Clonal Diversity and Genetic Differentiation of *Phytophthora infestans* Populations in Northern and Central Mexico

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ABSTRACT

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Two *Phytophthora infestans* populations in northern Mexico (northwestern and northeastern) were analyzed for genetic variation by use of mating type, two allozyme loci, and two DNA fingerprinting probes, and were compared to two populations from central Mexico. The genetic structure of the populations varied widely, even though both mating types occurred in all locations sampled. There was very little genetic diversity in northwestern Mexico; four genotypes were detected among 88 isolates tested, and only two of these were common. All A2 isolates in this population appeared to represent a single clone and were exclusively on potatoes, whereas the A1 isolates were primarily on tomatoes. As the A2 mating type was not known previously in northwestern Mexico, this clone probably represents a recent expansion in its range. In contrast to the low diversity in northwestern Mexico, isolates from northeastern and central Mexico were very diverse. In central Mexico, almost every isolate had a unique genotype, consistent with the hypothesis that sexual reproduction is frequent in this area. There was a moderate degree of genetic differentiation among populations from all four locations (Nei's $G_{ST} = 0.12$ for allozymes and 0.14 for DNA), indicating some restriction on gene flow. Although the DNA fingerprinting probes provided a higher degree of resolution, allozymes were surprisingly robust for estimating genetic diversity in many *P. infestans* populations. A previously unreported allele at the *Gpi* locus, 111, was found in northwestern and northeastern Mexico, but not in the samples from central Mexico. This allele occurred most often in a five-banded allozyme phenotype that was shown to be attributable to three alleles on different chromosomes in the same individual and, thus, provides the first genetic evidence for elevated ploidy in *P. infestans*.

Additional keywords: aneuploidy, gene diversity analysis, population genetics, potato late blight disease.

Central Mexico is believed to be the center of origin of the *Phytophthora infestans*-Solanaceae pathosystem. Until relatively recently, this was the only location in which both mating types of *P. infestans* were known to occur (17), and genetic diversity for allozyme (26) and virulence loci (11,14,19,27) is higher in central Mexico than elsewhere. However, almost nothing is known about the extent of genetic variation and degree of genetic differentiation among *P. infestans* populations in other areas of Mexico. There is also no information available about the clonal diversity of genotypes in any *P. infestans* populations. The capacity of sporangia for aerial dispersal over hundreds of kilometers during the course of a season (1) should lead to frequent migration and consequently little genetic differentiation among *P. infestans* populations within Mexico. Furthermore, the movement and exchange of seed potatoes among the different production areas of Mexico should facilitate the spread of the fungus from region to region. An analysis of the genetic structure of *P. infestans* populations is necessary to test hypotheses about the influence of migration on the population genetics and evolution of the *P. infestans*-Solanaceae pathosystem in Mexico.

In contrast to central Mexico, genetic diversity within *P. infestans* populations in the United States is extremely low (26). Until very recently (5), import and quarantine restrictions appear to have kept the A2 mating type out of the United States. One of the most likely avenues for the introduction of the A2 mating type into the United States would be through a cross-border migration from Mexico. The A2 mating type has been reported

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throughout the central highlands of Mexico and into northeastern Mexico, but not in northwestern Mexico or those areas closest to the U.S. border (18). Migration of *P. infestans* between northwestern Mexico and the other potato and tomato production areas of Mexico may be limited by deserts and high mountain ranges. It is not known if the northern Mexican *P. infestans* populations are more similar to those in other parts of Mexico or to those in the United States. Therefore, to assess the potential of cross-border migrations for augmenting genetic diversity in U.S. *P. infestans* populations, it is imperative to analyze the genetic structure and variability of *P. infestans* populations in northern Mexico.

Recently, moderately repetitive nuclear DNA "fingerprinting" probes have been developed for *P. infestans* (9,10). One of these probes, RG57, hybridizes to more than 25 distinct genetic loci (10) and thus provides a powerful tool for studying the genetic structure of *P. infestans* populations. The primary purpose of this paper was to elucidate the genetic structure of *P. infestans* populations in different parts of Mexico and to test the hypothesis that frequent migration (due to wind dispersal of sporangia and man-guided movement of tubers) leads to a low level of genetic substructuring among *P. infestans* populations in northern and central Mexico. The results may help determine the relative importance of sexual versus asexual reproduction in different *P. infestans* populations. Preliminary reports of some of the mating type and allozyme data appeared in Tooley et al (26), Fry and Spielman (8), and Spielman (21). A secondary goal was to compare the use of allozymes versus DNA fingerprinting for elucidating the genetic structure of *P. infestans* populations. Finally, in addition to providing information about the *P. infestans*-Solanaceae pathosystem in Mexico, knowledge of where the A2 mating type currently occurs will establish a baseline for monitoring its future spread into other areas of northern Mexico and the southern United States.

MATERIALS AND METHODS

Sources of isolates. More than 200 *P. infestans* isolates were obtained from naturally infected potato and tomato fields between 1983 and 1989. Four locations were sampled (Table 1). Los Mochis is in northwestern Mexico; Saltillo is in northeastern Mexico; and the Toluca Valley region and Chapingo are in central Mexico (Fig. 1). Lesions were sampled randomly from different infected plants within a field. Sample sizes varied (Table 1) depending on the prevalence of the disease. In some fields where there was not much disease, all available diseased plants were sampled. The number of infected fields sampled at each location varied. In Los Mochis, 21 fields were sampled (five potato, 16 tomato) within a 40-km radius (Fig. 2). Sample sizes within each field ranged from one to 13. Two fields approximately 15 km apart were sampled in Saltillo. Four additional isolates in the Saltillo collection came from infected tubers from a field near Santiago, Nuevo Leon (within 80 km of Saltillo), in February 1988. The 1983 collection from Toluca was from seven locations within the Toluca Valley region (26). Isolates from subsequent years were primarily from the potato germ plasm bank located near Toluca. The isolates from Chapingo came from a number of nearby sites. A preliminary study (allozymes and mating types only) of the 1983 and 1984 isolates from Toluca and Chapingo has been published (26). Tooley et al (26) pooled all central Mexican isolates. However, they were collected from many different locations so probably cannot be considered as part of the same population. Chapingo is separated from Toluca by high mountains, and the isolates from these two locations were collected in different years, therefore they are not a part of the same population and have been analyzed separately in this paper.

We made isolations from infected leaves, stems, or tubers by soaking lesion-containing tissue pieces in 70% ethanol for 15 s, surface-sterilizing in 0.525% sodium hypochlorite for 2–5 min, washing briefly in sterile distilled water, and plating onto 10% V8 juice agar (13) containing antibiotics (20 mg/L of rifamycin, 50 mg/L of Polymyxin B sulfate, 200 mg/L of ampicillin) and fungicides (67 mg/L of pentachloronitrobenzene 75WP, 100 mg/L of benomyl 50WP). All isolates were subsequently maintained on Rye A agar (4) or on 10% unclarified V8 juice agar plates at 18 C with transfers every 3–4 mo. Replicate cultures of each isolate are stored permanently in our culture collection at Cornell University by cryogenic storage at −135 C, in liquid nitrogen (25), and under oil at 18 C.

TABLE 1. Details of *Phytophthora infestans* collection sites in Mexico

Location	Date collected	Host	Sample size	Collected by
Los Mochis, Sinaloa	March 1989	Potato	30	S. N. Bergeron
(Northwest)	March 1989	Tomato	58	S. N. Bergeron
Saltillo, Coahuila	February 1988	Potato	4	J. Amador[a]
(Northeast)	August 1988	Potato	40	W. E. Fry
Toluca, Mexico	August 1983	Potato	41[b]	P. W. Tooley[c]
(Central)	August 1986	Potato	11	J. M. Parker[c] and A. Rivera[d]
	July 1987	Potato	8	J. M. Parker
Chapingo, Mexico (Central)	August 1984	Potato	9	J. Galindo[e] and P. W. Tooley

[a]Texas A&M University.
[b]For one isolate in this group, year and location of collection are unknown, but it was probably from Toluca.
[c]Cornell University.
[d]Instituto Nacional de Investigaciones Forestales y Agropecuaria, Mexico.
[e]Colegio de Postgraduados, Chapingo, Mexico.

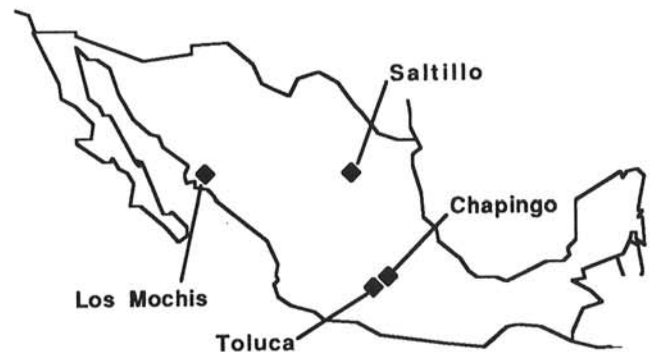

Fig. 1. Locations of the four major sampling sites in Mexico.

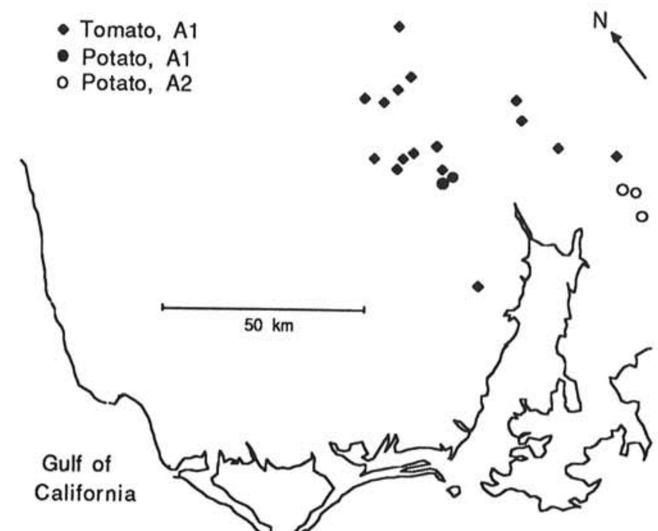

Fig. 2. Spatial arrangement of the five potato and 16 tomato fields sampled in Los Mochis (northwestern Mexico) and the mating types of isolates collected.

TABLE 2. Frequencies of mating types and glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) alleles by location

Location	Mating type			*Gpi*						*Pep*		
	A1	A2	SF[a]	83	86	90[b]	100	111	122	83	92	100
Los Mochis	0.716	0.284	...	...	0.025	...	0.726	0.127	0.137	...	0.345	0.655
Saltillo	0.317	0.659	0.024	...	...	...	0.878	0.061	0.061	...	...	1.000
Toluca 1983[c]	0.463	0.537	...	...	0.098	0.024	0.829	...	0.049	...	0.146	0.854
Toluca 1986	0.455	0.545	...	...	0.318	...	0.591	...	0.091	...	0.227	0.773
Toluca 1987	0.875	0.125	...	...	0.500	...	0.125	...	0.375	...	...	1.000
Chapingo[c]	0.556	0.444	...	0.056	0.056	...	0.722	...	0.167	0.056	0.444	0.500

[a] Self-fertile.
[b] Scored as 98 or 100 in previous studies (23,26).
[c] Modified from Tooley et al (26).

Characterization of isolates. We determined mating types by pairing each isolate with known A1 and A2 testers on 10% clarified V8 juice agar as described in Spielman et al (23). Allozyme genotypes for glucose-6-phosphate isomerase (GPI, EC 5.3.1.9) and peptidase (PEP, EC 3.4.3.1) were also determined according to the methods of Spielman et al (23). Methods of DNA extraction, restriction enzyme digestion, and Southern analysis were as described in Goodwin et al (10). DNA "fingerprints" were visualized by hybridizing blots with ^{32}P-labeled DNA of the two dispersed, moderately repetitive nuclear DNA probes, RG7 and RG57 (10). Most, but not all, of the isolates scored for allozymes and mating type were also used for the DNA fingerprinting analysis; occasional degradation of some DNA samples made it impossible to accurately score the higher molecular weight bands, so these isolates were excluded from further analysis. Genetic crosses were made as described in Spielman et al (23).

Data analysis. Allele frequencies were calculated for each locus at each location. For the two allozyme loci, alleles were designated as described in Tooley et al (26). The DNA fingerprinting probe RG57 reveals at least 25 bands in *P. infestans* (10). Genetic analyses have shown that each band represents a dominant allele at a different genetic locus; each locus segregates for the presence or absence of a band, and most of the loci are unlinked (10). The genotype of each isolate at each locus was deduced from the intensities of the bands; homozygotes with two allele doses were approximately twice as dark as heterozygotes with one dose (10). The higher molecular weight bands and certain other bands that did not show clear genetic segregations (10) were excluded from most analyses. Deviations from Hardy-Weinberg expectations and Workman and Niswander's (28) χ^2 contingency test for heterogeneity in allele frequencies were calculated by using the BIOSYS-1 program of Swofford and Selander (24). Gene diversity at each locus in each population was calculated by the method of Nei (15): $h_s = 1 - \Sigma p^2_i$, in which p_i is the frequency of the ith allele. The mean gene diversity ($\bar{h}_s$) was the mean of the h_s over loci in each population. Nei's G_{ST} (15) was used as a measure of population subdivision among locations. The equations of Nei and Chesser (16) were used to correct for bias in sampling within and among populations. Clonal diversity at each location was determined by using the multilocus genotype of each isolate. Isolates with the same restriction fragment length polymorphism (RFLP) banding pattern at all loci were considered the same multilocus genotype. Only the presence or absence of bands was considered for the genotypic diversity analyses. A normalized Shannon information statistic was used to measure genotypic diversity: $H_S = H/H_{MAX}$, in which H is the usual Shannon diversity measure (2) over genotypes, and H_{MAX} is ln(N), the maximum diversity for a sample of size N. This statistic has been found to be relatively stable and has provided the best measure of diversity (among three tested) when sample sizes varied (20).

RESULTS

Mating type and allozymes. Both mating types were found in all locations sampled (Table 2). The frequency of the A2 mating type was approximately 50% for the 1983 and 1986 Toluca samples and for the 1984 Chapingo sample. There was a significant departure from a 1:1 ratio for Los Mochis ($\chi^2 = 16.41$; $P < 0.005$) and Saltillo ($\chi^2 = 4.9$; $P < 0.025$). This discrepancy was even more evident when the samples from individual fields were analyzed separately. Three fields were sampled in the area near Saltillo. The four isolates from February 1988 (the "Santiago" collection) were all A1, 100/100, and 100/100 for mating type, glucose-6-phosphate isomerase (*Gpi*), and peptidase (*Pep*), respectively. Indeed, these isolates appeared to be all one clone according to DNA fingerprint analysis (see below). The August 1988 collection came from two fields near Saltillo, Bayonero and Vera Cruz. Seven isolates came from the Vera Cruz collection, and all were 100/100 at both allozyme loci. Six were A1, and one was A2. All of the A1 isolates appeared to be of the same clonal lineage as determined by DNA fingerprinting (see below). The remaining 34 isolates came from the Bayonero collection, and only seven of them were A1. Thus, there was a large excess of A2 in this field. Considering both allozyme loci together, there were four dilocus genotypes in total among the 34 isolates in the Bayonero collection (Table 3).

TABLE 3. Frequency of glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) allozyme genotypes and of mating types in Saltillo (August 1988 collection only), and the number of RFLP patterns identified by probe RG57 associated with each genotype[a]

Mating type	*Gpi*	*Pep*	Number of isolates	Frequency	Number of RFLP genotypes
A1	100/100	100/100	12	0.300	7
A2	100/100	100/100	20	0.500	7
A2	100/111	100/100	3	0.075	1
A2	100/122	100/100	2	0.050	1
SF[b]	100/122	100/100	1	0.025	1
A2	100/111/122	100/100	2	0.050	2

[a] The four isolates from February 1988 shared a single RFLP genotype. RFLP = restriction fragment length polymorphism.
[b] Self-fertile.

Only a single mating type was found in each of the 21 fields sampled in Los Mochis. The A2 isolates were exclusively from three potato fields that were in close proximity to each other on the southeastern border of the area sampled (Fig. 2). Isolates from the two remaining potato fields and from all of the tomato fields were mating type A1. On the basis of the data for mating type and the two allozyme loci, only three genotypes were found in Los Mochis. The most common genotype (*Gpi* 100/100, *Pep* 92/100, and A1 mating type) occurred in all 16 tomato fields sampled (57 isolates) (Table 4). Three isolates with this genotype also occurred in one potato field. All of the 25 A2 isolates had a five-banded allozyme phenotype for *Gpi* (Fig. 3) that is probably due to three different alleles in the same individual; these were therefore scored as 100/111/122. This five-banded phenotype was also found in two isolates from Saltillo. The 111 allele for *Gpi* has not been reported previously except in brief summaries of the northern Mexican data (7,20). All Los Mochis isolates with the five-banded *Gpi* phenotype were 100/100 for *Pep* and appear to represent a single clone according to DNA fingerprinting analysis (see below). There was a rare genotype that was A1,

86/122 for *Gpi* and 100/100 for *Pep* ; it occurred in two isolates from one potato field (Table 4).

When the Chapingo and Toluca isolates were analyzed separately, the pattern of variation was somewhat different from that reported by Tooley et al (26) (Table 2). Two of the rare alleles reported earlier (26), the *Gpi* 83 allele and the *Pep* 83 allele, were found in Chapingo and not in the Toluca region. The additional Toluca samples from 1986 and 1987 carried the 86, 100, and 122 alleles for *Gpi* found in the 1983 collection, but not the rare "98" (23) allele. When the isolates with the putative 98/98 genotype were retested with longer run times, which permit better separation of bands, they appeared to be identical to certain isolates with the 90/100 genotype from the Netherlands (7) and Poland (S. B. Goodwin, *unpublished*). The 90/100 heterodimer comigrates with the 98/98 phenotype, and in some isolates that are heterozygous for the 90 and 100 alleles neither of the homodimers are observed, only the 90/100 heterodimer. So, they appear homozygous rather than heterozygous. This condition has been documented by use of isoelectric focusing (7). Therefore, the isolates that were scored as 98/98 by Spielman et al (23) were misclassified and are really genotype 90/100. The differences in allele frequencies among the different years in Toluca are probably not meaningful because of the small sample sizes.

The allozyme genotypes were in Hardy-Weinberg equilibrium in all populations sampled except the 1987 Toluca (small sample size; $\chi^2 = 8.00$, $P < 0.046$) and Los Mochis ($\chi^2 = 174.0$ for *Gpi* and 24.1 for *Pep*) populations (the 100/111/122 genotypes were excluded from this analysis in Saltillo). There was significant heterogeneity in allele frequencies among locations at the *Gpi* ($\chi^2 = 66.9$, $P < 0.001$) and *Pep* ($\chi^2 = 69.5$, $P < 0.001$) loci.

To investigate the genetic basis for the five-banded phenotypes at the *Gpi* locus, we made two crosses involving isolates that appeared to be 100/111/122. In the first cross, central Mexican isolate 580 (mating type A1, *Gpi* 100/100) was crossed to Los Mochis isolate 660 (A2, 100/111/122). Isolate 660 has a DNA content near 2C (W.-K. Gu, *personal communication*), so it could be trisomic and have an extra chromosome containing the *Gpi* locus, rather than being polyploid. If isolate 660 is trisomic and there are no other meiotic abnormalities, both euploid and aneuploid gametes should result from each meiosis. There are six possible gametes that can be produced by the trisomic (100/111/122) parent: 100, 111, 122, 100/111, 100/122, and 111/122. These gametes will always combine with a gamete containing the 100 allele from the other parent (isolate 580), giving six possible genotypic classes in the progeny: 100/100, 100/111, 100/122, 100/100/111, 100/100/122, and 100/111/122. Although in theory it should be possible to distinguish the genotypes 100/111 and 100/100/111 on an allozyme gel, in practice it is often difficult and unreliable. Therefore, the genotypic classes 100/111 and 100/100/111 are combined into the one phenotypic class 100/111, whereas the genotypic classes 100/122 and 100/100/122 are collapsed into the single phenotypic class 100/122. This leaves four phenotypic classes among the six genotypic classes expected in the cross between isolates 580 and 660. Because they contain two genotypic classes, twice as many progeny are expected in the combined phenotypic classes. Among the four phenotypic classes expected in the progeny of this cross, three were recovered: 100/100 (11 progeny), 100/111 (three progeny), and 100/122 (11 progeny). The fourth class (100/111/122) was not observed. The proportion of progeny recovered in each class differed significantly from the 1:2:2:1 ratio expected, because of a large deficiency of transmission of the 111 allele. However, the segregation of the 111 and 122 alleles into separate progeny proved that the 111 allele is allelic to the others at this locus and that the five-banded phenotype was attributable to three alleles in a single individual.

The other cross, between Chapingo isolate 562 (A1, *Gpi* 86/100) and Los Mochis isolate 662 (A2, 100/111/122), behaved similarly. Isolate 662 has a DNA content intermediate between diploid and triploid (W.-K. Gu, *personal communication*) and thus is also probably an aneuploid and trisomic for the chromosome bearing the *Gpi* locus. At meiosis, this isolate will give the same six gamete classes described above. These will be combined with gametes carrying either the 86 or the 100 allele from the A1 parent to give 12 possible genotypic classes that will be reduced to 10 phenotypic classes. Among the 10 phenotypic classes expected from this cross, seven were recovered among only 14 progeny. The phenotypes of the isolates recovered were: 86/100/111 (one progeny), 86/111 (one progeny), 86/100/122 (one progeny), 86/122 (two progeny), 100/100 (two progeny), 100/111 (four progeny), and 100/122 (three progeny). No progeny were recovered from class 86/100, 86/111/122, or 100/111/122. Thus, this cross also appeared to segregate as predicted assuming one trisomic parent, particularly because twice as many progeny were expected in the two classes that had the largest numbers of progeny.

Moderately repetitive RFLP probes. Probe RG57 hybridized to 27 different bands (Table 5), each of which probably corresponds to a different genetic locus (9,10). This probe proved to be a powerful tool for analyzing the genetic structure of *P. infestans* populations. In some populations, the data generated from analysis with the probe confirmed the largely clonal nature of the population, which had been inferred from the allozyme data. However, in other locations the probe revealed a lot of diversity that was not evident from the allozyme data.

The RFLP bands that occurred in northern Mexico were a subset of those in central Mexico (Table 5). Twenty of the 27

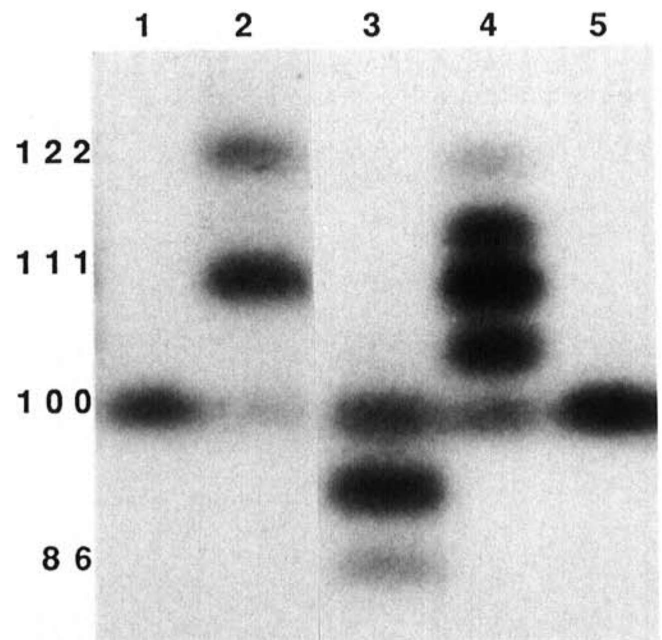

Fig. 3. Allozyme phenotypes at the glucose-6-phosphate isomerase (*Gpi*) locus. The genotypes of the isolates in each lane are as follows: 1, 100/100; 2, 100/122; 3, 86/100; 4, 100/111/122; 5, 100/100. The 100/111/122 genotype gives a five-banded phenotype because the 100/122 heterodimer comigrates with the 111/111 homodimer. Band intensities in the five-banded phenotype appear to approximate the 1:2:3:2:1 expected ratio.

TABLE 4. Frequency of mating types and of allozyme genotypes in Los Mochis, and the RFLP patterns for probe RG57 associated with each[a]

Host	Mating type	Allozyme genotype		RFLP pattern	Frequency
		Gpi[b]	*Pep*[c]		
Tomato	A1	100/100	92/100	A	0.648
	A1	100/100	92/100	B	0.011
Potato	A1	100/100	92/100	A	0.034
	A1	86/122	100/100	C	0.023
	A2	100/111/122	100/100	D	0.284

[a] RFLP = restriction fragment length polymorphism.
[b] Glucose-6-phosphate isomerase.
[c] Peptidase.

bands were in Saltillo, and 18 were in Los Mochis. With the exception of band 6, the bands in Los Mochis were a subset of those in Saltillo.

In Los Mochis, the largely clonal nature of the populations was confirmed from analysis of the fingerprint data. One additional genotype was found in a tomato isolate that had a different banding pattern from the other isolates. All of the A2 potato isolates had the same fingerprint pattern. In Los Mochis, the allozyme markers uncovered most of the variation present, and the DNA probes confirmed that isolates with the same allozyme genotypes were indeed members of a clone.

There were six genotypes in Saltillo according to the allozyme and mating type data (counting the self-fertile as a separate genotype) (Table 3). However, on the basis of only the DNA data, there were at least 19 different genotypes. The two isolates that had the five-banded phenotype for *Gpi* had the same mating type and peptidase genotype, but had different patterns according to the RFLP analysis (Table 3). Most of the RFLP patterns differed by at least two or three bands. Both isolates in Saltillo with the five-banded *Gpi* phenotype (the three-allele genotypes) were different from the five-banded isolates in Los Mochis. Two isolates from Saltillo that had the 100/111 *Gpi* genotype had an RFLP banding pattern identical to one of those with the 100/111/122 *Gpi* genotype and thus may be clonally derived from the latter by the loss of the 122 allele. The Los Mochis isolates with the 100/111/122 genotype had an RFLP banding pattern very similar to the 100/111/122 isolates from Saltillo, but very distinct from the other common pattern in Los Mochis (Fig. 4).

The levels of genotypic diversity for the populations in central Mexico were very different from those in northern Mexico. Almost every isolate in Toluca and Chapingo had a unique banding pattern and thus was a different genotype.

One isolate from Saltillo had the same RFLP banding pattern as an isolate from Toluca. This was the only RFLP genotype that occurred in more than one location. The two isolates had different mating types and *Gpi* genotypes, so they were not the same clone.

The other DNA fingerprinting probe, RG7, provided much the same information as probe RG57. Isolates that were identical for the RG57 fingerprint pattern were also identical for the RG7

TABLE 5. Band frequencies for probe RG57 by location

Band	Location					
			Toluca			
	Los Mochis	Saltillo	1983	1986	1987	Chapingo
1	1.00	1.00	0.97	1.00	1.00	0.25
2	. . .	. . .	0.15	0.11	. . .	. . .
3	0.68	0.07	0.88	0.78	0.75	0.63
4	1.00	1.00	1.00	0.89	1.00	1.00
5	1.00	1.00	0.74	0.89	1.00	1.00
6	0.67	. . .	0.15	0.22	. . .	0.14
7	0.68	0.07	0.88	0.78	0.75	0.43
8	. . .	. . .	0.09	0.11	. . .	0.88
9	. . .	0.11	0.06	0.22	. . .	. . .
10	1.00	0.26	0.85	0.78	1.00	0.29
11	. . .	. . .	0.03	. . .	. . .	. . .
12	. . .	. . .	0.03	. . .	. . .	0.14
13	1.00	1.00	1.00	1.00	1.00	1.00
14	1.00	1.00	0.97	1.00	1.00	1.00
15	. . .	. . .	0.15	0.22	. . .	0.29
16	0.32	0.86	0.68	1.00	1.00	0.75
17	0.02	0.36	0.21	0.11	. . .	. . .
18	0.67	0.19	0.29	0.11	. . .	. . .
19	0.02	0.93	0.27	0.56	0.25	0.25
20	1.00	1.00	1.00	0.89	1.00	1.00
21	1.00	1.00	1.00	0.89	1.00	0.88
22	. . .	0.19	0.12	0.22	. . .	0.14
23	0.32	0.19	0.06	0.22	. . .	0.14
24	1.00	0.89	0.94	0.89	1.00	1.00
24a	. . .	. . .	0.03	. . .	. . .	. . .
25	1.00	1.00	1.00	1.00	1.00	1.00
25a	. . .	. . .	0.003	. . .	. . .	. . .

pattern. However, because the genetic interpretation of the RG7 bands is not as straightforward as those for RG57 (10), it was not used on all the isolates, and the data were not analyzed further. The primary use of this probe was in confirming the clonal nature of genotypes that were identified with the other markers.

Overall genotypic diversity was highest in central Mexico and lowest in Los Mochis (Table 6). The large number of loci scored with the RG57 probe greatly increased the resolution of genotypic diversity, and this was particularly evident for Saltillo and the 1983 Toluca collection.

Gene diversity analysis. Gene diversities varied from location to location and depended on the type of marker used (Table 7). Toluca and Chapingo had the highest overall gene diversities, followed by Los Mochis. Saltillo had the lowest gene diversity for both sets of markers. This result is misleading because genotypic (in contrast to gene) diversity is much higher in Saltillo than in Los Mochis. The high gene diversities in Los Mochis are due to the presence of two very different genotypes in high frequency, giving an even frequency of polymorphic markers.

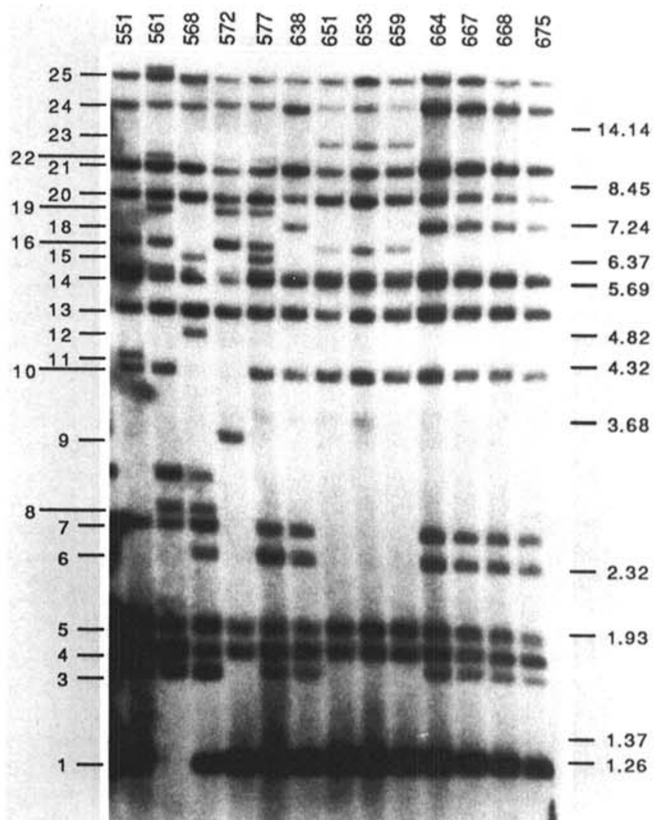

Fig. 4. DNA fingerprint patterns revealed by probe RG57 for representative isolates from central Mexico (where almost every individual had a unique genotype) and Los Mochis in northwestern Mexico (where two clonal genotypes dominated the sample). Band numbers are indicated on the left. The band between 8 and 9 in two isolates is due to plasmid contamination of the two DNA samples. Isolate 551 was from the 1983 Toluca collection; 561 and 568 were from the 1984 Chapingo collection; 572 and 577 were from the 1986 Toluca collection; and 638--688 were from Los Mochis in 1989. The Toluca and Chapingo isolates were from wild *Solanum* species or from cultivated potatoes. All of the Los Mochis isolates except 667 and 688 were from different fields. Isolates 651, 653, and 659 were from potatoes and have restriction fragment length polymorphism (RFLP) pattern "D" (see Table 3), the genotype possessed by all A2 isolates in Los Mochis. Isolates 638, 664, 667, and 688 were from tomatoes and have RFLP pattern "A", the most common pattern on tomatoes. Isolates 664 and 667 came from fields that were 50 km apart. Isolate 675 was from a potato field, has RFLP pattern "A", and except for the host is identical to the isolates from tomato with this RFLP pattern. Approximate migration distances and fragment sizes for phage lambda DNA digested with the restriction enzyme *Bst*EII are indicated on the right.

G_{ST} is the proportion of the total gene diversity that is due to differentiation among locations. There was a moderate level of genetic differentiation among the four locations (Table 7). Both the allozyme and DNA fingerprinting data sets gave very similar values for G_{ST}.

DISCUSSION

The hypothesis that a low level of genetic substructuring in northern and central Mexico exists was not supported by the electrophoretic data. *P. infestans* populations in northern and central Mexico are moderately differentiated. Although these locations are widely separated, this result was somewhat surprising given the high capacity for dispersal provided by the asexual sporangial stage of this fungus. Mountain ranges and deserts probably provide effective barriers to the migration of sporangia in Mexico. Most of the variation in northern Mexico was a subset of that in central Mexico, and this was particularly evident from the DNA markers. The exception was the 111 allele for *Gpi*, which occurred in both Los Mochis and Saltillo but has not yet been detected in central Mexico. *P. infestans* populations in northern Mexico also have a different mitochondrial DNA haplotype from those in Toluca (9; S. B. Goodwin, *unpublished*), and this raises the possibility that these populations may be even more differentiated than indicated by the present data.

The genetic structure of *P. infestans* populations in Mexico varied widely, even though both mating types occur in close proximity in all four locations sampled. The very low genotypic diversity in northwestern Mexico (Los Mochis) compared to the other locations probably means that Los Mochis is the only location where sexual reproduction does not occur; thus, reproduction in this population appeared to be completely clonal, whereas in central Mexico (Toluca and Chapingo) almost every individual had a unique genotype, indicating that sexual reproduction probably plays or has played an important part in the life cycle. In northeastern Mexico (Saltillo), there was a large number of distinct genotypes, but some genotypes were much more frequent than others. This population could be reproducing sexually, with selection during asexual reproduction leading to the dominance of particular clones at the end of the season. It is also possible that local environmental conditions in Saltillo favor the survival of many different clones from season to season. Monitoring these populations over a number of years will be required to confirm the existence of sexual reproduction in Saltillo.

Because they provided data on a much larger number of loci, the DNA fingerprinting probes were much more precise for elucidating population structure than allozymes. The allozyme loci were surprisingly robust for detecting genetic variation in some *P. infestans* populations. However, there was much more diversity in Saltillo than was indicated by the allozyme data, so allozyme uniformity does not necessarily imply a lack of genetic variation in populations of *P. infestans*. Improvements in technique (primarily changes in gel pH and run times) have increased the resolution of allozyme alleles. Two of the isolates that were initially reported to be *Gpi* 100/100 by Tooley et al (26) were subsequently scored as 98/98 by Spielman et al (23) after the pH of the gel buffer was lowered from 7 to 6. Lengthening of the run time from 3 to 10 h on pH 6 gels and use of appropriate controls provide even greater resolution, and this revealed that these isolates are really genotype 90/100. This is particularly interesting because the 90/100 genotype is one component of a "new" population of *P. infestans* that is rapidly becoming established and replacing the "old" genotypes in much of the world (22). This is the first report of the 90/100 genotype in Mexico, and it now looks as if it was present in the Toluca Valley as early as 1983.

The origin of the five-banded GPI phenotypes is unknown. The genetic analyses confirmed that they were due to three alleles in the same individual, and the segregation of each allele separately rules out tandem gene duplication. Some possible explanations are that they arose from unreduced gametes or meiotic nondisjunction during sexual reproduction, or from somatic fusion during vegetative growth. If they arose from unreduced gametes, all of these isolates should be triploid, but instead they appear to have DNA contents similar to those of diploids (W.-K. Gu, *personal communication*) and, thus, are probably aneuploids. New pathotypes of another oomycete, *Bremia lactucae*, in California appeared to have originated from somatic fusion (12), and the resulting "hyperploid" isolates were found in many different locations, indicating that this phenomenon may be common in that fungus. However, the lower ploidy levels of the five-banded *P. infestans* isolates would seem to argue against the somatic fusion hypothesis. The most likely explanation is that they are trisomics that arose from nondisjunction at meiosis of the chromosome containing the *Gpi* locus. Progeny with three alleles for *Gpi* have been obtained occasionally in laboratory crosses between diploid isolates of *P. infestans* (L. J. Spielman, *unpublished*), and these probably arose by meiotic nondisjunction. Furthermore, meiotic abnormalities appeared to be common in progeny from crosses between isolates of *P. parasitica* according to RFLP data (6). If meiotic nondisjunction is the origin of the Los Mochis and Saltillo isolates with the five-banded phenotypes, it probably occurred independently in each location because the isolates had different DNA genotypes that are unlikely to have arisen from instability in a single trisomic line.

A possible case of instability was observed in Saltillo. Isolates with the 100/111 *Gpi* genotype had the same mating type and DNA fingerprint pattern as one of the isolates with the 100/111/122 genotype. These 100/111 isolates may have been derived from the 100/111/122 genotype by the loss of the 122 allele. This could occur without changing the RFLP pattern because alleles at the DNA fingerprinting loci are dominant (10); furthermore, none of the RFLP loci is known to be linked to the *Gpi* locus. These three-allele genotypes provide the first genetic evidence for elevated ploidy in *P. infestans*. It is interesting that all of the field isolates that had the 111 allele were of the A2 mating type.

There are at least three factors that could explain the apparent lack of sexual reproduction in Los Mochis. First, host specialization may have isolated the two mating types. A1 isolates were primarily found on tomatoes, and the A2 isolates were exclusively on potatoes. A1 and A2 isolates were never found in the same field. A similar situation has been reported in Brazil where there

TABLE 6. Genotypic diversity as measured by the normalized Shannon information measure (H_S) for the two allozyme loci, glucose-6-phosphate isomerase and peptidase, and DNA fingerprinting loci revealed by probe RG57 for *Phytopthora infestans* populations in northern and central Mexico

	Genotypic diversity		
Location	Allozymes	DNA	Combined
Los Mochis	0.157	0.172	0.190
Saltillo	0.177	0.769	0.831
Toluca 1983	0.429	0.906	0.965
Toluca 1986	0.770	0.930	0.930
Toluca 1987	0.270	0.871	0.750
Chapingo	0.720	0.871	0.917

TABLE 7. Mean gene diversities over loci in each location ($\bar{h}_s$) and the overall genetic differentiation among locations (G_{ST}) for the two allozyme loci, DNA fingerprinting loci revealed by probe RG57, and the combined data set

Location	Allozymes	DNA	All loci
Los Mochis	0.460	0.180	0.205
Saltillo	0.111	0.126	0.125
Toluca 1983[a]	0.275	0.258	0.259
Chapingo	0.498	0.205	0.230
G_{ST} among locations	0.118	0.140	0.137

[a]The 1986 and 1987 Toluca samples were excluded from this analysis.

was a single A1 genotype on tomatoes and a different single A2 genotype on potatoes (3). Preliminary data from Los Mochis indicate that the potato genotypes had equal fitness on both hosts (measured by lesion size and sporulation capacity), whereas the tomato isolates had a much greater fitness on tomatoes (8; L. J. Spielman, *unpublished*).

Second, the A1 and A2 genotypes in Los Mochis may have been sexually or genetically incompatible. We have frequently observed failures in sexual reproduction in controlled crosses between particular isolates in the laboratory, even though both isolates were sexually competent in combination with other isolates. Even if oospores are formed, they may not germinate because of genetic incompatibilities. These incompatibilities may preclude sexual recombination in nature, even though both mating types occupy the same general area.

Third, the introduction of the A2 mating type may have been so recent that there has not yet been time for the progeny of sexual recombination to increase in frequency and become detectable. Collections of *P. infestans* from 1950 to 1970 revealed only A1 isolates from Los Mochis and areas to the north (18). Furthermore, the A2 genotype in Los Mochis was the only one with the 111 *Gpi* allele, and this clone differed from the other common clone at six of the RFLP bands for probe RG57. It also has a different mitochondrial DNA haplotype from the other isolates in Los Mochis (S. B. Goodwin, *unpublished*). This provides indirect evidence that the A2 genotype in Los Mochis may have been introduced recently from another area.

If the A2 mating type has spread to northern Mexico recently, then the probability of its introduction into the United States has increased. This could pose an increased burden on U.S. agriculture, because oospores are a potential long-lasting source of inoculum and reservoir of genetic variability. Additional sampling and monitoring of *P. infestans* populations in northern Mexico and the southern United States are needed for determining the current range and rate of establishment of A2 isolates; more stringent quarantine measures may be needed to prevent or at least delay their introduction to other potato- and tomato-growing areas.

LITERATURE CITED

1. Bourke, P. M. A. 1964. Emergence of potato blight, 1843-46. Nature (London) 203:805-808.
2. Bowman, K. O., Hutcheson, K., Odum, E. P., and Shenton, L. R. 1971. Comments on the distribution of indices of diversity. Stat. Ecol. Ser. 3:315-359.
3. Brommonschenkel, S. H. 1988. Pathogenicity, compatibility, cytogenetics and isoenzyme patterns of Brazilian isolates of *Phytophthora infestans* (Mont.) de Bary. M.S. thesis. Universidade Federal de Viçosa. 82 pp.
4. Caten, C. E., and Jinks, J. L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Can. J. Bot. 46:329-348.
5. Deahl, K. L., Goth, R. W., Young, R., Sinden, S. L., and Gallegly, M. E. 1991. Occurrence of the A2 mating type of *Phytophthora infestans* in potato fields in the United States and Canada. Am. Potato J. 68:717-726.
6. Förster, H., and Coffey, M. D. 1990. Mating behavior of *Phytophthora parasitica*: Evidence for sexual recombination in oospores using DNA restriction fragment length polymorphisms as genetic markers. Exp. Mycol. 14:351-359.
7. Fry, W. E., Drenth, A., Spielman, L. J., Mantel, B. C., Davidse, L. C., and Goodwin, S. B. 1991. Population genetic structure of *Phytophthora infestans* in The Netherlands. Phytopathology 81:1330-1336.
8. Fry, W. E., and Spielman, L. J. 1991. Population biology. Pages 171-192 in: *Phytophthora infestans*, The Cause of Late Blight of Potato. Advances in Plant Pathology. Vol. 7. D. J. Ingram and P. H. Williams, eds. Academic Press, London.
9. Goodwin, S. B. 1991. DNA polymorphisms in *Phytophthora infestans*: The Cornell experience. Pages 256-271 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. Cambridge University Press, Cambridge.
10. Goodwin, S. B., Drenth, A, and Fry, W. E. 1992. Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. Curr. Genet. 22:107-115.
11. Graham, K. M., Niederhauser, J. S., and Romero, S. 1959. Observations on races of *Phytophthora infestans* in Mexico during 1956-1957. Am. Potato J. 36:196-203.
12. Hulbert, S. H., and Michelmore, R. W. 1988. DNA restriction fragment length polymorphism and somatic variation in the lettuce downy mildew fungus, *Bremia lactucae*. Mol. Plant-Microbe Interact. 1:17-24.
13. Miller, P. M. 1955. V-8 juice agar as a general purpose medium for fungi and bacteria. Phytopathology 45:461-462.
14. Mills, W. R., and Niederhauser, J. S. 1953. Observations on races of *Phytophthora infestans* in Mexico. Phytopathology 43:454-455.
15. Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70:3321-3323.
16. Nei, M., and Chesser, R. K. 1983. Estimation of fixation indices and gene diversities. Ann. Hum. Genet. 47:253-259.
17. Niederhauser, J. S. 1956. The blight, the blighter, and the blighted. Trans. NY Acad. Sci. 19:55-63.
18. Niederhauser, J. S. 1991. *Phytophthora infestans*: The Mexican connection. Pages 25-45 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. Cambridge University Press, Cambridge.
19. Niederhauser, J. S., Cervantes, J., and Servin, L. 1954. Late blight in Mexico and its implications. Phytopathology 44:406-408.
20. Sheldon, A. L. 1969. Equitability indices: Dependence on the species count. Ecology 50:466-467.
21. Spielman, L. J. 1991. Isozymes and the population genetics of *Phytophthora infestans*. Pages 231-241 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw, and L. R. Cooke, eds. Cambridge University Press, Cambridge.
22. Spielman, L. J., Drenth, A., Davidse, L. C., Sujkowski, L. J., Gu, W.-K., Tooley, P. W., and Fry, W. E. 1991. A second world-wide migration and population displacement of *Phytophthora infestans*? Plant Pathol. 40:422-430.
23. Spielman, L. J., Sweigard, J. A., Shattock, R. C., and Fry, W. E. 1990. The genetics of *Phytophthora infestans*: Segregation of allozyme markers in F_2 and backcross progeny and the inheritance of virulence against potato resistance genes R2 and R4 in F_1 progeny. Exp. Mycol. 14:57-69.
24. Swofford, D. L., and Selander, R. B. 1981. BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281-283.
25. Tooley, P. W. 1988. Use of uncontrolled freezing for liquid nitrogen storage of *Phytophthora* species. Plant Dis. 72:680-682.
26. Tooley, P. W., Fry, W. E., and Villarreal Gonzalez, M. J. 1985. Allozyme characterization of sexual and asexual *Phytophthora infestans* populations. J. Hered. 76:431-435.
27. Tooley, P. W., Sweigard, J. A., and Fry, W. E. 1986. Fitness and virulence of *Phytophthora infestans* isolates from sexual and asexual populations. Phytopathology 76:1209-1212.
28. Workman, P. L., and Niswander, J. D. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Am. J. Human Genet. 22:24-49.