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Identification and Characterization of Chromosome Length Polymorphisms Among Strains Representing Fourteen Races of *Ustilago hordei*

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Electrophoretic karyotypes were determined for monosporidial strains that represent each of the 14 races of *Ustilago hordei*. A unique karyotype was observed for each strain; the number of chromosome-sized DNA bands varied from a minimum of 15 to a maximum of 19, and their approximate length distribution ranged from 170 to 3,150 kilobases. Strains were also obtained from one ordered and six unordered meiotic tetrads representing four of the races. Karyotype was typically conserved among members of individual tetrads and between tetrads representing the same race. The strains from one tetrad from race 14, however, showed 2:2 segregation for one chromosome-sized DNA band. Conversely, the total soluble protein from 14 of these strains fractionated by sodium dodecyl sulfate-PAGE did not vary to the same extent as the karyotypes. Southern hybridization was used to identify chromosome length polymorphisms and to map genomic fragments and heterologous conserved genes to the chromosomes.

Additional keywords: contour-clamped homogeneous electric field electrophoresis, heterobasidiomycetes, karyotypic analysis, pulsed-field gel electrophoresis, smut fungi.

Ustilago hordei Pers. (Lagerh.) is the causal agent of covered smut of barley (*Hordeum vulgare* L.), a disease of continuing importance where barley is commercially grown (Thomas 1984). The importance of this pathogen has prompted classical genetic investigations (Thomas 1988) and, more recently, molecular genetic analysis (Holden *et al.* 1988). Heterokaryotic infectious hyphae produced by the fusion of compatible haploid basidiospores penetrate the coleoptile of a germinating barley seedling and reside in the developing meristem. The host plant remains essentially symptomless until heading, when the teliospores are formed and replace the kernels on diseased plants (Thomas 1988). There is no vegetative or asexual reproduction of *U. hordei*, and the proximity of compatible basidiospores on the probasidium of a germinated teliospore increases the probability that reproduction will occur by inbreeding (Caten 1987). A well-characterized physiologic race structure comprising at least 14 races has been described for *U. hordei* (Tapke 1945; Pedersen and Kiesling 1979; W. L. Pedersen, personal communication), and the races are sexually compatible (Emara 1972). Moreover, a gene-for-gene relationship (Flor 1955) has been described for this host-parasite system by Sidhu and Person (1972).

The ability to fractionate chromosome-sized DNA molecules by pulsed-field (agarose) gel electrophoresis (PFGE, Schwartz and Cantor 1984) has led to new approaches for karyotypic analysis of fungi, whose cytogenetics are often intractable. More recently, the development of contour-clamped homogeneous electric field (CHEF) PFGE has made feasible the comparison of the chromosomes of numerous organisms or isolates in a single gel (Chu *et al.* 1986). Using PFGE, the haploid number of chromosomes of laboratory strains of *U. maydis* (DeCandolle) Corda was determined to be at least 20, and 10 highly conserved heterologous genes from fungi and animals were mapped by Southern hybridization (Southern 1975) to chromosome-sized DNAs of this fungus (Kinscherf and Leong 1988). Karyotypic variability was shown to correlate with pathogenic variation in *Nectria haematococca* Berk. & Broome (Miao and VanEtten 1989) and has been used as a criterion for confirming the distinction between diploid and polyploid isolates of *Phytophthora megasperma* Drechs. (Howlett, in press). Other fungi in which genes have been mapped to chromosome-sized DNAs include *Saccharomyces cerevisiae* Hansen (Carle and Olson 1985), *Neurospora crassa* Shear & Dodge (Orbach *et al.* 1988), and *Candida albicans* (Robin) Berkhout (Magee *et al.* 1988). Furthermore, chromosome length polymorphisms have been observed among different laboratory strains of *S. cerevisiae* (Ono and Ishino-Arao 1988).

In this study we report the electrophoretic karyotype of strains representing 14 races of *U. hordei*. A high degree of karyotypic variability was observed among individual strains representing each of the races but not among strains derived from individual meiotic tetrads, although the segregation of a chromosome length polymorphism in one meiotic tetrad was observed. The strains showed a high degree of similarity of the total soluble protein complement using sodium dodecyl sulfate (SDS)-PAGE. We have begun to define linkage groups using random DNA fragments.

MATERIALS AND METHODS

Fungal and bacterial strains, plasmids, and media. Teliospores from collections representing the 14 races of *U. hordei* (Table 1) were kindly provided by W. L. Pedersen, University of Illinois, Urbana, and are derived from the original isolates described by Tapke (1945). Monosporidial strains of *U. hordei* were isolated from teliospores that

were germinated on potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI) at room temperature. Germinated teliospores containing four primary basidiospores were removed on a small block of agar with a fine glass needle and vortexed in 500 μl of potato-dextrose broth (PDB, Difco). The entire cell suspension was spread onto fresh PDA with a glass rod, and the individual colonies that emerged were transferred to PDA slants where they were maintained and transferred bimonthly. Alternatively, ordered tetrads were isolated with a Sensaur micromanipulator as described by Thomas (1988).

Table 1. Strains and plasmids used in this study

Designation	Relevant characteristics[a]	Source or reference
Ustilago hordei		
1.3	Product of unordered tetrad of race 1	W. L. Pedersen[b]; Tapke 1945
2.1	Product of unordered tetrad of race 2	Tapke 1945
3.1	Product of unordered tetrad of race 3	Tapke 1945
4.1	Product of unordered tetrad of race 4	Tapke 1945
5.1	Product of unordered tetrad of race 5	Tapke 1945
6.1	Product of unordered tetrad of race 6	Tapke 1945
7.1	Product of unordered tetrad of race 7	Tapke 1945
8.1	Product of unordered tetrad of race 8	Tapke 1945
9.1	Product of unordered tetrad of race 9	Tapke 1945
10.1	Product of unordered tetrad of race 10	Tapke 1945
11.1	Product of unordered tetrad of race 11	Tapke 1945
12.1	Product of unordered tetrad of race 12	Tapke 1945
13.1	Product of unordered tetrad of race 13	Tapke 1945
14.1a	Product of unordered tetrad of race 14	Pedersen and Kiesling 1979[c]
14.1b	Product of unordered tetrad of race 14	Pedersen and Kiesling 1979
14.1c	Product of unordered tetrad of race 14	Pedersen and Kiesling 1979
14.1d	Product of unordered tetrad of race 14	Pedersen and Kiesling 1979
U. maydis	Minnesota field isolate, 1987	K. B. Johnson[d]
Escherichia coli DH5α		Focus 8(2):9, 1986[e]
Plasmids		
pUC18	Ap^r cloning vector	Norrander *et al.* 1984
pSF8	*Aspergillus nidulans* actin gene	Fidel et al. 1988
pZHS3	*Saccharomyces cerevisiae cox* III	Muller *et al.* 1984
pOSU4011	6-kb *U. hordei Eco*RI fragment in pUC18	This study
pOSU4012	1.2-kb *U. hordei Eco*RI fragment in pUC18	This study
pOSU4015	3.2-kb *U. hordei Eco*RI fragment in pUC18	This study
pOSU4117	6-kb *U. hordei Eco*RI fragment in pUC18	This study

[a] Ap^r, ampicillin-resistant; kb, kilobase.
[b] All *U. hordei* samples were provided by W. L. Pedersen, Univ. of Illinois, Urbana, as teliospores.
[c] Also listed as 14.1 in the figures.
[d] Oregon State Univ., Corvallis.
[e] Published by Bethesda Research Laboratories, Gaithersburg, MD.

Escherichia coli DH5α was used as the host for plasmid transformations. Selections were conducted in Luria-Bertani medium with 50 or 100 μg/ml of ampicillin, when appropriate. The plasmids used and constructed in this study are presented in Table 1.

Chromosomal sample preparation. Chromosome-sized DNA was prepared for electrophoresis by a modification of the method of Kinscherf and Leong (1988). Briefly, haploid sporidia grown for 18 hr in 100 ml of PDB at 28° C were harvested by centrifugation in a Sorvall SS34 rotor at 5,000 × *g* for 10 min. The cell pellet was suspended in 10 ml of a solution containing 10 mM ethylenediaminetetraacetic acid (EDTA), pH 8, 25 mM 2-mercaptoethanol and incubated with gentle shaking for 20 min at room temperature. The cells were centrifuged as before, and the pellet was suspended in 4.5 ml of 0.8 M sorbitol, 50 mM sodium citrate, pH 5.8 (SC50). A 0.5-ml aliquot of SC50 containing 50 units per milliliter of Novozyme 234 (Calbiochem, La Jolla, CA) and 200 mM 2-mercaptoethanol was added to each sample, and the cell suspension was incubated at room temperature for approximately 2 hr with gentle shaking. The protoplast mixture was centrifuged at 300 × *g* for 10 min, and the pellet was gently suspended in 100–200 μl of SC50 to a final concentration of approximately 5×10^8 protoplasts per milliliter. An equal volume of 2.5% low-melting temperature agarose (FMC Bioproducts, Rockland, ME) dissolved in SC50 was added to each sample, and the entire mixture was gently pipetted into a well of a precooled plug casting mold (Bio-Rad Laboratories, Richmond, CA). The plugs were incubated from 18 to 24 hr at 50° C in a solution containing 1 mg/ml of protease (type XIV, Sigma, St. Louis, MO), 0.45 M EDTA, pH 8, and 1% SDS. They were subsequently rinsed with 0.5 M EDTA (pH 8) and thereafter stored at 5° C in the same solution.

CHEF electrophoresis conditions. Electrophoresis was conducted in a Bio-Rad CHEF DR-II electrophoresis system. Bands in the 100- to 1,500-kilobase (kb) range were resolved by electrophoresis through 1% agarose (Sigma type II, medium electroendosmosis) gels at 14° C in 0.5× TBE buffer (Maniatis *et al.* 1982) for 15 hr at 200 V with a 70-sec switch interval, followed by 11 hr at 200 V with a 120-sec switch interval. Resolution of bands in the 1,500- to 3,500-kb range was accomplished using a modification of the parameters described by Howlett (1989) to resolve chromosomes from *Phytophthora*. The chromosome-sized DNA bands were visualized by staining with 0.5 μg/ml of ethidium bromide in 0.5× TBE for 30 min and photographed after destaining overnight in 0.5× TBE. *S. cerevisiae* and *Schizosaccharomyces pombe* Lindner chromosome size standards were obtained from Bio-Rad Laboratories.

Source of DNA probes. Total genomic DNA was isolated from a haploid strain of race 8 using the method of Specht *et al.* (1982). The DNA was digested to completion with *Eco*RI and ligated into pUC18, and the entire mixture was used to transform *E. coli* DH5α according to the methods of Maniatis *et al.* (1982). Plasmid DNA mini-preps were obtained by alkaline lysis according to the method of

Birnboim and Doly (1979). The inserts in randomly selected recombinant plasmids were released by digestion with *Eco*RI and purified from low-melting temperature agarose gels using Elutip-d (Schleicher & Schuell, Keene, NH) according to the manufacturer's instructions. Radiolabeled DNA was prepared using the random priming technique of Feinberg and Vogelstein (1983) and labeled to a specific activity of 10^8 to 10^9 cpm per microgram of DNA.

DNA hybridizations. Gels were blotted to nylon membranes (Genetran, Plasco, Woburn, MA) according to the methods of Orbach *et al.* (1988). Blots were prehybridized overnight in 20 ml of 3× SSPE (Maniatis *et al.* 1982), 50% formamide, 5% dextran sulfate, 5× Denhardt's solution, 0.1% SDS, and 100 μg/ml salmon sperm DNA at 42° C. Hybridization reactions were conducted in 10 ml of the same solution without the salmon sperm DNA, but containing 10^7 to 10^8 dpm of labeled probe DNA at 42° C for 16 to 36 hr. Blots were washed in 120 ml of 3× SSPE containing 50% formamide and 0.1% SDS for 15 min at 42° C, followed by two successive washes of 30 min at 60° C in 2× SSPE and 1× SSPE, each also containing 0.1% SDS. Membranes were blotted dry and placed on a clean sheet of 3MM paper and then wrapped in plastic film. X-ray film (X-Omat AR, Kodak, Rochester, NY) was exposed overnight at −70° C in Kodak cassettes containing regular intensifying screens.

Protein sample preparation. Monosporidial cultures grown to stationary phase in PDB were harvested by centrifugation at 6,000 × *g* for 10 min. Each cell pellet was washed once in 40 ml of 10 mM Tris-HCl, 1 mM EDTA, 25 mM NaCl, pH 7.5, and the final pellet was frozen at −20° C. A 250-μl aliquot of the same buffer was added to each sample, which was kept on ice, and the entire mixture was sonicated for 30 sec at one-half maximum power in a "sonifier cell disruptor" (Model W185, Heat Systems Ultrasonics, Farmingdale, NY). Cellular debris was removed by centrifugation at 10,000 × *g* for 10 min, and the supernatant was heated to 100° C for 10 min and then frozen at −20° C. The proteins were boiled in sample buffer containing 1% SDS, separated by SDS-PAGE (Laemmli 1970), and visualized with Coomassie Brilliant Blue stain (type R, Sigma).

RESULTS

Protoplast preparation. Protoplasts were observed after incubation of secondary sporidia of *U. hordei* in Novozyme for 1–2 hr using a Wild EB-11 light microscope. Protoplasts were typically observed to emerge from one end of a cell, and complete digestion of the sporidial walls was not observed even after several hours of digestion. Approximately 80 to 95% of the sporidia had formed protoplasts under these conditions as determined by plating samples diluted either in water or an osmotically buffered solution (SC50). Approximately 4% of these protoplasts were regenerable.

Molecular karyotypic analysis. The electrophoretic karyotypes of one of the meiotic products of a germinated teliospore from collections of each of the 14 races of *U. hordei* are presented in Figure 1. Visual inspection of gels stained with ethidium bromide revealed a dissimilar banding pattern for each strain. The chromosome-sized DNA bands resolved with these parameters ranged from approximately 170 kb for the smallest band in strain 1.3 to a broad high molecular weight band that is present in all strains. A preliminary report of this work (McCluskey

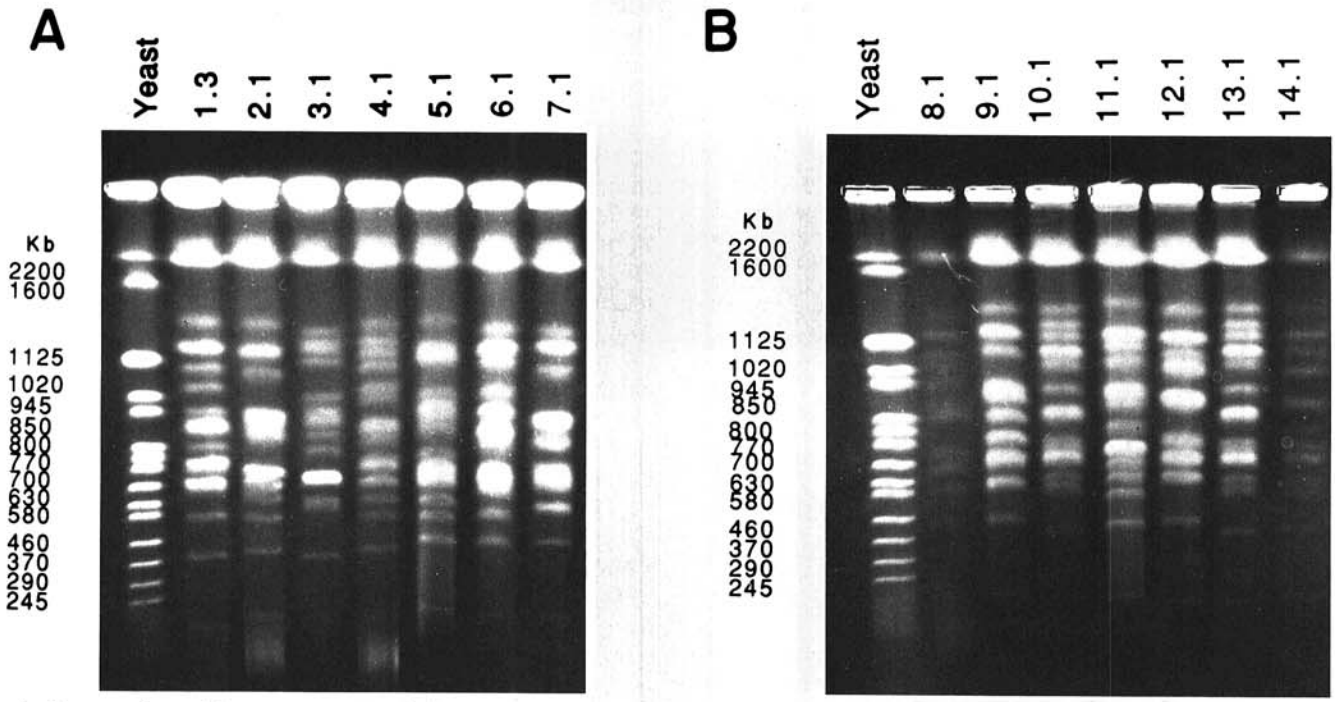

Fig. 1. Contour-clamped homogeneous electric field pulsed-field gel electrophoresis of chromosome-sized DNA molecules of *Ustilago hordei*. **A**, Strains obtained from races 1–7; **B**, Strains obtained from races 8–14. The left lane in each panel contains chromosomes from *Saccharomyces cerevisiae* used as standards.

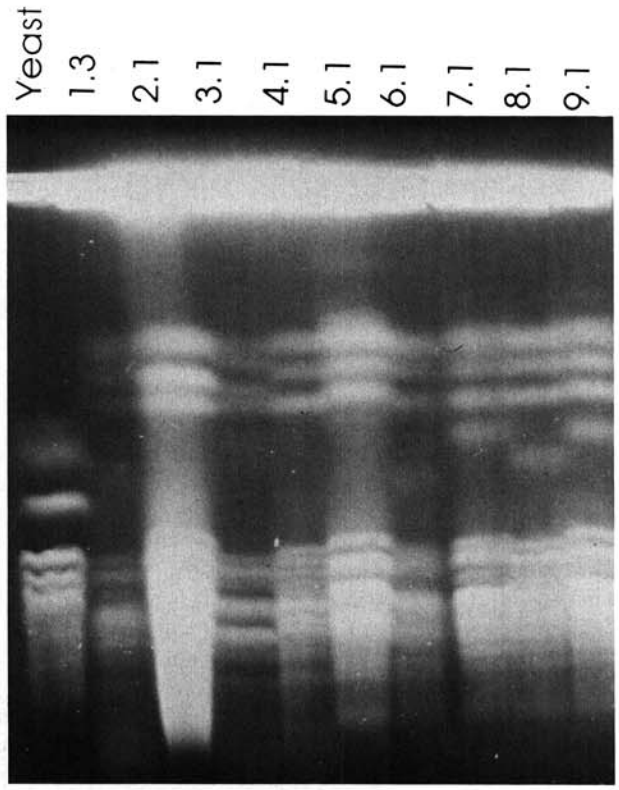

Fig. 2. Contour-clamped homogeneous electric field pulsed-field gel electrophoretic resolution of the largest chromosome-sized DNA molecules of *Ustilago hordei*. The broad band at 2,200 kilobases in all strains (Fig. 1) was resolved into three to five bands ranging in length from 2,000 to 3,150 kilobases (top bands in gel) and is shown here for strains from races 1–9. Chromosomes from *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (not shown) were used as standards. Conditions of electrophoresis: 0.8% agarose; 1) initial 24 hr, 60-min pulse at 50 V; 2) then 48 hr ramped 1,900–900 sec with the initial 20 hr at 60 V, followed by 28 hr at 85 V; 3) followed by 24 hr ramped 900–480 sec at 90 V; and finally, 4) 23 hr with a 420-sec pulse at 100 V.

and Mills 1989) did not include resolution of the higher molecular weight bands. The diffuse band at approximately 2,200 kb was shown to include three to five bands ranging in length from approximately 2,000 to 3,150 kb (Fig. 2 and Table 2).

All of the strains had at least three bands of approximately 2,700, 2,850, and 3,150 kb; all strains except 1.3, 2.1, 4.1, and 5.1 had an additional band that was at least 2,000 kb long. Strain 3.1 was unusual in that it had five bands that were 2,400 kb or longer. A faint band of approximately 5,000 kb was also detected in strain 5.1 (Fig. 2). Because this band is not present in all strains or in all preparations of strain 5.1, it is not included in the summary of the estimates of the numbers and lengths of chromosome-sized DNA bands of each race (Table 2).

Although the banding pattern revealed certain bands whose lengths were unique to a particular strain (for example, the 2,750-kb band of strain 3.1, Table 2), other bands of similar length recurred in several strains (for example, the 1,060-kb band in strains 4.1, 6.1, and 13.1). The minimum number of bands detected was 15 in strains 2.1 and 7.1, although a band of 1,190 kb in strain 2.1 and three bands in strain 7.1 are assumed to be doublets. The maximum number of bands detected was 19 in strains 10.1, 11.1, and 13.1, although two of the bands in strain 10.1 and 13.1 were presumed to be doublets.

To ascertain whether the molecular karyotype determined for these strains varied from preparation to preparation, or was affected by maintenance in storage, samples were prepared from strain 11.1 over the course of a year and run in parallel. The banding pattern was identical for all of these samples (data not shown).

Molecular karyotypic analysis of unordered and ordered tetrads. Teliospores from collections of several races were germinated and meiotic tetrads were isolated. The strains derived from these tetrads were analyzed with CHEF PFGE to ascertain whether the karyotype for strains from one

Table 2. Chromosome-sized DNAs of 14 strains of *Ustilago hordei*

1.3	2.1	3.1	4.1	5.1	6.1	7.1	8.1	9.1	10.1	11.1	12.1	13.1	14.1a
3,150[a]	3,150	3,100	3,150	3,150	3,150	3,150	3,150	3,150	3,150	3,150	3,150	3,150	3,150
2,850	2,850	2,900	2,850	2,850	2,850	2,850	2,850	2,850	2,850	2,850	2,850	2,850	2,850
2,700	2,700	2,750	2,700	2,700	2,700	2,700	2,700	2,700	2,700	2,700	2,700	2,700	2,700
1,290	1,290	2,700	1,310	1,290	2,000	2,450	2,200	2,450	2,200	2,000	2,200	2,200	2,200
1,190[b]	1,190[b]	2,400	1,230	1,200[b]	1,300	1,290	1,200	1,260	1,260	1,280	1,260	1,260	1,260
1,110	1,100	1,260	1,190	1,180	1,210[b]	1,230[b]	1,150[b]	1,180[b]	1,210	1,180	1,150[b]	1,210	1,170[b]
1,050	960	1,210	1,140	1,040	1,160	1,140	1,090	1,100	1,150	1,130	1,100	1,150	1,100
960	940	1,200	1,060	1,000	1,060	975	1,040	980	1,100[b]	1,075	1,075	1,100[b]	1,040
910	890	1,150	1,025	950	990[b]	900	980	925	1,050	970	1,020	1,060	920[b]
900	760	1,025	940[b]	910	960[b]	850[b]	920	850[b]	960	920	960	970	830
840	700	975	860	840	875	800	850[b]	780[b]	890	890	890	890	760
770	650	940	790[b]	800	810	710	720	720	850	830	800	850	720[b]
750	570[c]	890	710[b]	700	700	650[b,c]	650	700	770	770	760	770	630
700	460	840	650	650	610[c]	510	590[b,c]	620[b,c]	735	750	720	735	590[c]
685	220	720[d]	585[c]	585[c]	510	245	450	470	710[b]	700	660	710[b]	460
570[c]		650	460	500	245		200	200	640	630	640[c]	640	190
430		620[c]	210	245					590[c]	580[c]	490	590[c]	
170		450							450	460	190	450	
		220							170	200		190	

[a] Lengths more than 2,200 kilobases are estimates. Lengths less than 2,200 kilobases were calculated by Cricket Graph (Cricket Graphics, Inc., Philadelphia).

[b] Bands interpreted as doublets based on intensity of ethidium bromide staining.

[c] Identified by pSF8 as containing sequences homologous to an actin gene from *Aspergillus nidulans*.

[d] Band interpreted as a triplet based on intensity of ethidium bromide staining.

teliospore was variable or conserved. The molecular karyotype was determined for three unordered tetrads from teliospores of the race 8 collection, one tetrad from a teliospore of the race 2 collection, two tetrads from teliospores of the race 14 collection, and for an ordered tetrad from a teliospore of the race 11 collection. All 12 monosporidial strains derived from the race 8 collection had a karyotype that was identical to the karyotype of strain 8.1 (Fig. 1B). The karyotypes of the four monosporidial strains from the race 2 and the race 11 collections were identical to strains 2.1 and 11.1, respectively (Fig. 1A, B). Six of the eight monosporidial strains from the race 14 collection had identical karyotypes, but among the four monosporidial strains from one tetrad, a chromosome length polymorphism segregated 2:2 (Fig. 3).

Analysis of total soluble proteins. Because the molecular karyotypes of the representative strains for 14 races were dissimilar, it was of interest to determine whether another technique frequently used to distinguish different fungal strains might resolve the races into common groups. The electrophoretic pattern of total soluble proteins, which was previously used to distinguish between phytopathogenic species of *Phytophthora* (Hamm and Hansen 1983), was also used in this study. However, the protein banding pattern was very similar for each strain (data not shown), excluding the use of this technique for differentiating strains of *U. hordei*.

Southern hybridization analysis. Evidence for the presence of chromosome length polymorphisms was verified by Southern blot hybridization of CHEF PFGE-fractionated chromosomes with randomly selected homologous DNA probes. A radiolabeled probe made of a 1.2-kb *Eco*RI fragment (pOSU4012) obtained from strain 8.1 (Table 1) had homology with a single band in each of the other strains that ranged in length from approximately 1,200 to 1,300 kb (Fig. 4). Although the mobility of this band differs among the strains, it clearly represents the same linkage group and provides evidence for chromosome length polymorphisms of as much as 100 kb.

This approach was also used to map fragments to other linkage groups and to develop a better understanding of length polymorphisms. The inserts in pOSU4015 and pOSU4117 (Table 1) do not cross-hybridize, but hybridize to the same chromosome-sized DNA band in strains 9.1 and 13.1 (Fig. 5). However, the 3.2-kb insert (pOSU4015) and the 6.0-kb insert (pOSU4117), respectively, hybridized to a 750- and a 700-kb band of strain 11.1 (Fig. 5). These probes also hybridized to bands of slightly different mobility in strains 10.1 and 12.1, suggesting that the probes are either identifying different bands in a doublet or hybridizing to sequences that are linked in strains 9.1 and 13.1, and unlinked in strains 10.1, 11.1, and 12.1.

The 6-kb insert of pOSU4011 showed intense hybridization to sequences in the sample well and weak hybridization to small, diffuse DNA near the bottom of the blot (data not shown). The *cox* III mitochondrial gene from *S. cerevisiae* (Table 1) hybridized with yeast and *U. hordei* DNA in the sample well and to a diffuse band of DNA near the bottom of the blot only in the lane containing yeast chromosomes. Relaxed forms of circular DNA molecules, such as the mitochondrial genome, do not

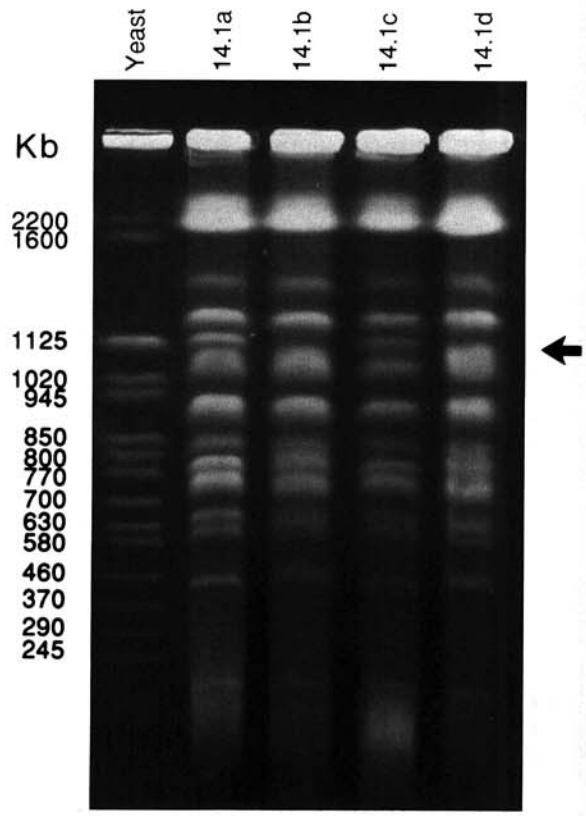

Fig. 3. Electrophoretic karyotype of members of an unordered tetrad of race 14 of *Ustilago hordei*. The arrow identifies a chromosome length polymorphism that segregates 2:2 among the progeny. Electrophoresis was conducted using the conditions that are described in the text.

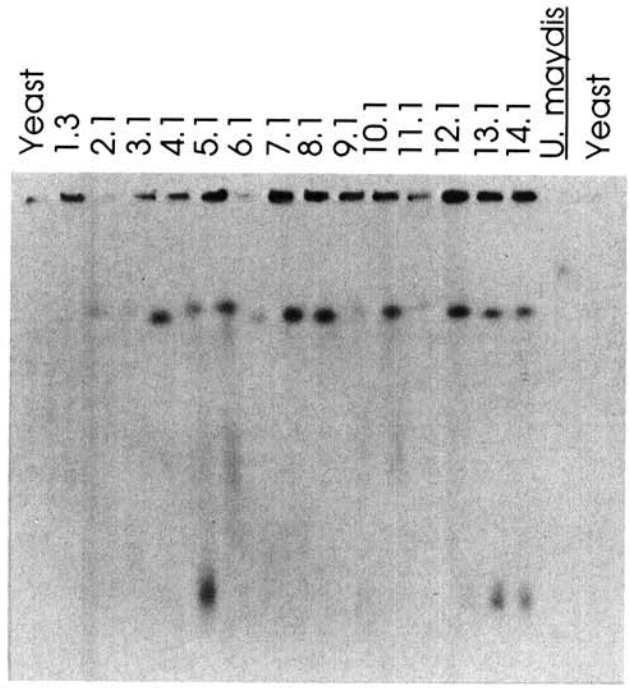

Fig. 4. Detection of chromosome length polymorphisms in *Ustilago hordei*. A gel containing chromosome-sized DNA molecules from a strain of each race was Southern blotted and probed with a 1.2-kilobase *Eco*RI fragment (pOSU4012) from *U. hordei*.

readily enter the gel under these conditions (Hightower and Santi 1989), which suggests that the insert in pOSU4011 may be of mitochondrial origin. Moreover, these results indicate that the smallest band in each strain is not the mitochondrial genome, because it did not hybridize with either the yeast mitochondrial DNA probe or the insert in pOSU4011 (data not shown).

To show the feasibility of mapping heterologous conserved genes to electrophoretically separated chromosomes of *U. hordei*, a probe made of the *Aspergillus nidulans* (Eidam) Winter actin gene (Table 1) hybridized to a band in each strain that migrates between 570 and 650 kb (Table 2), indicating a chromosome length polymorphism of approximately 80 kb for this chromosome.

DISCUSSION

CHEF PFGE was used to examine the karyotypes of strains representing 14 races of *U. hordei*. The karyotype of strains from each race varied with respect to both the number and length of chromosome-sized DNA bands. The karyotypic variability could not be attributed to artifacts of the extraction procedure, although the possibility that some bands arise by specific cleavage at fragile sites on the chromosomes could not be ruled out (Sutherland and Hecht 1985). The similarity of total soluble proteins extracted from these strains and separated by SDS-PAGE precluded the use of this technique for distinguishing isolates of *U. hordei*.

The number of chromosome-sized DNA bands varied from a minimum of 15 for strains 2.1 and 7.1, with one or more bands being interpreted as a doublet, to a maximum of 19 in strains 10.1 and 11.1, with three bands in strain 10.1 being interpreted as doublets (Table 2). These results

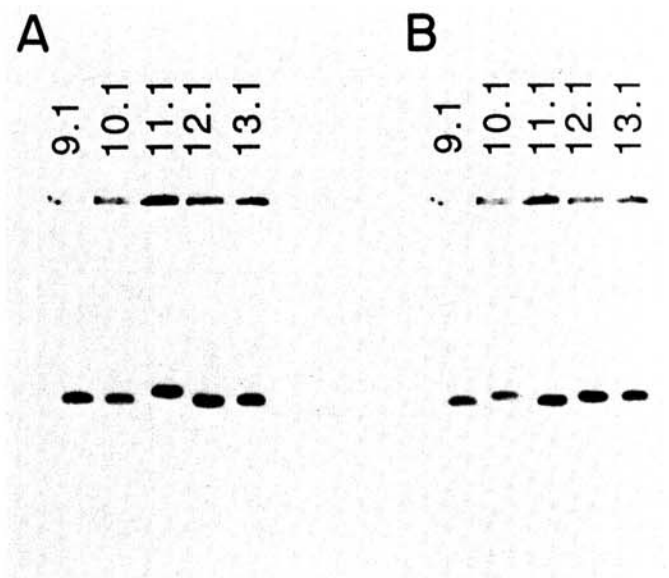

Fig. 5. A Southern blot of chromosome-sized DNA molecules of *Ustilago hordei* fractionated by contour-clamped homogeneous electric field electrophoresis and probed with two genomic *Eco*RI fragments. The gel presented in Figure 1B was blotted to a nylon membrane. **A**, Blot probed with a 3.2-kilobase fragment (pOSU4015). **B**, Same blot stripped and probed with a 6.0-kilobase fragment (pOSU4117).

indicated that CHEF PFGE analysis alone cannot provide an accurate number of the haploid complement of chromosomes for two reasons. First, a doublet band could be comprised either of disomic chromosomes or of heterologous chromosomes of similar size. Second, Southern blot analysis revealed chromosome length polymorphisms for some chromosomes (Fig. 4). Consequently, homologous disomic chromosomes that differ in size would be indistinguishable from heterologous chromosomes by CHEF PFGE analysis. In the absence of corroborative Southern blot linkage data for all bands, the occurrence of chromosome length polymorphisms among disomic chromosomes would lead to an inflated base number of the haploid complement of chromosomes. The highly variable number of chromosome-sized DNA bands reported here is not unique to this smut fungus; similar results have been reported for laboratory strains of *U. maydis* (Kinscherf and Leong 1988).

Although a larger number of DNA probes will be required to establish the precise number of linkage groups, the CHEF PFGE analysis of these strains (Figs. 1 and 2) allows an estimate of the maximum number of chromosomes and the size of the haploid genome of *U. hordei*. Strain 2.1 with the fewest bands should be most representative of a strain with the haploid complement of chromosomes. If each band represents an intact chromosome, and the lone doublet (Table 1) is arbitrarily assumed to be comprised of different chromosomes, the maximum number of chromosomes per haploid cell would not exceed 16. The combined length of these bands is approximately 19,600 kb, which would make the haploid genome of *U. hordei* approximately 1.6 times larger than *S. cerevisiae* (Mortimer and Schild 1985). Strain 10.1 has the largest number of bands including doublets, which have a combined length of approximately 26,100 kb and a DNA content 1.35-fold greater than strain 2.1. The possibility that the variable bands presented here are B chromosomes is impossible to exclude without further study. B chromosomes are unknown in basidiomycetous fungi and are unstable in inbreeding plant species (Jones and Rees 1982), although a preliminary report of unstable chromosomes in an ascomycetous fungus has appeared (Miao and VanEtten 1989).

Chromosome length polymorphisms have been described for sexually compatible strains of *S. cerevisiae* (Ono and Ishino-Arao 1988), for strains of *C. albicans* (Magee *et al.* 1988), and for laboratory strains of *U. maydis* (Kinscherf and Leong 1988). Moreover, in crosses of strains of *S. cerevisiae* having chromosome length polymorphisms for specific homologous chromosomes, a high percentage of viable offspring were obtained, and both parental and variable-sized recombinant chromosomes were recovered (Ono and Ishino-Arao 1988). These results are consistent with our observations, which clearly indicate that the karyotypes of strains representing each race of *U. hordei* are highly variable, although individual strains from different races are sexually compatible (Emara 1972).

Several bands in each strain were consistently present. The uniformity in size among the highest molecular weight bands (Fig. 2) may be either a function of the resolution of DNA molecules in this size range by the CHEF system

or a manifestation of an ability of smaller bands to tolerate a higher degree of variability. The band at 1,100 to 1,175 kb is present in every strain (Fig. 1), but caution is warranted in assuming that bands of identical size represent homologous DNA molecules. In several strains, only some of the similar-sized bands had homology with a DNA probe (Fig. 5). Moreover, two *U. hordei* genomic *Eco*RI fragments used as probes appear to belong to the same linkage group in strains 9.1 and 13.1 because they have homology to a common band, but they have homology with different bands in strains 10.1 and 11.1. These results cannot be definitively interpreted. The two fragments could have become linked or unlinked by reciprocal translocation, or they have homology with dissimilar DNA molecules of identical size. The development of chromosome-specific DNA clone libraries (K. McCluskey and D. Mills, unpublished results) and their use as molecular probes will be useful in resolving these questions.

Heterologous DNA probes made of highly conserved genes were used in Southern blot analysis to map homologous genes to chromosomes of *U. maydis* and to show that most of the mitochondrial DNA remains in the sample well (Kinscherf and Leong 1988). Using a radiolabeled probe of the actin gene of *A. nidulans*, we have determined that a homologous gene resides on a single band in each strain that ranges in length from 570–620 kb (Table 2). Furthermore, using *cox* III (*oxi* 2), a mitochondrial gene from *S. cerevisiae* (Muller *et al.* 1984), we have determined that homologous sequences remain in the sample well and that the smallest band in each strain is apparently not of mitochondrial origin. Heterologous probes will allow both the rapid development of genetic linkage maps and the cloning and description of important genes from *U. hordei.*

The upper limit of length of *U. hordei* chromosomes appears to be about 3,000 kb (Fig. 2). The size limit of these chromosomes is similar to that seen for *U. maydis* (Kinscherf and Leong 1988), but somewhat smaller than observed for *P. megasperma* (Howlett 1989) and *Tilletia* spp. (B. W. Russell and D. Mills, unpublished data). Moreover, the variability in size and number of bands among these strains is of the magnitude observed in *U. maydis* (Kinscherf and Leong 1988).

A valuable feature of *U. hordei* as an experimental system is the production of ordered tetrads of basidiospores. This feature will facilitate studies of the distribution of genes on individual chromosomes and analyses of polymorphisms associated with specific chromosomes. Although the karyotypes appeared to be essentially invariant for strains derived from one or more tetrads of a particular race, a chromosome length polymorphism segregated 2:2 among the four members of a tetrad from race 14 (Fig. 3). The apparent variability in chromosome number among the strains suggests that there has been a need to maintain a disomic state for some chromosomes. The variability in size of individual bands may be a physical manifestation of genome rearrangements that require the maintenance of disomic chromosomes. Work is currently in progress to determine whether this hypothesis accurately describes genetic mechanisms acting in the genesis of races of *U. hordei.*

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