World Journal of Microbiology & Biotechnology **16**: 49–55, 2000.

Genetic diversity of *Ustilago maydis* strains

María Elena Valverde[1], George J. Vandemark[1,3], Octavio Martínez[2] and Octavio Paredes-López[1,*]
[1]*Departamento de Biotecnología y Bioquímica*
[2]*Departamento de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados del IPN, Unidad Irapuato. km. 9.6 Libramiento Norte, Carretera Irapuato-León. Apdo. Postal 629, Irapuato 36500, Gto., México.*
[3]*Present address: Agricultural Research Service, Pacific West Area 24106 N. Bunn Road, Prosser WA 99350-9687, USA*
**Author for correspondence: E-mail: oparedes@irapuato.ira.cinvestav.mx*

Received in revised form 21 September 1999; accepted 7 October 1999

Keywords: Genetic diversity, RFLP fingerprinting, *Ustilago maydis*

Summary

Thirty wild isolates belonging to five different locations in Mexico plus two laboratory strains of *Ustilago maydis* were characterized by restriction fragment length polymorphism (RFLP) analysis using 23 different clones as probes derived from a *Pst*I library and two restriction enzymes. All loci analysed presented a high level of polymorphism, including one locus with thirty one different alleles. Geographical grouping of the populations was based on Nei's genetic distance and there was no correlation between genetic and geographic distances among these isolates. Our results suggest that DNA fingerprinting is a useful method for detecting genetic variation in populations of *U. maydis*. This work demonstrated that considerable genetic variation may be present within field populations of *U. maydis*.

Introduction

The basiodiomycete *Ustilago maydis* is the causal agent of common smut of maize. Besides its importance as a pathogen, the teliospore-containing tumors (galls) induced by the fungus on young maize ears are edible, and are gaining appreciation in the North American markets as a delicacy called 'huitlacoche'.

The ability of *U. maydis* to cause disease in maize is directly dependent on the capability for compatible mating. This fungus has two mating loci, *a* and *b*, both of which must be heterozygous in mating haploids for compatibility. The *a* locus has two alleles; it encodes components of a pheromone response pathway and controls cell fusion and filamentous growth. The *b* locus, which is represented by up to 25 different compatibility types, encodes a combinatorial regulatory protein and regulates development of infectious filamentous cells and tumor formation, in other words, pathogenicity. The interdependence of infection and sexual compatibility arises because mating-type loci that control compatibility also control subsequent transition from the saprophytic to the pathogenic phase of the life cycle (Rowell & De Vay 1954; Rowell 1955; Puhalla 1970; Banuett & Herskowitz 1989; Banuett 1992; Banuett 1995). On the other hand, the fungus is heterothallic and presents an undetermined number of lines or biotypes. In addition, new biotypes arise by hybridization in sexual generations or by mutation. Also, outbreeding contributes to diversity because teliospores are disseminated over long distances by wind (Christensen 1963). However, *U. maydis* has no naturally occurring morphological or known physiological markers, and there are very few studies of its genetic diversity in natural populations. Recently, a high level of *b* locus diversity within populations from four Minnesota locations has been found and common *b* mating types were found across broad geographic distance (Zambino *et al.* 1997).

Qualitative and quantitative information on diversity is an essential aspect of many fields in biology, both fundamental and applied. Various genetic markers can be used to study fungal phytopathogen populations, but they are not easy to assay. However, the advent of genetic markers based on differences in DNA sequences has made it possible to distinguish among all the individuals in a fungal population. This has allowed for basic studies on population and evolutionary biology in fungi; thus lack of allelic information is less of a problem in these organisms, where the haploid state prevails (Christiansen & Giese 1990; Girardin *et al.* 1993; Hamelin *et al.* 1994; Weising & Kahl 1997). The main application of DNA markers in fungi has been to differentiate among individuals in a population or collection of isolates. For example, PCR techniques have been a powerful tool for identification of the major species in the genus *Phytophthora*. They have also defined phenotypic groups in *Trichoderma* and have been useful for analysing the genetic structure of populations in many other fungi (Banuett 1992; Castle

et al. 1998; Griffith & Shaw 1998; Ristaino *et al*. 1998). The most widely used tool for DNA fingerprinting in fungi is restriction fragment length polymorphism (RFLP) analysis, also called multilocus fingerprinting. This technique is based on hybridization of probes to anonymous repetitive DNA sequences cloned from the species under investigation. Advantages of using these probes cloned from the fungus under study include a strong hybridization signal under high stringency hybridization and washing conditions, due to the high degree of homology between probe and target sequences. This usually results in reproducible hybridization patterns with the high degree of resolution needed to easily distinguish different genotypes (Weising *et al*. 1995). RFLP fingerprinting is rapidly becoming the tool of choice for analysing fungal genetics because it is precise, selectively neutral, codominant, easy to assay, and can provide an unlimited number of genetic markers (Michelmore & Hulbert 1987; Ueng *et al*. 1992; Rosewich & McDonald 1994). Its high sensitivity and the possibility of screening the genome rapidly with few probes has prompted the use of multilocus fingerprinting for linkage analysis and genome mapping (Weising & Kahl 1997).

Genetic markers allowed for the study of the amount and distribution of genetic variability of the phytopathogenic fungus *Mycosphaerella graminicola*, and showed that fungal populations may contain a high degree of genetic diversity that is often distributed on a very fine scale (McDonald & Martínez 1991; Xia *et al*. 1993; McDonald *et al*. 1994). Different genotypes of the wheat pathogen *Septoria tritici* were found existing in the same lesion or on the same leaf (Boeger *et al*. 1993). In the rice pathogen *Magnaporthe grisea*, hierarchical diversity analysis demonstrated that most of the variability was distributed within locations in a field (Xia *et al*. 1993). These data were very similar to studies of *S. tritici*, where 93% of the diversity was distributed within sampling locations in a field (Boeger *et al*. 1993). DNA fingerprinting in *S. tritici* and *Stagonospora nodorum* was used to show that the majority of individuals in a population were unique genotypes, and no clone was widespread, while populations of *Sclerotinia sclerotiorum* are predominantly clonal (McDonald & Martínez 1991; McDonald *et al*. 1994; Kohli and Kohn 1998). RFLP fingerprinting has also been used to assess the distribution over space and time of genetic variation in populations of *Phaeosphaeria nodorum* (Ueng *et al*. 1992), and for resolving the population dynamics and the evolution of *M. grisea* in the USA (Levy *et al*. 1991). For *Phytophthora infestans*, it was shown that the Toluca Valley in Mexico harbored a higher degree of genetic variation than other regions in Mexico, Europe and North America, supporting the hypothesis that the Toluca Valley is the center of origin for this pathogen (Fry *et al*. 1992; Goodwin *et al*. 1992a, b). Additionally, RFLP fingerprinting has been used to assess the potential for gene flow between geographically separated populations of *Mycosphaerella graminicola* (Boeger *et al*. 1993).

In view of the scarce knowledge on the population genetics of *U. maydis*, and given the proven usefulness of RFLP fingerprinting in studying fungal populations, we have become interested in analysing the genetic relationships in *U. maydis* isolates from different geographic areas of Mexico. Our objective was to develop molecular genetic markers suitable for characterizing the biodiversity of *U. maydis* and evaluate the population genetic structure of this fungus. This study provides information regarding the amount and distribution of genetic variability, and in the future, this knowledge can be useful in studies of gene flow, natural selection, and host-pathogen coevolution.

Materials and Methods

Fungal isolates

U. maydis strains used in this study are listed in Table 1. Teliospores were collected from smut galls of naturally-infected maize from different locations of Mexico during the rainy season in 1997. Within each location, a single ear fully infected with huitlacoche was removed from each of three different plants, all from only one field, and they were mixed. Spore masses were lyophilized, stored separately by location at 4 °C and were used to obtain the isolates for each group.

Sporidia were isolated as previously described (Thakur *et al*. 1989; Pope & McCarter 1992; Valverde 1992).

Table 1. Ustilago maydis strains.

Group	Origin	Location*	Altitude (m)	Reference
1 S1, S2	Obtained from the same teliospore, standard laboratory strains	USA		Banuett & Herskowitz (1989)
2 I1, I2, I3, I4, I5, I6	Wild isolates	Irapuato, Central Mexico	1730	Valverde (1992)
3 O1, O2, O3, O4, O5, O6	Wild isolates	Oaxaca, South West Mexico	230	Our lab
4 P1, P2, P3, P4, P5, P6	Wild isolates	Pachuca, Central Mexico	2000	Our lab
5 C1, C2, C3, C4, C5, C6	Wild isolates	Culiacan, North West Mexico	20	Our lab
6 T1, T2, T3, T4, T5, T6	Wild isolates	Toluca, Central Mexico	2660	Valverde (1992)

* All locations differ greatly in annual precipitation.

Teliospores were germinated on potato-dextrose agar at 28 °C. Individual haploid isolates were obtained as isolated colonies after plating cell dilutions on the same media.

Genomic library

Sporidia were grown in potato-dextrose broth in an Erlenmeyer flask agitated in a reciprocal shaker at 200 rev/min. Cells were collected by centrifugation and washed twice in a solution containing 100 mM Tris/HCl, pH 8.0, 5 mM EDTA and 150 mM NaCl. After freeze-drying, cells were frozen in liquid nitrogen, ground to a powder and lysed for 1 h at 50 °C in a solution containing 200 mM Tris/HCl, pH 8.0, 100 mM EDTA, 1% Sarkosyl and 1 mg/ml proteinase K. Total DNA was purified by several phenol/chloroform extractions, ethanol-precipitated and resuspended in TE (10 mM Tris/HCl, pH 8.0, 1 mM EDTA) (Sánchez-Alonso *et al*. 1996). All DNA samples were quantitated spectrophotometrically at 260 nm. A partial genomic library of *U. maydis* was constructed from isolate I3 (Table 1) from Irapuato. Genomic DNA was digested with *Pst*I and resolved by gel electrophoresis using 1% (w/v) agarose. DNA fragments ranging from 1 to 4 kb were isolated by purification from sliced agarose using the GeneClean II Kit (Bio 101) according to manufacturer's suggestions, and cloned into the *Pst*I site of vector pBluescript KS$^-$ (Sambrook *et al*. 1989; Ueng *et al*. 1992). Competent cells of *E. coli* DH5α were transformed, and recombinants appeared as white colonies on Luria–Bertani agar media containing ampicillin (50 μg/ml), isopropyl β-D-thio-galactopyranoside (IPTG) (70 μg/ml), and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) (70 μg/ml).

Probes

Plasmid DNA was purified preparatively by alkaline lysis (Sambrook *et al*. 1989). Probes were obtained from the *U. maydis* genomic library by digestion of the clones with *Pst*I, and separation by gel electrophoresis using 1% (w/v) agarose in 1× TAE. Purification of the DNA from the gel was carried out with the GeneClean II Kit, and radioactive labeling with α-^{32}P-dCTP using the Prime-It II Random Primer Labeling Kit.

DNA hybridization

To ensure complete digestion, at least 5 to 8 units of restriction enzyme/μg microgram of genomic DNA should be used (Weising *et al*. 1995). Genomic DNA (10 μg) extracted from the 32 *U. maydis* isolates was digested individually at 37 °C during 12 h with 50 U of *Eco*RI or *Pst*I. A small portion of every digested sample was analysed by electrophoresis on an agarose minigel and stained with ethidium bromide; a visual examination of the stained gels was carried out to confirm full digestion and approximately the same amount of DNA in all lanes. The digested DNAs were resolved by 1% (w/v) agarose gel electrophoresis at constant voltage (25 V) overnight. The gel was pretreated with 0.25 M HCl for 10 min, washed twice with water, denatured with 0.5 M NaOH/0.6 M NaCl, and then the gel was transferred using the same solution to a SureBlot nylon membrane (ONCOR), according to the manufacturer's suggestions. Hybridizations were carried out at 42 °C overnight in Hybrisol I (ONCOR) with a probe concentration of 10^6 c.p.m./ml. Membranes were washed once with a high-stringency solution containing 2× SSC (0.3 N NaCl, 0.03 M sodium citrate, pH 7) and 1% SDS, and twice with a solution containing 0.1× SSC and 0.1% SDS at 42 °C, and were exposed to X-ray film.

Statistical analyses

The first step in the analyses was to obtain a data matrix of ones and zeros indicating the presence or absence, respectively, of each RFLP band in each isolate. Simple matching coefficients were obtained to estimate the genetic relationship among *U. maydis* isolates. This data matrix was then re-coded to detect all the alleles for each RFLP locus. According to the convention of RFLP fingerprinting analysis, the different restriction fragments detected by each probe were treated as alleles at a single RFLP locus. Since haploid sporidia were employed for the DNA extraction, the genetic frequencies of each allele at each locus were directly estimated. Consecutive integers were assigned to each band pattern within a probe and represented a specific RFLP allele, thus obtaining an allelic matrix containing the integer code for each allele within each RFLP locus for all the individuals. The allele matrix was then recorded to conform to the format of the Genetic Data Analysis program, and the distribution of genetic variation within and among populations as well as Nei's genetic distances (Nei 1978) among populations were calculated (Lewis & Zaykin 1999).

Results and Discussion

To analyse the genetic diversity, a *U. maydis* partial genomic library was constructed from one isolate, and the size of the genomic DNA fragments cloned ranged from 0.8–2.8 kb. About 100 clones were picked randomly to use as probes for RFLP fingerprinting analysis and were tested for the ability to generate polymorphisms; approximately 50% of these probes detected polymorphisms and produced a complex pattern of hybridization, suggesting that this fungus contains a substantial amount of repetitive DNA. The amount of dispersed repetitive DNA varies among species, but it is generally thought to be lower in fungi than in plants and animals. For example, *Aspergillus nidulans*, *Aspergillus fumigatus* and *Neurospora crassa* have very small amounts of repetitive DNA (<1%) (Timberlake 1978; Krumlauf & Marzluf 1980; Girardin *et al*. 1993).

Moderate levels of repetitive DNA are found in the rice pathogen *Magnaporthe grisea* and the water mould *Achlya bisexualis* (8%) (Hudspedh *et al.* 1977; Hamer *et al.* 1989), while *S. tritici* and *P. infestans* have much higher levels of repetitive DNA (26–29%) (McDonald & Martínez 1991; Goodwin *et al.* 1992a,b). Large amounts of repetitive DNA have been reported in biotrophic parasite fungi. This repetitive DNA or multiple copy sequences indicate the occurrence of frequent polymorphisms in fungal populations (Christiansen & Giese 1990; Ueng *et al.* 1992).

From the preliminary screen, 23 probes were chosen to characterize the populations of *U. maydis* isolates. In general, those probes produced highly variable banding patterns that were reproducible, easy to score, and produced 507 bands in total. Figure 1 shows an example of the banding patterns obtained with probe pKS-56. The simple matching coefficient; this is, the number of band differences (1–0 or 0–1) divided by the total number of comparisons performed, was used to estimate genetic dissimilarity among isolates and a dendrogram was obtained by the UPGMA alogarithm (Sneath & Sokal 1973; Skroch *et al.* 1992; Avise 1994). Because each probe resulted in a different number of bands, a matrix of dissimilarity was obtained for the bands of each probe and then these matrices were averaged to obtain the estimate of genetic dissimilarity among isolates (Figure 2). This approach is very sensitive because each probe is a locus, and thus it must have the same relevance when calculating the dissimilarity among isolates, irrespective of the number of bands. In general, standard isolates were separated from Mexican isolates; Irapuato and Oaxaca isolates formed independent groups, and Culiacan, Pachuca and Toluca isolates were scattered.

Together, these RFLP loci produced 32 haplotypes, each isolate showed a specific band pattern. Many polymorphisms were observed, and *U. maydis* showed an extensive diversity among loci, from tetramorphic to hypervariable loci (31 alleles). Nearly all isolates had different hybridization patterns with some probes, suggesting that they are useful for DNA fingerprinting of *U. maydis*. In other fungi like *M. grisea*, *M. graminicola* and *S. nodorum*, adequate probes have been found for the same purpose (Levy *et al.* 1991; Skinner *et al.* 1993; McDonald *et al.* 1994).

Table 2 shows the locus name, number of alleles and diversity analysis obtained for each probe. On average,

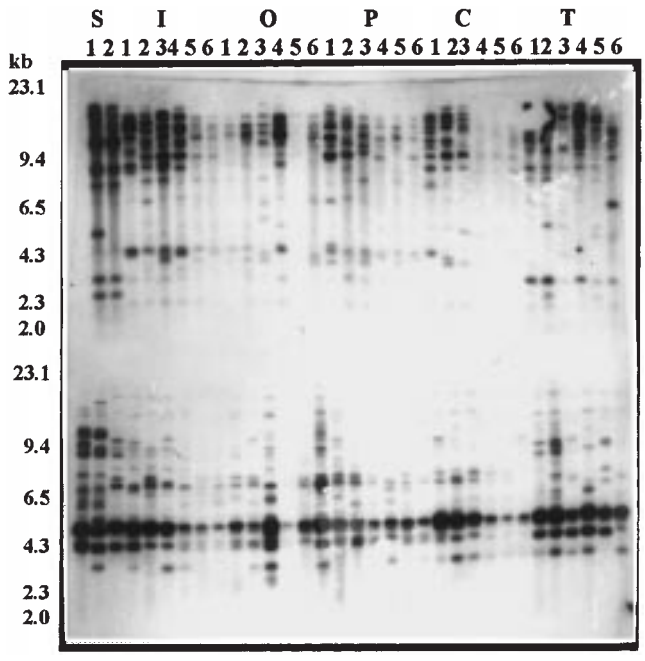

Figure 1. RFLP fingerprinting analysis of *Ustilago maydis* isolates listed in Table 1. (S = 1, 2 Standard isolates, I = 1, 2, 3, 4, 5, 6 from Irapuato, O = 1, 2, 3, 4, 5, 6 from Oaxaca, P = 1, 2, 3, 4, 5, 6 from Pachuca, C = 1, 2, 3, 4, 5, 6 from Culiacan, and T = 1, 2, 3, 4, 5, 6 from Toluca). DNA samples (0.5 μg) were digested with *Eco*RI (top of the gel) and *Pst*I (bottom of the gel), size fractionated by agarose gel, transferred to a nylon membrane and tested with probe pKS-56. Size standards: Gibco-BRL λ/*Hind*III DNA.

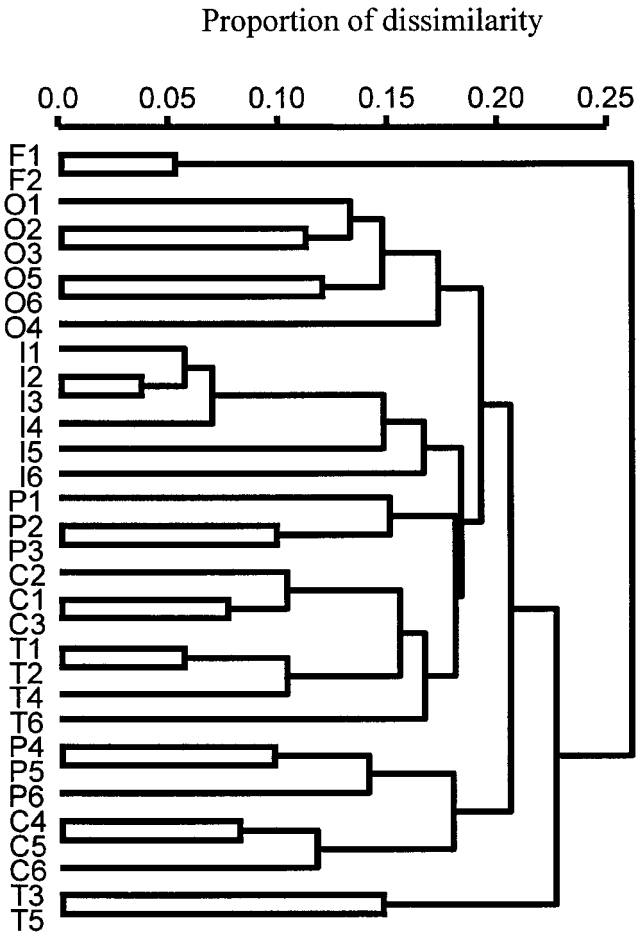

Figure 2. Dendrogram showing genetic relationships among 30 Mexican *Ustilago maydis* wild isolates and two laboratory strains. RFLP data obtained with 23 different probes were used to estimate genetic dissimilarity among isolates using the simple matching coefficient (number of band differences divided by the total number of comparisons performed); dendrogram was obtained by the UPGMA alogarithm. S = 1, 2 Standard isolates, I = 1, 2, 3, 4, 5, 6 Irapuato isolates, O = 1, 2, 3, 4, 5, 6 Oaxaca isolates, P = 1, 2, 3, 4, 5, 6 Pachuca isolates, T = 1, 2, 3, 4, 5, 6 Toluca isolates and C = 1, 2, 3, 4, 5, 6 Culiacan isolates.

each RFLP locus exhibited about 22 bands and 19 alleles. However, four probes (pKS-11, pKS-36, pKS-48 and pKS-56) yielded a complex pattern of hybridization with a high number of bands (37–58) and alleles (27–31).

The mean variation within populations was 77.46% and among populations was 10.37% over all loci analysed. The analysis of diversity within a single locus indicated that most of the variability was distributed within populations, with a small proportion distributed among populations. These results suggest that most of the genetic variation in *U. maydis* may be distributed on a local scale, rather than on a macrogeographic scale, and these results are consistent with those found in other fungi like *Cronartium quercuum*, *M. graminicola* and *Rhynchosporium secalis* (Boeger *et al.* 1993; Goodwin *et al.* 1994; Hamelin *et al.* 1994). Recently, genetic diversity at the *U. maydis b* mating-type locus was determined in samples from four Minnesota locations, and a high level of *b* diversity within populations was also revealed (Zambino *et al.* 1997).

Table 3 shows, for the 23 RFLP loci, the number of alleles per locus in each population and basic statistics for this quantity, as well as the percentage of shared alleles among populations. Each band of the RFLP pattern detected approximately one allele, indicating the high genetic diversity present in the sampled populations. The minimum number of alleles was one and the maximum six (the number of individuals sampled per population), with means that ranged from 3.48 in the Irapuato population to 4.43 in the Oaxaca and Pachuca populations. The fact that in all five populations and in many loci there are as many alleles as individuals indicates that the estimated mean of alleles per loci will most probably increase if more individuals are sampled in each population. Eleven of the 23 loci (~48%) did not share any allele among populations, indicating a complete genetic disconnection for these loci among the populations. Furthermore, 12 loci (~52%) share alleles among populations, indicating some degree of population divergence. This is also supported by the results in Table 2.

Table 2. Distribution of genetic variation within and among populations of *Ustilago maydis.*

No	Locus	Number of alleles	Within population diversity	Among population diversity
1	pKS -11	28	0.9744	0.0208
2	pKS -13	23	0.8077	0.1914
3	pKS -14	26	0.8846	0.1148
4	pKS -21	5	0.3590	0.0832
5	pKS -28	24	0.9825	0.0172
6	pKS -33	20	0.8270	0.1730
7	pKS -35	13	0.6491	0.3475
8	pKS -36	27	0.9818	0.0183
9	pKS -46	14	0.7429	0.2209
10	pKS -48	27	1.0000	0.0000
11	pKS -51	26	0.8846	0.1196
12	pKS -53	18	0.7985	0.1376
13	pKS -55	10	0.6240	0.2686
14	pKS -56	31	0.9872	0.0128
15	pKS -67	14	0.7821	0.0883
16	pKS -68	18	0.8718	0.0800
17	pKS -69	4	0.2051	0.0363
18	pKS -70	25	0.8846	0.1148
19	pKS -78	12	0.6154	0.0804
20	pKS -86	26	0.8974	0.1068
21	pKS -88	6	0.5000	0.0190
22	pKS -89	9	0.6923	0.0400
23	pKS -91	19	0.8636	0.0949
	Mean	18.48	0.7746	0.1037

Table 3. Number of alleles per locus per population and descriptive statistics.

Locus	Irapuato	Oaxaca	Pachuca	Culiacan	Toluca
pKS-11	5	6	6	6	5
pKS-13	3	6	4	5	3
pKS-14	6	3	5	5	5
pKS-21	2	3	2	3	1
pKS-28	–	5	5	6	6
pKS-33	4	5	4	2	5
pKS-35	4	3	5	1	–
pSK-36	5	5	6	5	6
pKS-46	3	4	5	4	2
pKS-48	6	6	5	5	5
pKS-51	3	6	4	6	5
pKS-53	3	4	5	3	6
pKS-55	3	4	3	3	3
pKS-56	6	6	6	5	6
pKS-67	4	5	4	3	4
pKS-68	3	5	4	4	5
pKS-69	1	1	2	1	2
pKS-70	4	4	6	5	5
pKS-78	2	2	2	5	3
pKS-86	5	6	6	6	4
pKS-88	2	3	3	3	2
pKS-89	2	6	5	3	2
pKS-91	4	4	5	3	5
All/loc*	3.48	4.43	4.43	3.96	3.91
P/ P/L*	0.88	0.92	0.96	0.88	0.91

* All/Loc = Alleles per locus, P/P/L = Proportion of polymorphic loci.

The proportions of polymorphic RFLP loci and number of alleles per locus in *U. maydis* isolates from all regions of Mexico were high; on average, the proportion of polymorphic loci was 0.91 and there were 4.04 alleles per RFLP locus. Oaxaca, Pachuca and Toluca presented the highest level of polymorphic loci, while the lowest level was found in Irapuato and Culiacan. Similar results were observed in the number of alleles per locus (Table 3).

Table 4 shows Nei's genetic distance (below diagonal) and Nei's identity (above diagonal) among the six groups of isolates. The identity is a ratio of the proportions of alleles that are alike between and within populations (identity = proportion of alleles alike between/proportion of alleles alike within), while genetic distance is a measurement of divergence with respect to the ancestral population (Nei 1978; Weir 1996). All populations presented more genetic distance than genetic identity. A dendrogram was constructed from

Table 4. Identity and distance *Nei's matrix.

	Standard	Irapuato	Oaxaca	Pachuca	Culiacan	Toluca
Standard	0	0.3407	0.2509	0.2834	0.2096	0.3368
Irapuato	1.0769	0	0.5199	0.5350	0.4164	0.5148
Oaxaca	1.3825	0.6541	0	0.5584	0.5467	0.5379
Pachuca	1.2609	0.6255	0.5826	0	0.4820	0.5505
Culiacan	1.5625	0.8761	0.6038	0.7299	0	0.4966
Toluca	1.0882	0.6640	0.6201	0.5970	0.7000	0

* Genetic distance (below diagonal) and identity (above diagonal). The identity is a ratio of the proportions of alleles that are alike between and within populations, while genetic distance is a measurement of divergence with respect to the ancestral population.

Nei's distances presented in Table 4 (Figure 3). The standard isolates formed the most distant group; this correlates with the geographic origin of this population, given that it consists of isolates from the USA whereas the other populations are native to Mexico. This agrees with results previously reported (Sánchez-Alonso *et al.* 1996). Oaxaca and Pachuca showed the highest among-population identity, which makes them the closest populations. Irapuato and Culiacan presented the highest within-population genetic diversity, which makes them the most separate populations. There was no correlation between genetic distance and geographic distance or altitude, or annual precipitation; this may be the result of small sample sizes or a high variability of the fungus due to dispersion patterns and sexual reproduction (Christensen 1963).

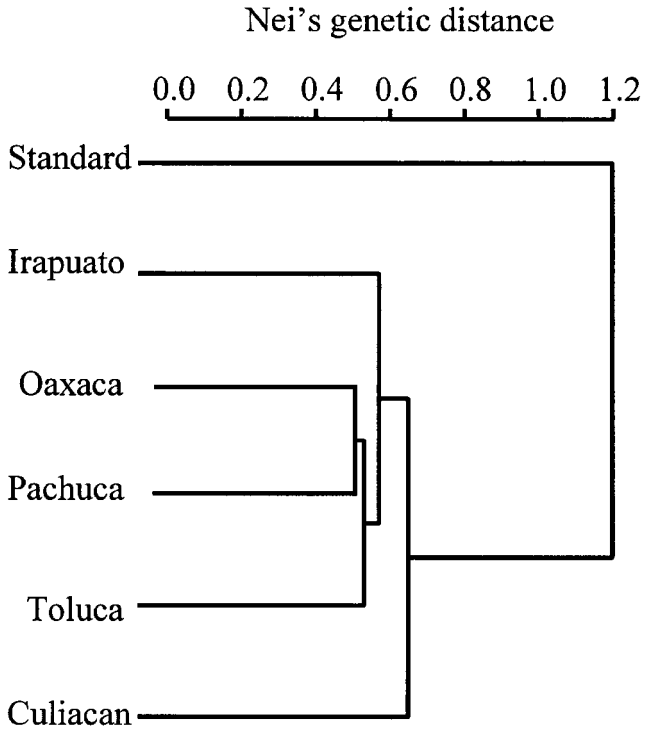

Figure 3. UPGMA cluster dendrogram obtained from Nei's genetic distance matrix from RFLP data of *Ustilago maydis* isolates from five different locations in Mexico (Irapuato, Oaxaca, Pachuca, Toluca and Culiacan) and two laboratory strains (Standard).

We are aware that sample size is small and that the data presented are insufficient to discriminate the causes of population divergence; however, *U. maydis* presented a huge diversity. Given that a large proportion of the genome of most eukaryotes is composed of non-coding DNA, and that the probes were selected at random, we can speculate that it is likely that many of the RFLP loci are selectively neutral. If this is the case, then the main forces that determine the genetic frequencies of these loci are genetic drift and migration.

Genetic structure refers to the amount and distribution of genetic variation within and among populations. The definition of the genetic structure of populations is a logical first step in studies of fungal population genetics, because the genetic structure of a population reflects its evolutionary history and its potential to evolve (McDonald 1997). The use of genetic markers may offer insight about genetic variation and distribution of alleles and genotypes (population structure) and the challenge is to relate molecular marker data to the biology of the organism (Francis & St.Clair 1997).

Our results demonstrated that RFLP fingerprinting is a useful method for detecting genetic variation in populations of *U. maydis* and can be utilized as a reliable tool to discriminate populations of this fungus and also to study the genetic relationships among and within them. This work also indicates that field populations of *U. maydis* are composed of genetically diverse isolates.

Acknowledgment

This research was supported by grants from Consejo Nacional de Ciencia y Tecnología-México.

References

Avise, J.C. 1994 *Molecular Markers, Natural History and Evolution*. London: Chapman & Hall. ISBN 0-412-03771-8.

Banuett, F. 1992 *Ustilago maydis*, the delightful blight. *Trends in Genetics* **8**, 174–180.

Banuett, F. 1995 Genetics of *Ustilago maydis*, a fungal pathogen that induces tumors in maize. *Annual Review of Genetics* **29**, 179–208.

Banuett, F. & Herskowitz, I. 1989 Different *a* alleles of *U. maydis* are necessary for maintenance of filamentous growth but not for meiosis. *Proceedings of the National Academy of Sciences of the USA* **86**, 5878–5882.

Boeger, J.M., Chen, R.S. & McDonald, B.A. 1993 Gene flow between populations of *Mycosphaerella graminicola* (Anamorph *Septoria tritici*) detected with restriction fragment length polymorphism markers. *Phytopathology* **83**, 1148–1154.

Castle, A., Speranzini, D., Rghei, N., Alm, G., Rinker, D. & Bissett, J. 1998 Morphological and molecular identification of *Trichoderma* isolates on North American mushroom farms. *Applied and Environmental Microbiology* **64**, 133–137.

Christensen, J.J. 1963 Corn smut caused by *U. maydis*. Monograph No 2. The American Phytopathological Society, p. 41.

Christiansen, S.K. & Giese, H. 1990 Genetic analysis of the obligate parasitic barley powdery mildew fungus based on RFLP and virulence loci. *Theoretical and Applied Genetics* **79**, 705–712.

Francis, D.M. & St.Clair, D.A. 1997 Population genetics of *Pythium ultimum*. *Phytopathology* **87**, 454–461.

Fry, W.E., Goodwin, S.B., Matuszak, J.M., Spielman, L.J., Melgroom, M.G. &. Drenth, A. 1992 Population genetics and intercontinental migration of *Phytophthora infestans*. *Annual Review of Phytopathology* **30**, 107–129.

Girardin, H., Latgé, J.P., Srikantha, T., Morrow, B. & Soll, D.R. 1993 Development of DNA probes for fingerprinting *Aspergillus fumigatus*. *Journal of Clinical Microbiology* **31**, 1547–1554.

Griffith, G. & Shaw, D.S. 1998 Polymorphisms in *Phytophthora infestans*: Four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host lesions. *Applied and Environmental Microbiology* **64**, 4007–4014.

Goodwin, S.B., Drenth, A. & Fry, W.E. 1992a Cloning and genetic analysis of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Current Genetics* **22**, 107–115.

Goodwin, S.B., Spielman, L.J., Matuszak, J.M., Bergeron, S.N. & Fry, W.E. 1992b Clonal diversity and genetic differentiation of *Phytophthora infestans* population in Northern and Central Mexico. *Phytopathology* **82**, 955–961.

Goodwin, S.B., Cohen, B.A. & Fry, W.E. 1994 Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Sciences of the USA* **91**, 11591–11595.

Hamelin, R.C., Doudrick, R.L. & Nance, W.L. 1994 Genetic diversity in *Cronartium quercuum* f. sp. *fulsiforme* on loblolly pines in Southern US *Current Genetics* **26**, 359–363.

Hamer, J.E., Farrall, L., Orbach, M.J., Valent, B. & Chumley, F.G 1989 Host species-specific conservation of family of repeated DNA sequences in the genome of a fungal plant pathogen. *Proceedings of the National Academy of Sciences of the USA* **86**, 9981–9985.

Hudspedh, M.E.S., Timberlake, W.E. & Goldberg, R.B. 1977 DNA sequences organization in water mold *Achlya*. *Proceedings of the National Academy of Sciences of the USA* **74**, 4332–4336.

Kohli, Y. & Kohn, L.M. 1998 Random association among alleles in clonal populations of *Sclerotinia sclerotiorum*. *Fungal Genetics and Biology* **23**, 139–149.

Krumlauf, R. & Marzluf, G.A. 1980 Genome organization and characterization of the repetitive and inverted repeat DNA sequences in *Neurospora crassa*. *Journal of Biological Chemistry* **255**, 1138–1195.

Levy, M., Romao, J., Marchetti, M.A. & Hamer, J.E. 1991 DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. *Plant Cell* **3**, 95–102.

Lewis, P.O. & Zaykin, D. 1999 Genetic Data Analysis: Computer program for the analysis of allelic data. Version 1.0 (d12). Free program distributed by the authors over the internet from the GDA Home Page at http://chee.unm.edu/gda/.

McDonald, B.A. & Martínez, J.P. 1991 DNA fingerprinting of the plant pathogenic fungus *Mycosphaerella graminicola* (Anamorph *Septoria tritici*). *Experimental Mycology* **15**, 146–158.

McDonald, B.A., Miles, J., Nelson, L.R & Pettway, R.E. 1994 Genetic variability in nuclear DNA in field population of *Stagonospora nodorum*. *Phytopathology* **84**, 250–255.

McDonald, B.A. 1997 The population genetics of fungi: tools and techniques. *Phytopathology* **87**, 448–453.

Michelmore, R.W. & Hulbert, S.H. 1987 Molecular markers for genetic analysis of phytopathogenic fungi. *Annual Review of Phytopathology* **25**, 383–404.

Nei, M. 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.

Pope, D.D. & McCarter, S.M. 1992 Evaluation of inoculation methods for inducing common smut on corn ears. *Phytopathology* **82**, 950–955.

Puhalla, J.E. 1970 Genetic studies of the *b* incompatibility locus in *Ustilago maydis*. *Genetical Research Cambridge* **16**, 229–232.

Ristaino, J.B., Madritch, M., Trout, C.L. & Parra, G. 1998 PCR amplification of ribosomal DNA for species identification in the plant pathogen genus *Phytophthora*. *Applied and Environmental Microbiology* **64**, 948–954.

Rosewich, U.L. & McDonald, B.A. 1994 DNA fingerprinting in fungi. *Methods in Molecular and Cellular Biology* **5**, 41–48.

Rowell, J.B. & De Vay, J.E. 1954 Genetic of *Ustilago zeae* in relation to basic problems of its pathogenicity. *Phytopathology* **44**, 356–362.

Rowell, J.B. 1955 Functional role of compatibility factors and *in vitro* test for sexual compatibility with lines of *Ustilago zeae*. *Phytopathology* **45**, 370–374.

Sambrook, J., Fritsch, E.F. & Maniatis, T. 1989 *Cloning: a laboratory manual 2nd ed*. New York: Cold Spring Harbor Laboratory Press. ISBN 0-87969-309-6.

Sánchez-Alonso, P., Valverde, M.E., Paredes-López, O. & Guzmán, P. 1996 Detection of genetic variation in *U. maydis* strains by probes derived from telomeric sequences. *Microbiology* **142**, 2931–2936.

Skinner, D.Z., Budde, A.D., Farman, M.L., Smith, J.R., Leung, H. & Leong, S.A. 1993 Genome organization of *Magnaporthe grisea*: genetic map, electrophoretic karyotype, and occurrence of repeated DNAs. *Theoretical and Applied Genetics* **87**, 545–557.

Skroch, P., Tivang J. & Nienhuis, J. 1992 *Analysis of genetic relationships using RAPD marker data*. In Joint Plant Breeding Symposia. pp. 26–30. Minneapolis.

Sneath, P.H.A. & Sokal, R.R. 1973 *Numerical Taxonomy*. San Francisco: W.H. Freeman and Company. ISNB 0-7167-0697-0.

Thakur, R.P., Leonard, K.J. & Pataky, J.K. 1989 Smut gall development in adult corn plants inoculated with *U. maydis*. *Plant Disease* **73**, 921–925.

Timberlake, W.E. 1978 Low repetitive DNA content in *Aspergillus nidulans*. *Science* **202**, 973–975.

Ueng, P.P., Bergstrom, G.C., Slay, R.M., Geiger, E.A., Shaner, G. & Svharen, A.L. 1992 Restriction fragment length polymorphisms in the wheat glume blotch fungus, *Phaeosphaeria nodorum*. *Phytopathology* **82**, 1302–1305.

Valverde, M.E. 1992 Studies on *Ustilago maydis* (Huitlacoche) infection and its nutritional characteristics. M.Sc. Thesis. Centro de Investigación y de Estudios Avanzados del IPN, Unidad Irapuato, México.

Weir, B.S. 1996 *Genetic Data Analysis II*. California: Sinauer Associates, Inc. ISNB 0-87893-902-4.

Weisisng, K.H. & Kahl, G. 1997 Hybridization-based microsatellite fingerprinting of plants and fungi. In: *DNA Markers, Protocols, Applications and Overviews*. ed. Caetano-Anollés, G & Gresshoff, P.M. New york: Wiley-VCH. ISNB 0-471-16067-9.

Weising, K., Nybom, H., Wolff, K. & Meyer, W. 1995 *DNA fingerprinting in plants and fungi*. Boca Raton, Florida: CRC Press, Inc. ISNB 0-8493-8720-8.

Xia, J.Q., Correll, J.C., Lee, F.N., Marchetti, M.A. & Rhoads, D.D. 1993 DNA fingerprinting to examine microgeographic variation in the *Magnaporthe grisea* (*Pyricularia grisea*) population in two rice fields in Arkansas. *Phytopathology* **83**, 1029–1035.

Zambino, P., Groth, J.V., Lukens, L., Garton, J.R. & May, G. 1997 Variation at the *b* mating type locus of *U. maydis*. *Phytopathology* **87**, 1222–1239.