, MD). Many conditions were tested to improve the separation of chromosomes in different size ranges. The following sets of electrophoretic conditions described by duration, voltage, and linear gradient of switching time cover most of the chromosome size range of the *L. maculans* isolates. Set A separates the large chromosomes from 3 up to 6 Mb: 115 h, 50 V, 3,600–1,800 sec; 24 h, 50 V, 1,800–1,367 sec; 30 h, 60 V, 1,367–825 sec; and 30 h, 80 V, 825–600 sec. Set B separates most of the chromosomes smaller than 3 Mb: 92 h, 50 V, 2,700–1,500 sec; 26 h, 50 V, 1,500–1,175 sec; 28 h, 60 V, 1,175–825 sec; 24 h, 80 V, 825–600 sec; and 24 h, 80 V, and 600 sec. Set C separates chromosomes smaller than 2.5 Mb: 100 h, 80 V, 300–825 sec; 41 h, 60 V, 825–1,180 sec; 37 h, 50 V, 1,180–1,500 sec; and 18 h, 50 V, 1,500–2,700 sec.

Estimation of chromosome sizes. Photographic negatives of ethidium bromide-stained gels were scanned with a LKB Ultroscan XL enhanced laser densitometer, and chromosome sizes were estimated by interpolation from the size standards using the Pharmacia GelScan XL software (version 2.1) (Pharmacia LKB, Piscataway, NJ). The chromosome size standards used were: *Schizosaccharomyces pombe* strain 972 and *Saccharomyces cerevisiae* strain YNN295 obtained from Bio-Rad (Richmond, CA), and *Candida albicans* strain ATCC 14053 purchased from Clontech (Palo Alto, CA). The sizes of chromosomes from *S. pombe* and *S. cerevisiae* were obtained from Bio-Rad. The sizes of the *C. albicans* chromosomes were estimated by interpolation from the mobility of the chromosomal bands from *S. pombe* and *S. cerevisiae* in CHEF gels run under different conditions.

DNA manipulations. DNA from groups of chromosomal bands from the isolate Leroy were purified from CHEF low melting point agarose gels by digestion with agarase (Calbiochem, La Jolla, CA) (40). The purified DNA was digested with *Hin*dIII,

TABLE 1. List of *Leptosphaeria maculans* isolates used in this study

Isolate name	Isolate name abbreviation	Virulence to rapeseed	Origin	Source
Leroy	L	Highly virulent	Rapeseed	R. K. Gugel and G. A. Petrie
PHW1276	H6	Highly virulent	Rapeseed	P. H. Williams
PHW1275	H5	Highly virulent	Rapeseed	P. H. Williams
WR5	W	Highly virulent	Rapeseed	R. K. Gugel and G. A. Petrie
Unity	U	Weakly virulent	Rapeseed	R. K. Gugel and G. A. Petrie
North Battleford 2	N	Weakly virulent	Rapeseed	R. K. Gugel and G. A. Petrie
Outlook 2	O	Weakly virulent	Rapeseed	R. K. Gugel and G. A. Petrie
Thl4	T4	Weakly virulent	*Thlaspi arvense*	R. K. Gugel and G. A. Petrie
Thl5	T5	Weakly virulent	*Thlaspi arvense*	R. K. Gugel and G. A. Petrie

TABLE 2. Plasmids used as probes for hybridization

Plasmid	Insert size (kb)	Gene(s)	Organism of origin	Reference	Source
Heterologous probes					
pMF2	6.7	17,5.8 and 26S rDNA	*Neurospora crassa*	10	R. L. Metzenberg
pJD81	2.6	β-Tubulin	*Neurospora crassa*	29	J. C. Dunlap
pPpA35	1.4	Actin	*Physarum polycephalum*	14	Luc Adam
pRP43-1	4.4	*gpd*	*Curvularia lunata*	34	H. D. Osiewacz
pHA1	3.3	UB14 (ubiquitin)	*Saccharomyces cerevisiae*	9	R. K. Storms
pJF1	3.7	UB13	*Saccharomyces cerevisiae*	9	R. K. Storms
pMW30	4.0	*oli*C31	*Aspergillus nidulans*	49	M. Ward
pAO4-2	4.0	*pyr*G	*Aspergillus oryzae*	39	C. A. M. J. J. van den Hondel
pUC-C31	0.95	Cutinase	*Fusarium solanii*	43	P. E. Kolattukudy
pMSK381	7.6	Shikimate dehydrogenase	*Neurospora crassa*	12	Fungal Genetic Stock Center (FGSC)
pMSK338	3.2	Dehydrokinase	*Neurospora crassa*	12	FGSC
pSTA10	7.5	*nii*A and *nia*D	*Aspergillus niger*	48	J. R. Kinghorn
pLAM7	7.2	*lam*A and *lam*B	*Aspergillus nidulans*	22	M. E. Katz
pCYW	2.3	*crp*-1	*Neurospora crassa*	25	J. E. Heckman
pH1.1	2.0	H2A core histone	*Aspergillus nidulans*	5	G. May
pH3	0.7	H3 core histone	*Aspergillus nidulans*	5	G. May
$pGBH_2B.1$	4.0	H2A and H2B core histones	*Aspergillus nidulans*	5	G. May
pH4C.2	0.7	H4 core histone	*Aspergillus nidulans*	5	G. May
Homologous probes cloned from *Leptosphaeria maculans*					
L217	2.8	Unknown	Isolate Leroy	This work	
TB133	0.533	Unknown	Isolate Leroy	This work	
TB306	5.5	Unknown	Isolate Leroy	This work	
T17	2.8	Unknown	Isolate Leroy	This work	
T23	4.7	Unknown	Isolate Leroy	This work	

ligated to *Hin*dIII-linearized vector pTZ19R (Pharmacia LKB, Piscataway, NJ) and transformed into DH5αmcr cells that had been made competent by a modified calcium-rubidium method (26).

Plasmid DNA was isolated by the alkaline lysis method of Maniatis et al (27) either as a miniprep or a scaled-up version of this procedure.

Gels to be blotted were exposed to UV light (300 nm) for 2 min followed by denaturation for 2 × 15 min in 0.5 M NaOH, 1.5 M NaCl, and neutralization for 2 × 15 min in 1 M Tris HCl, pH 7.4, 1.5 M NaCl. DNA transfer onto nylon membranes (Nytran, Schleicher & Schuell, Keene, NH) was done by the capillary method using 10× SSPE (0.18 M NaCl, 10 mM $NaPO_4$, pH 7.7, 10 mM EDTA) as the blotting buffer.

Hybridization probes were labeled with ^{32}P by the random primers technique (7,8). Blots were prehybridized overnight at 42 C or 32 C in 50% formamide, 6× SSPE, 0.5% SDS, 5× Denhardt's solution, and 100 mg/ml denatured low-molecular-weight salmon sperm DNA. Hybridization was performed in the same buffer and temperature for 24 h (44). The low-stringency wash for blots hybridized at 42 C was performed at 37 C. For high stringency the blots received an additional washing at 65 C. Blots hybridized at 32 C were washed at 32 C.

RESULTS

Conditions for chromosome separation. Low voltages and long switching times were required for good resolution of chromosomal DNAs. Although voltages above 80 V shortened the running times, they produced diffused and poorly resolved chromosomal bands. Because of the lower background of degraded DNA, the preparation of sample blocks without the use of $MgSO_4$ improved the resolution slightly. The banding pattern for a given isolate was very reproducible for different runs as well as for different batches of sample blocks. The brightness of the bands, however, did not follow the simple relation of brightness to size of DNA fragment that is commonly observed in conventional linear electrophoresis. Some bands were consistently brighter or dimmer than expected from their apparent size. Some of these bright bands could be resolved into two or more bands by changing the PFGE conditions, but others ran as a single bright band in all conditions tested. Differences in band brightness are commonly observed in PFGE gels of fungal chromosomes. It is generally assumed that the bright bands are formed by two or more chromosomes of similar sizes. However, some of the bright bands observed in our gels also gave a strong hybridization signal as if they contained double or triple the amount of a single DNA fragment.

Chromosomal banding patterns after PFGE. No two isolates had identical banding patterns (Fig. 1). These results were expected from the findings by Taylor et al (47) who screened a larger number of isolates using TAFE. CHEF electrophoresis, however, allowed the separation of chromosomes of a wider range of sizes with better band resolution. The number of chromosomes separated from the highly virulent isolates increased (for example in isolate WR5, from 8 to 14). The better band resolution and the use of a wider range of chromosome standards permitted a more accurate estimation of total chromosome numbers and sizes. There were three general patterns that corresponded to the three groups of isolates. These three patterns are distinctive because of their size ranges and the distribution of bands within these ranges. The size of the smallest chromosome is similar in all the isolates (0.7 and 1.0 Mb). The highly virulent isolates have the largest chromosomes and the distribution of bands is approximately homogeneous throughout the size range (0.7–3.7 Mb). The weakly virulent and *Thlaspi* isolates have chromosomes in a narrower size range (0.8–3.2 Mb) and with a heavy concentration of bands in the lower end of this range. Figure 2 presents a graphic summary of the banding patterns which also includes information obtained using electrophoretic conditions different from those shown in Figure 1.

The sizes of the genome, estimated from eight electrophoretic runs, range from 23.2 to 32.0 Mb for the highly virulent isolates,

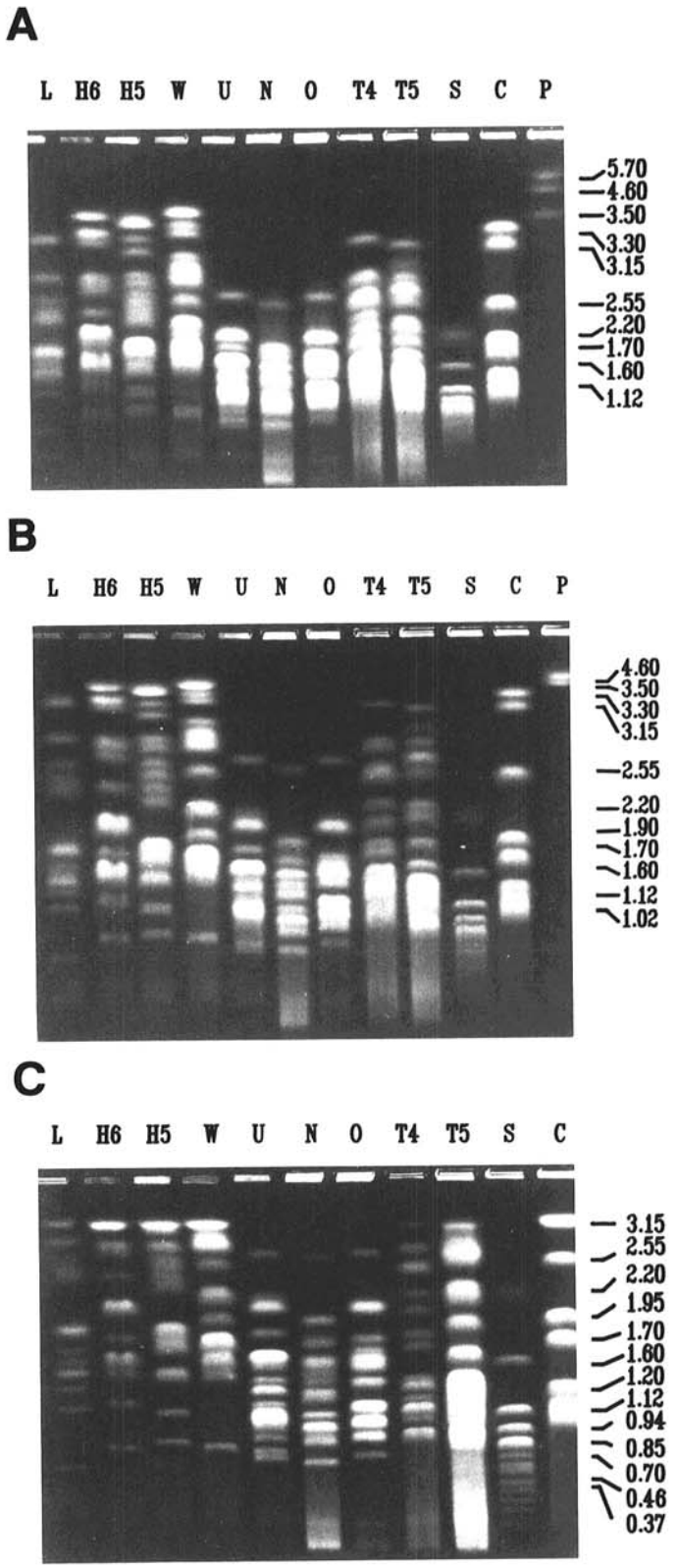

Fig. 1. CHEF electrophoresis of isolates under different electrophoretic conditions. **A,** Separation obtained using the set A conditions; **B,** Separation obtained using the set B conditions; **C,** Separation obtained using the set C conditions (see Materials and Methods for a description of the electrophoretic conditions). Isolate name abbreviations are: L, Leroy; H6, PHW1276; H5, PHW1275; W, WR5; U, Unity; N, North Battleford 2; O, Outlook 2, T4, Thl4; and T5, Thl5. For size standards: S, *Saccharomyces cerevisiae* YNN295; C, *Candida albicans* ATCC 14053 and P, *Schizosaccharomyces pombe* strain 972. The numbers on the right represent the chromosome sizes of the standards in Mb.

from 16.6 to 17.1 Mb for the weakly virulent isolates, and from 25.8 to 30.1 Mb for the *Thlaspi* isolates. For these estimates each band was assumed to be formed by a single chromosome; therefore they are minimal estimates of genome size.

Hybridization results. Fewer than half of the heterologous probes hybridized to *L. maculans* DNA blots of CHEF gels under the conditions used, even though some of the genes encoded by the probes are regarded as well conserved among fungi (Table 3). These results were all obtained with a hybridization temperature of 42 C, except for pHA1 and PJF1, which only hybridized at 32 C. Those plasmids listed in Table 2 that do not appear in Table 3 did not hybridize to any of the *L. maculans* chromosomes. We did not search exhaustively for hybridization conditions suitable for the probes that failed under our normal procedures. Lowering the stringency of washings or the temperature of hybridization resulted in faint nonspecific signals.

Most of the probes that hybridized did so to a single chromosome in each isolate. One exception was plasmid T17 which hybridized to apparently all of the chromosomes of the highly virulent isolates and to none of the other groups (Fig. 3). Evidently this clone contains repetitive sequences specific for the highly virulent isolates. Several other clones have been obtained from the highly virulent isolates that contain repetitive sequences and their characteristics will be published elsewhere. Most of the probes hybridized to chromosomes of relatively similar sizes (±0.5 Mb) within a pathogenicity group (see Table 3), but often to very dissimilar chromosomes when pathogenicity groups were compared. An example of this is shown in Figure 4 with probe TB306, which carries a DNA fragment that was cloned from isolate Leroy. There are, however, two types of exceptions to this pattern: Some genes are present on chromosomes of very different sizes in isolates of the same pathogenicity groups and two probes that hybridize to the same chromosome in one isolate may hybridize to separate chromosomes in another isolate of the same pathogenicity group. An example of the first type of exception are the genes coding for the ribosomal RNA (probe pMF2) which are present on chromosomes of very different sizes among the highly virulent isolates (Fig. 5). The second type of exception occurs with probes pJD81 and L217. These two probes hybridize to the same chromosome in two of the highly virulent isolates but are separated in the other two highly virulent isolates (see Table 3).

DISCUSSION

Large variation in chromosome sizes was observed among the nine isolates of *L. maculans* tested; however, three chromosomal patterns could be discerned that corresponded with the three pathogenicity groups. The hybridization results showed that the variation in chromosome size is much smaller within than across pathogenicity groups. However, the two types of exceptions to this generality mentioned in the results indicate a relatively high frequency of chromosomal rearrangements.

The relative brightness and sharpness of a given band changes little with electrophoretic conditions or between batches of sample blocks. Some of the bands were either sharp or diffused, faint or bright in all the electrophoretic conditions that gave a good quality separation. The second largest band from Thl5, for example, was always very faint and was not regarded as a true chromosomal band until it was shown to code for the ribosomal RNA genes (Fig. 5). Bands fainter than the average probably result from a tendency toward entrapment of those chromosomes in the spore debris or to degradation during the sample block preparation. The extent of entrapment or degradation may reflect differences in the physiological states of the chromosomes, such as the level of gene transcription or DNA-protein association, at the time of spore wall digestion. Some of the bright bands may represent two nonhomologous chromosomes with very

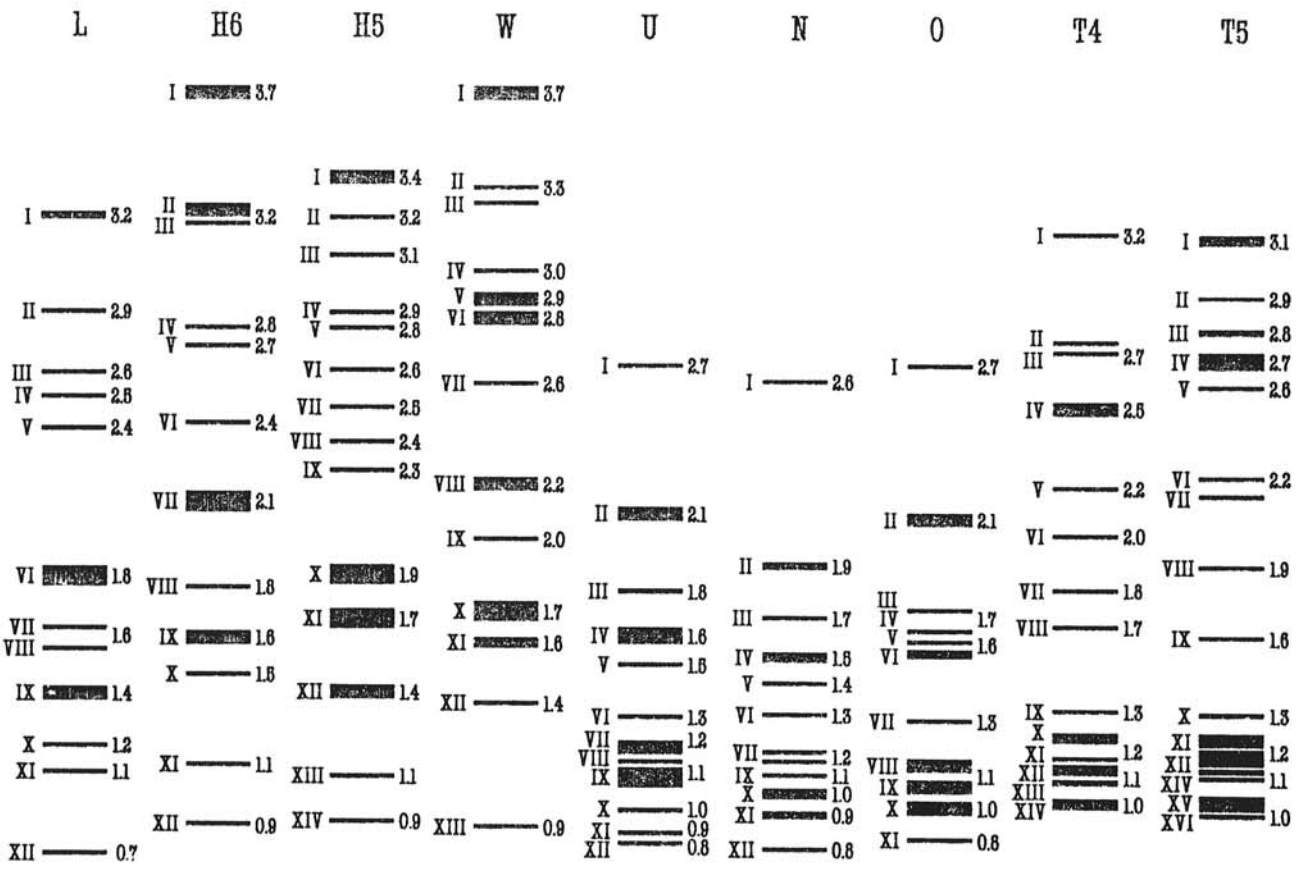

Fig. 2. Summary of chromosomal banding patterns for *Leptosphaeria maculans* isolates. The data used were collected from gel scans from eight electrophoretic runs under different PFGE conditions. The numbers on the right of the band represent the apparent size in Mb. These size estimations were rounded to the nearest first decimal. The characteristic thickness and migration of the bands were used in the drawing to convey a more accurate representation of the stained gel. Isolate identification as in Figure 1.

similar sizes. They may also be chromosomes that are well protected from nucleases during the spore wall digestion or that are present in more copies than normal (hyperploid chromosomes). More information is needed before the composition of the bright bands can be certain.

The total number of chromosomes cannot be determined because the composition of the bright bands is uncertain. The minimal number of chromosomes is 14 for the highly virulent and *Thlaspi* isolates and 12 for the weakly virulent isolates. The *Thlaspi* isolates may have the largest number of chromosomes

TABLE 3. Assignment of genes and cloned fragments to chromosomes by hybridization to homologous and heterologous probes

		Homology with chromosomal band in isolate[b]								
Probe[a]	Gene	L	H6	H5	W	U	N	O	T4	T5
pMF2	rDNA subunit	VI[c]	I	I	VI	II	III	II	IV	II
		1.8[d]	3.7	3.4	2.8	2.1	1.7	2.1	2.5	2.9
pJD81	β-Tubulin	VII or VIII	VII	X	IX	IV	IV	VI	IV	IV
		1.6	2.1	1.9	2.0	1.6	1.5	1.6	2.5	2.7
pPpA35	Actin	I	II	I	I	VII	VII or VIII	VII	IX	XI
		3.2	3.2	3.4	3.7	1.2	1.2	1.3	1.3	1.2
pRP43-1	*gpd*	X	IX	XII	XII	VII	IX	VIII	X	XII
		1.2	1.6	1.4	1.4	1.2	1.1	1.1	1.2	1.2
pH3	Histone H3	IV	VI	VIII	VII	II	II	II	I	I
		2.5	2.4	2.4	2.6	2.1	1.9	2.1	3.2	3.1
pH4C.2	Histone H4	IV	VI	VIII	VII	II	II	II	I	I
		2.5	2.4	2.4	2.6	2.1	1.9	2.1	3.2	3.1
pJF1	UBI3	NH[e]	II	II	I	NH	NH	NH	NH	II
			3.2	3.2	3.7					2.9
pHA1	UBI4	I	II	II	I	NH	NH	NH	II	IV
		3.2	3.2	3.2	3.7				2.7	2.7
L217	Unknown	VI	VII	X	X	II	II	II	IV	VII
		1.8	2.1	1.9	1.7	2.1	1.9	2.1	2.5	2.2
TB133	Unknown	III	IV	IV	IV	II	II	II	VI	VII
		2.6	2.8	2.9	3.0	2.1	1.9	2.1	2.0	2.2
T23	Unknown	I	I	I	I	IV	V	IV or V	VII	IX
		3.2	3.7	3.4	3.7	1.6	1.4	1.6	1.8	1.6
TB306	Unknown	I	I	I	I	VIII	XI	IX	XII	XII
		3.2	3.7	3.4	3.7	1.1	0.9	1.1	1.1	1.2
T17	Unknown	AC[f]	AC	AC	AC	NH	NH	NH	NH	NH

[a] For information on the plasmids used as probes see Table 2.
[b] Abbreviations of the isolates names are in Table 1.
[c] Chromosomal band numbers were assigned in order of decreasing size as in Figure 2.
[d] Estimated size of chromosomal bands in Mb.
[e] No hybridization.
[f] All chromosomal bands.

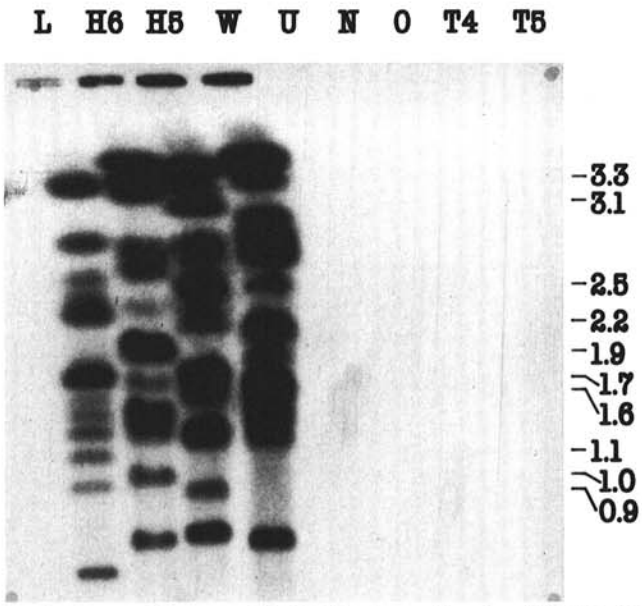

Fig. 3. Autoradiogram of hybridization with plasmid T17. The hybridization conditions were low stringency. The blot was obtained from a PFGE gel run under set B conditions (see Methods). The numbers on the right represent the chromosome sizes of the standards in Mb. Isolate abbreviations as in Figure 1.

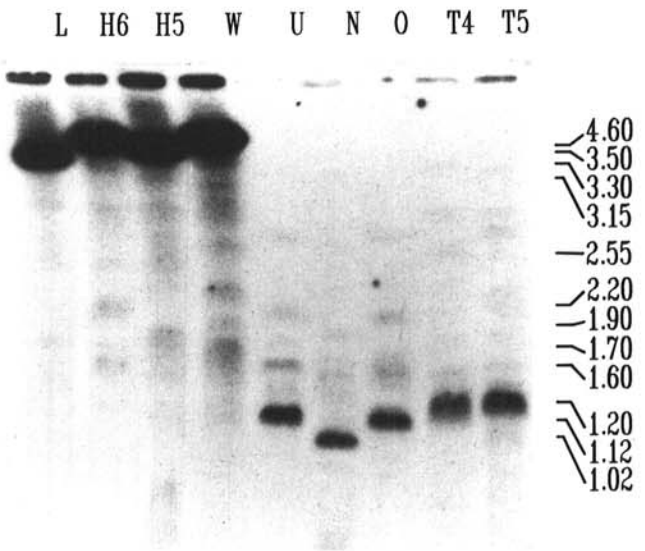

Fig. 4. Autoradiogram of hybridization with plasmid TB306. Low-stringency hybridization conditions were used on a blot obtained from a gel run under the set B conditions. The numbers on the right represent the chromosome sizes of the standards in Mb. Isolate abbreviations as in Figure 1.

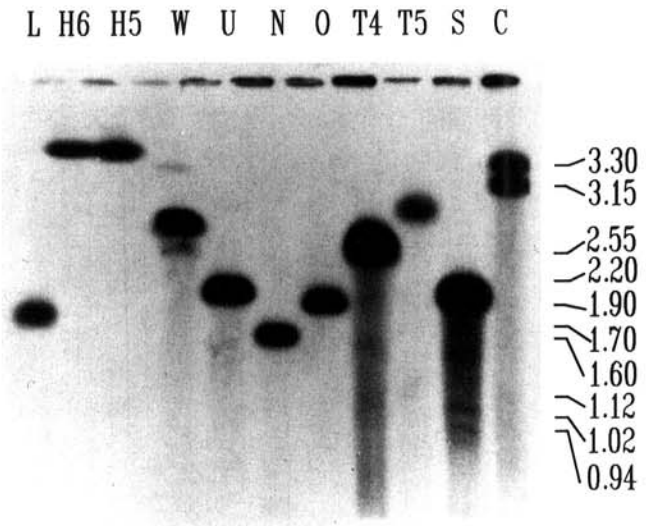

Fig. 5. Autoradiogram of hybridization with plasmid pMF2. High-stringency conditions were used on a blot obtained from a gel run under the set B conditions. The numbers on the right represent the chromosome sizes of the standards in Mb. Isolate abbreviations as in Figure 1.

because Thl5 has at least 16 chromosomes and possibly more, because the presence of unresolved heavy bands at the bottom of the gels. It is also possible that variation in the total genome content occurs within, as well as across, pathogenicity groups. The weakly virulent isolates may have the smallest genome of the three groups.

The extent of chromosomal polymorphism found among isolates of *L. maculans* indicates the existence of an active mechanism for chromosome rearrangements. This mechanism may involve repetitive sequences such as those present in clone T17. It would be interesting to know if repetitive sequences are also common among isolates from other pathogenicity groups. The effect of this high degree of chromosomal variation on sexual reproduction remains to be investigated. Since *L. maculans* is a heterothallic fungus and ascocarps of the different pathogenicity groups are a common occurrence in crop residue (37), chromosome polymorphisms may not affect sexual reproduction to the extent suggested by Cooley and Caten (4) for *Septoria nodorum*.

The clear differences in karyotypes and gene allocations to chromosomes between the highly and weakly virulent groups of *L. maculans* are further indications that they represent different species. Although the differences observed between the *Thlaspi* and the weakly virulent isolates suggest that they are distinct groups, the establishment of a more precise relationship requires more information, particularly on sexual compatibility. Unfortunately, successful matings have been obtained in the laboratory only among highly virulent isolates (17,37). Therefore, the study of gene exchange among the different groups may require the use of molecular identification techniques to study the composition of natural populations of ascospores found on rapeseed stubble.

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