Rapid Evolution of Pathogenicity Within Clonal Lineages of the Potato Late Blight Disease Fungus

Stephen B. Goodwin, Ludwik S. Sujkowski, and William E. Fry

Department of Plant Pathology, 334 Plant Science, Cornell University, Ithaca, NY 14853.
This work was supported in part by Cornell Hatch project 153437, USDA NRI-CRGP grant 93-37303-8982, and by Ciba-Geigy Corporation.
We thank J.-H. Ok, E. Habegger, D. Legard, and B. Makepeace for assisting in the completion of this project.
Accepted for publication 30 January 1995.

ABSTRACT

Goodwin, S. B., Sujkowski, L. S., and Fry, W. E. 1995. Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. Phytopathology 85:669-676.

Seventy-seven isolates of the potato and tomato late blight disease pathogen, *Phytophthora infestans*, from the United States, Canada, and northwestern Mexico, were tested for pathogenicity to nine potato and three tomato cultivars carrying different genes for resistance. Based on previous analyses of mating type, allozyme, and DNA fingerprint data, these isolates had been assigned to eight different clonal lineages. When the total pathogenic variation was partitioned into within- and among-lineage components using the Shannon information statistic, most (63%) of the variation was due to differentiation among lineages; only 37% of the total pathogenic diversity was due to variation within lineages. Older lineages had more pathogenic variation than did those that were more recently introduced into the United States and Canada. Isolates pathogenic to all of the potato differential cultivars were found within two lineages; four isolates infected all of the potato and tomato differentials tested. Variation within lineages is probably the result of rapid evolution after migration, and suggests that mutation rates at pathogenicity loci are higher than those at the molecular loci that defined each genotype. Mutation, selection, and genetic drift have probably all contributed to the pathogenic variation observed within clonal lineages of *P. infestans* in the United States and Canada.

The ability to identify clonal genotypes of plant pathogenic fungi with neutral genetic markers has provided new insights into the evolution of pathogenicity. For example, DNA fingerprint analysis revealed that asexually reproducing populations of the rice blast disease pathogen, *Magnaporthe grisea*, in the United States and Colombia were composed of a limited number of clonal lineages. Although the amount of pathotype diversity in the population as a whole could be large (18), pathotype diversity within clonal lineages was generally much less (18,19,34). Each clonal lineage was associated with a particular subset of pathotypes. Similar associations between pathotype and electrophoretic genotype due to asexual reproduction were reported for cereal rust fungi (2) and for the barley scald disease pathogen, *Rhynchosporium secalis* (7). In contrast, sexually reproducing populations of rust fungi generally exhibit a much higher diversity of pathotypes (3,25).

There may be constraints on the evolution of pathogenicity within clonally reproducing populations of plant pathogenic fungi that do not apply to sexually reproducing populations. One advantage of sexual reproduction is the recombination of new mutations into different genetic backgrounds, which may result in individuals with higher fitness. Sexual recombination can also facilitate the spread of favorable mutations throughout a population, because all individuals essentially share a common gene pool. In contrast, asexual clonal lineages do not share a common gene pool, so a mutation in one lineage does not affect other lineages. A new mutation will only spread throughout an asexual population if the clone in which it occurs displaces all other genotypes in the population. Thus, the rate of evolution in asexual populations may be slower than in sexual populations.

The potato and tomato late blight disease pathogen, *Phytophthora infestans* (Mont.) de Bary, has both sexual and asexual populations (14,29). An advantage of working with *P. infestans* is that its center of origin has been located: this oomycete fungus is endemic to a limited area in the highlands of central Mexico (23), and all other populations worldwide originated by migration during the past 150 years. There were probably extreme genetic bottlenecks associated with the original migrations of *P. infestans* out of Mexico that resulted in a single clonal lineage distributed throughout the world (10,12). This lineage has been designated US-1 (9). Populations founded during these initial migrations contained only one of the two mating types, so these populations were limited to asexual reproduction.

Recent analyses confirmed that populations of *P. infestans* in the United States and Canada were founded by a very small number of genotypes. Only 18 multilocus genotypes (based on the mating type, two allozyme, and 25 DNA fingerprint loci) were detected among 120 isolates collected from 1979 through 1991 (9), and 11 of these (61%) belonged to only two clonal lineages. The US-1 clonal lineage was detected throughout the United States and Canada and was present in the earliest samples analyzed. This is the genotype that occurs throughout the world; it may have been present in the United States since the first reports of late blight disease in 1843 (9,28). The other common clonal lineage contained the US-6 genotype (9). US-6 was probably introduced into California from northwestern Mexico in 1979 (9). Migrations of additional clonal lineages into the United States and Canada, probably from northwestern Mexico, occurred in the early 1990s (15). Analyses of over 380 isolates collected in the United States and Canada in 1992 and 1993 revealed only 11 multilocus genotypes. Nine of these genotypes had not been detected previously and were probably either recent migrants (five genotypes), sexual recombinants (three genotypes), or (for one genotype) a new mutation in a previously occurring clonal lineage (15).

The existence of a small number of clearly defined clonal genotypes of *P. infestans* in the United States and Canada provides an opportunity to study the evolution of pathogenicity

within lineages and within a defined time frame. For the purposes of this study, "pathogenicity" will refer both to specific compatibility (virulence) and to host genus (potato or tomato) pathogenicity. Although potato pathogenicity appears universal in *P. infestans*, not all isolates infect tomato (5,16,33). Genetic analyses both of the hosts (1,32) and the pathogen (26,27) have demonstrated the probable existence of classic gene-for-gene interactions in the *P. infestans*–Solanaceae pathosystem. A hypersensitive response occurs in potato cultivars carrying any of the 11 known resistance genes (designated R1 through R11) when inoculated with pathogen isolates lacking any of the corresponding virulence genes. Two tomato resistance genes (Ph1 and Ph2) are also known. However, the interaction of *P. infestans* with these genes has been less well studied. Previous surveys of virulence diversity have demonstrated that isolates from asexually reproducing populations (in North America, Wales, and Peru) were less diverse than those from a sexual population in central Mexico (30,31). However, introduced resistance genes were rapidly broken down in Europe (e.g., 21), indicating the potential for rapid evolutionary changes even in asexual populations.

The purpose of this paper was to test three hypotheses about the evolution of pathogenicity in asexually reproducing populations of *P. infestans*. The first hypothesis is that pathogenicity can evolve rapidly within clonal lineages. A prediction from this hypothesis is that there will be pathogenic diversity among isolates with the same multilocus genotype for molecular markers. A second hypothesis is that asexual reproduction will lead to strong associations between pathotype and multilocus genotype. A prediction from this hypothesis is that there will be less pathogenic diversity within than among clonal lineages. Because mutations should accumulate over time, a third hypothesis is that older clonal lineages should contain more pathogenic diversity than those that were recently introduced into the United States and Canada (assuming founder populations were small for all introductions).

MATERIALS AND METHODS

Sources of isolates. Over 600 isolates of *Phytophthora infestans* were collected throughout the United States and Canada from 1989 through 1994 (9,15, S. B. Goodwin and W. E. Fry, *unpublished*). Each isolate originated from a single lesion or infected tuber. These isolates had been analyzed previously for mating type and for genotype at the two allozyme loci *glucose-6-phosphate isomerase* (*Gpi*) and *peptidase* (*Pep*). These analyses revealed that populations of *P. infestans* in the United States and Canada were composed of a small number of presumably clonal genotypes (9,15). Some genotypes were widely distributed and occurred on both potato and tomato, whereas others were only found in a single location on a single host. Among these, 73 isolates representing the most commonly occurring genotypes were chosen for pathogenicity analysis. Because the goal of this study was to analyze pathogenic variation within and among genotypes, not to estimate virulence frequencies, we included representative isolates of each genotype from as many different geographical areas as possible. For less widely distributed genotypes, representative isolates were chosen from different fields or sampling sites. No more than three isolates from the same field were included. Four additional isolates from northwestern Mexico (near Los Mochis, Sinaloa) were also included. Northwestern Mexico contains the probable source population for recent migrations of *P. infestans* into the United States and Canada (9,15). The four isolates were A2 mating type, and had dilocus allozyme genotypes that were recently detected in the United States and in British Columbia. They were collected by Ruben Felix (Campbell's Sinalopasta, Guasave, Sinaloa, México) in 1992.

Nuclear DNA "fingerprint" analysis with probe RG57 (11) for most isolates was not completed until after the pathogenicity analyses. Probe RG57 reveals more than 25 different bands in *P. infestans*, most of which represent alleles at independent genetic loci (11,13). The DNA fingerprint data confirmed the clonal nature of most genotypes identified previously by mating type and allozyme analyses (15), and revealed one additional genotype. In total, there were eight multilocus genotypes among the 77 isolates tested (Table 1). Isolates that were identical for all markers were considered to be the same clonal genotype. The probability that these genotypes could have arisen by random recombination in sexually reproducing Mexican populations has been estimated at 2 or 3 $\times 10^{-5}$ for US-1 and US-7, respectively, and 2 $\times 10^{-6}$ for US-8 (10,15). The probability that they could have arisen independently in the United States is essentially zero because only one mating type was present until very recently, precluding sexual recombination. It is thus highly likely that isolates sharing the same multilocus genotype are members of a single clonal lineage. Complete descriptions of the genotypes found previously in the United States and Canada have been published (9,15).

Pathogenicity testing. Pathogenicity testing was with the detached leaflet method (13, 30). Plants of the potato cultivar Norchip (no known resistance genes), Rosa (resistance gene R1), PI 203905 (R2), PI 203902 or PI 423653 (R3), PI 203900 (R4), PI 303146 (R5), PI 303148 (R7), PI 303149 (R8), PI Hodgson 2573 (R9), and PI 423656 (R10) were grown from virus-free tissue-culture stock maintained in the laboratory of S. A. Slack (Department of Plant Pathology, Cornell University), and were multiplied further by cuttings. Occasionally, plants were also grown from tubers saved from the previous year. The tomato cultivars Vendor

TABLE 1. Multilocus genotypes detected among 77 isolates of *Phytophthora infestans* collected in the United States, Canada, and Mexico from 1989 through 1994 that were tested for pathogenicity to nine potato and three tomato differential cultivars

Genotype[a]	Mating type	Allozyme genotype		DNA fingerprint pattern[d]	Number of isolates
		Gpi[b]	*Pep*[c]		
US-1	A1	*86/100*	*92/100*	10111010110011010001100 11	25
US-6	A1	*100/100*	*92/100*	10111110010011000101100 11	19
US-7	A2	*100/111*	*100/100*	10011000010011010101100 11	10
US-8	A2	*100/111/122*[e]	*100/100*	10011000010011010001101 11	16
US-12	A1	*100/111*	*92/100*	. . .	1
BC-2	A2	*100/100*	*100/100*	10001100000011010001100 11	1
BC-3	A2	*100/100*	*100/100*	10100010010011000101100 11	1
US-7 (Mexico)	A2	*100/111*	*100/100*	10011000010011010101100 11	2
ND[f] (Mexico)	A2	*100/100*	*100/100*	. . .	2

[a] Genotype designations are as indicated in Goodwin et al. (9,15).
[b] *Glucose-6-phosphate isomerase.*
[c] *Peptidase.*
[d] Presence (1) or absence (0) of probe RG57 DNA "fingerprint" bands 1–25 are indicated from left to right.
[e] The US-8 genotype is probably trisomic for the chromosome containing the *Gpi* locus (14).
[f] Not determined.

(no known resistance genes), New Yorker or Nova II (resistance gene Ph1), and Caline (Ph2) were grown from seed. All plants were grown in a greenhouse and sprayed as needed to control pests and diseases.

Inoculum was prepared by washing sporangia with sterile distilled water from 10- to 20-day-old cultures grown on rye A or rye B agar (4). Inoculum concentrations were standardized to approximately 40,000 sporangia per milliliter (actual range 12,000 to 133,000) with a hemacytometer. Following harvest, sporangial suspensions were placed at 4 C for 30 to 60 min to induce zoospore formation. Drops (approximately 50 µl each) of inoculum were placed on four to six leaflets of each cultivar in inverted 14-cm petri dishes. Each petri dish had approximately 35 ml of 1.5% water agar in the bottom to provide humidity (13,30).

Disease symptoms were scored after 5 to 7 (usually 6) days of incubation in an 18 C incubator. Lesions were scored for size: –, no lesion; HS, hypersensitive response; SL, small lesion less than 1 cm in diameter; L, lesion between 1 and 2 cm in diameter; or BL, big lesion greater than 2 cm in diameter. Sporulation of lesions was rated from 0 to 4, where 0 = no sporulation, 1 = a few sporangia visible with magnification, 2 = sparse sporulation visible with magnification, 3 = profuse sporulation easily visible without magnification, and 4 = extremely profuse sporulation throughout the lesion. Only those tests in which there were large, profusely sporulating lesions on the susceptible cultivar Norchip were used. A final rating of plus (compatible) or minus (incompatible) was given to each cultivar/isolate interaction by evaluating the response of all leaflets of each cultivar inoculated with a given isolate. Inoculations that produced large, profusely sporulating lesions (i.e., BL3 or BL4 scores) on all inoculated leaflets were rated compatible. If there was no infection on all inoculated leaflets (i.e., HS or – scores) the interaction was rated incompatible. Occasionally, some inoculated leaflets of a cultivar were infected but others were not. These inoculations were repeated until consistent results were obtained. To minimize the effect of environmental variation, all inoculations were performed in a limited time, from 31 January through 6 February 1992, from 12 February through 16 March 1993, and from 1 to 17 January 1995. Additional details of the inoculation procedures can be found elsewhere (13,30).

Octal nomenclature. An octal nomenclature was used as an efficient, convenient method of summarizing the data. For this nomenclature, differentials were assigned to groups of three, and each group of three differentials was one octal digit. The 11 known potato resistance genes (R1 through R11) and the three tomato cultivars (Vendor, New Yorker or Nova, Caline) formed five octal digits. The first digit on the left has information about the potato differentials R1, R2, R3; the second digit, R4, R5, R6; the third digit, R7, R8, R9; the fourth digit, R10 and R11; followed by a comma and the fifth digit, tomato cultivars Vendor, New Yorker or Nova II (Ph1), and Caline (Ph2). The fourth digit only has data for two resistance genes, R10 and R11, so is incomplete. If an additional resistance gene is discovered in the future, it could be included in the fourth digit. We did not have access to differentials containing the potato resistance genes R6 and R11, so the digits in which they occurred were underscored to indicate missing data, as suggested elsewhere (8). All isolates that were scored infected the susceptible control, so the potato cultivar Norchip was not included in the nomenclature. However, not all isolates infected tomato, so the susceptible tomato cultivar Vendor was included.

To use this nomenclature, the scores for all differentials were listed from left to right; a 0 was used to denote an incompatible interaction and a 1 to denote a compatible interaction. This list of zeros and ones was then transformed into an octal number by taking each group of three and converting it to its octal equivalent: 000 = 0; 001 = 1; 010 = 2; 011 = 3; 100 = 4; 101 = 5; 110 = 6; and 111 = 7. Thus, values for octal digits range from 0 to 7. Missing data were counted as if they were zeros and the digits in which they occurred were underlined. For example, an isolate that is pathogenic to the potato differentials R3, R5, R7, R8, R10 and the tomato cultivar Vendor, nonpathogenic to potato differentials R1, R2, R4, R9 and cultivars carrying the tomato resistance genes Ph1 and Ph2, and not tested on differentials R6 and R11, would be listed as 00101011010,100 in zeros and ones, and would be 1$\underline{2}$6$\underline{4}$,4 in octal. The comma in the octal nomenclature separates the four potato digits from the tomato digit, and the second and fourth digits are underlined to indicate that some information is missing (in this case for differentials R6, R11, and the nonexistent differential R12). For the differential set used in this study, digits two and four were always underlined to indicate the missing differentials R6 and R11. Thorough discussions about octal nomenclatures in general, and about the advantages of octal systems over other forms of nomenclature in particular, can be found elsewhere (6,8).

Statistical analyses. Pathogenic diversity within and among clonal genotypes was quantified with the Shannon information statistic. This statistic was chosen because it can be partitioned into within- and among-genotype components (7,20,35), analogous to Nei's gene diversity analysis (22), but it is more suitable for phenotypic data. Within each clonal lineage, pathogenic diversity was calculated for each differential cultivar as $h_0 = -\Sigma p_i ln p_i$ where the p_i are the frequencies of compatibility and incompatibility. Diversity within each lineage was the mean of the h_0 over all differential cultivars. These values were divided by the maximum possible diversity ($ln2 = 0.693$), to give a normalized pathogenic diversity. The total pathogenic diversity (H_{TOTAL}) was partitioned into within- and among-genotype components (7,20, 35) with a previously used C program (7). The within-genotype component was the proportion of the total pathogenic diversity that was due to variation within genotypes ($\overline{H}_{GENOTYPE} / H_{TOTAL}$); the among-genotype component was the proportion of the total pathogenic diversity that was due to differentiation among genotypes $[(H_{TOTAL} - \overline{H}_{GENOTYPE}) / H_{TOTAL}]$.

RESULTS

Pathogenicity testing. Most of the pathogenicity responses were clear and easily scored; all inoculated leaflets usually had the same reaction as long as inoculum concentrations were greater than 20,000 sporangia per milliliter. Some interactions (particularly the US-1 genotype on potato differentials R7 and R10) gave lesions that were smaller and less profusely sporulating than those on the susceptible cultivar Norchip. However, because these reactions were repeatable, they appeared to be characteristic of particular isolate/cultivar combinations, and were thus scored as compatible. Compatible interactions on tomato also produced large, profusely sporulating lesions that often encompassed the entire inoculated leaflet. Incompatible interactions on tomato sometimes caused a hypersensitive response, but more often were characterized by small, sparsely sporulating (sporulation ratings of 0, 1, or 2) lesions a little larger than the inoculation droplet. These reactions also were repeatable.

Pathogenicity by clonal genotype. In total, the 77 isolates tested represented eight clonal genotypes (Table 1), and had 27 different pathotypes (Table 2). Most of the within-genotype variation occurred in the US-1 and US-6 clonal lineages, with seven and nine different pathotypes, respectively (Table 2). Three pathotypes, 4$\underline{2}$4$\underline{4}$,7 (in US-12 and BC-3), 5$\underline{2}$40,7 (in US-1, US-7 and BC-2), and 5$\underline{2}$4$\underline{4}$,7 (in US-1 and US-7) occurred in more than one genotype; all other pathotypes were restricted to a single clonal lineage.

The recently immigrated US-7 and US-8 genotypes (first detected in the United States in 1992) had fewer pathotypes than were present in the US-1 and US-6 genotypes. Isolates from the same field always had the same genotype as determined by mo-

lecular markers, and in four fields also had the same pathotype. In five other fields the isolates were identical except for reaction to a single differential cultivar, usually R10.

Most isolates in the US-1 and US-8 clonal lineages did not infect the three tomato cultivars tested (Table 2). However, two US-1 isolates that were isolated from tomato did infect the three tomato cultivars. These isolates also infected a number of additional potato differentials, including R3, a cultivar that was not infected by any other isolate with the US–1 genotype. In contrast, all other genotypes infected at least one of the tomato cultivars in addition to potatoes. Isolates that infected tomato usually infected all three tomato cultivars (Table 2).

TABLE 2. Pathogenicity phenotypes of 77 isolates of *Phytophthora infestans* collected from 1989 through 1994 by clonal genotype[a]

Genotype	Pathogenicity phenotype		Original host	Location	Number of isolates
	Race nomenclature	Octal nomenclature[b]			
US-1	0	0000,0	Potato	Oregon	1
	10	0004,0	Potato	Alberta	1
			Potato	Florida	1
			Potato	Michigan	3
			Potato	North Dakota	2
			Potato	Oregon	3
	7,10	0044,0	Potato	Alberta	1
			Potato	Florida	3
			Potato	Maine	2
			Potato	North Dakota	2
			Potato	Oregon	1
			Potato	Prince Edward Island	1
	1,5,7,10	4244,0	Potato	Oregon	1
	1,5,7,10,ph,Ph2	4244,5	Potato	Prince Edward Island	1
	1,3,5,7,ph,Ph1,Ph2	5240,7	Tomato	New York	1
	1,3,5,7,10,ph,Ph1,Ph2	5244,7	Tomato	New York	1
US-6	2,4,5,ph,Ph1,Ph2	2600,7	Potato	Washington	1
	1,4,5,7,ph,Ph1,Ph2	4640,7	Potato	British Columbia	1
			Potato	Washington	1
	1,3,5,7,8,10,ph,Ph1,Ph2	5264,7	Potato	Florida	2
	1,2,4,5,7,8,10,ph,Ph2	6664,5	Potato	Washington	1
	1,2,3,4,5,7,8,ph,Ph1,Ph2	7660,7	Potato	British Columbia	1
			Potato	Washington[c]	1
	1,2,3,4,5,7,10,ph,Ph1[d]	7644,6[d]	Tomato	California[e]	1
	1,2,3,4,5,7,8,10,ph,Ph1[d]	7664,6[d]	Tomato	California[e]	1
	1,2,3,4,5,7,8,10,ph,Ph1,Ph2	7664,7	Tomato	Florida[f]	1
			Potato	Washington[c]	3
			Tomato	Washington[c]	1
	1,2,3,4,5,7,8,9,10,ph,Ph1,Ph2	7674,7	Tomato	Florida[f]	4
US-7	1,7,ph,Ph1,Ph2	4040,7	Tomato	South Carolina	1
	1,5,7,ph,Ph1,Ph2	4240,7	Potato	New York	2
			Tomato	New York	2
			Tomato	North Carolina	1
			Tomato	Tennessee	1
			Tomato	Virginia	1
	1,3,5,7,ph,Ph1,Ph2	5240,7	Tomato	Tennessee	1
	1,3,5,7,10,ph,Ph1,Ph2	5244,7	Tomato	New York	1
US-7 (Mexico)	0,ph,Ph1,Ph2	0000,7	Tomato	Guasave, SIN	1
	1,7,ph,Ph1,Ph2	4040,7	Tomato	Ahome, SIN	1
US-8	1,2,3,4,7,10	7444,0	Potato	North Dakota	1
	1,2,3,4,5,7,10	7644,0	Potato	Maine	1
			Potato	Michigan	1
			Potato	New Brunswick	1
			Nightshade[g]	New York	1
			Potato	New York	1
			Tomato	New York	2
			Potato	North Carolina	1
			Potato	Oregon	1
			Potato	Pennsylvania	2
	1,2,3,4,5,7,9,10	7654,0	Potato	North Carolina	1
	1,2,3,4,5,7,8,9,10	7674,0	Potato	New York	2
	1,2,3,4,5,7,8,9,10,Ph1	7674,2	Potato	New York	1
US-12	1,5,7,10,ph,Ph1,Ph2	4244,7	Tomato	New York	1
BC-2	1,3,5,7,ph,Ph1,Ph2	5240,7	Potato	British Columbia	1
BC-3	1,5,7,10,ph,Ph1,Ph2	4244,7	Potato	British Columbia	1
ND[h] (Mexico)	2,4,7,ph	2440,4	Potato	Los Mochis, SIN	1
	1,2,3,4,10,ph,Ph2	7404,5	Tomato	Ahome, SIN	1

[a] Most isolates were collected during the 1992/1994 growing seasons with a few indicated exceptions.
[b] These isolates were not tested on potato differentials R6 and R11; the second and fourth octal digits were underlined to indicate missing data.
[c] Collected in 1990.
[d] Not tested on potato resistance genes R6, R9, R11 and tomato gene Ph2; the corresponding octal digits were underlined to reflect this.
[e] Collected in 1989.
[f] Collected in 1991.
[g] Hairy nightshade, *Solanum sarachoides* Sendtner.
[h] Not determined.

An additional 35 isolates were analyzed by combining previously published virulence (30,31) and molecular (9,10) data (Tables 3 and 4). Both types of data were available for nine United States isolates collected from 1979 through 1984 (30), one from Wales (year of collection unknown) (30), and 25 Peruvian isolates collected in 1984 and 1985 (31). Most of these isolates (28/35 = 80%) had the US-1 genotype, six isolates were identical to US-1 except for single changes and were considered to belong to the US-1 clonal lineage (US-1.1 through US-1.6), and the remaining isolate had the US-5 genotype (Table 3). Summaries of the mating type, allozyme, and DNA fingerprint data for these isolates were reported previously (9,10,30,31).

Virulence analyses of these 35 isolates (30,31) revealed only seven different pathotypes, six of which occurred within the US-1 clonal lineage (Table 4). The United States isolate with the most complex pathotype in the previous study (30) infected six of the nine differential potato cultivars and had the US-5 genotype (Table 4).

Pathogenicity within and among genotypes. Most US-1 isolates had very simple pathotypes, infecting 1.5 of the nine potato differential cultivars on average (Table 5); only five of the 53 US-1 isolates tested in these studies infected more than three potato differentials. The variation was mostly on the R1, R5, R7, and R10 differentials. Most isolates with this genotype did not infect the tomato cultivars tested; however, the two US-1 isolates that were isolated from tomato did infect the tomato cultivars. They were also different from most other US-1 isolates in having more complex pathotypes on potato (infecting 4.5 potato cultivars on average) (Table 2). Molecular variants within the US-1 clonal lineage (genotypes US-1.1 through US-1.6) also had very simple pathotypes.

In contrast, isolates with the US-5, US-6, and US-8 genotypes had very complex pathotypes, infecting six or more of the nine potato differential cultivars (Table 5). Three of the 16 US-8 isolates and four of the 19 US-6 isolates could overcome all nine potato resistance genes tested; the US-6 isolates also infected all of the tomato differentials. US-7 isolates and the single isolates representing the US-12, BC-2, and BC-3 genotypes had moderately complex pathotypes on potato, infecting three or four potato differential cultivars on average, in addition to all three tomato cultivars.

The "older" clonal lineages, US-1 and US-6, had much more pathogenic diversity than the more recently immigrated genotypes US-7 and US-8 (Table 6). The number of pathotypes within a lineage ranged from four in US-7 (in the United States) to nine in US-6 (Table 2). For contrast, pathogenic diversity was calculated among isolates from genetically heterogeneous, sexually reproducing populations (24,30) in central Mexico (Table 6). There was less pathogenic diversity in each clonal lineage than in the central Mexican populations. One of the Mexican populations had 87% of the maximum possible diversity, compared with less than 50% for the most variable clonal lineage (Table 6). The total pathogenic variation was partitioned into components using the Shannon information statistic for the 104 isolates in the US-1, US-6, US-7, and US-8 clonal lineages. There was less diversity within than among lineages: 37% of the total pathogenic diversity

TABLE 3. Multilocus genotypes of *Phytophthora infestans* identified among 35 isolates (from the United States, Wales, and Peru) that were previously characterized for pathogenicity to eight potato differential cultivars (30,31)

Genotype[a]	Mating type	Allozyme genotype		DNA fingerprint pattern[d]	Number of isolates
		Gpi[b]	*Pep*[c]		
US-1	A1	*86/100*	*92/100*	10111010110011010000110011	28
US-1.1	A1	*86/100*	*100/100*	10111010110011010000110011	1
US-1.2	A1	*86/100*	*92/100*	10111010100011010000110011	2
US-1.3	A1	*86/100*	*92/100*	10111010010011010000110011	2
US-1.6[e]	A1	*86/100*	*92/100*	10111010110011010000111011	1
US-5	A1	*100/100*	*92/100*	10111010010011010111110011	1

[a] Genotype designations are as indicated in Goodwin et al. (9,15).
[b] *Glucose-6-phosphate isomerase.*
[c] *Peptidase.*
[d] Presence (1) or absence (0) of probe RG57 DNA "fingerprint" bands 1–25 are indicated from left to right.
[e] This genotype was found only in Peru. It is identical to US-1 except for one additional DNA fingerprint band so is a member of the US-1 clonal lineage.

TABLE 4. Pathogenicity phenotypes of 35 previously characterized isolates of *Phytophthora infestans* from the United States, Wales, and Peru according to clonal genotype

Genotype	Pathogenicity phenotype[a]		Location	Year isolated	Number of isolates
	Race nomenclature	Octal nomenclature[b]			
US-1	0	0000	Wales	. . .[c]	1
			New York	1979	2
			Maine	1980	1
			New York	1982	1
			Peru	1984/1985	8
	1	4000	Peru	1984/1985	6
	1,5	4200	Peru	1985	4
	1,7	4040	Peru	1985	1
	1,5,10	4204	Peru	1984/1985	3
	1,2,3,4,10	7404	New York	1984	1
US-1.1	0	0000	New York	1979	1
US-1.2	1	4000	Peru	1985	1
	1,5,10	4204	Wisconsin	1982	1
US-1.3	1	4000	Peru	1985	1
	1,5,10	4204	Wisconsin	1982	1
US-1.6	0	0000	Peru	1984	1
US-5	1,2,3,4,5,8	7620	. . .	1979	1

[a] These isolates were not tested on potato differentials R6, R9, and R11, nor on any of the tomato cultivars (30,31).
[b] The last three digits were underlined to indicate the missing differentials R6, R9, and R11; the tomato digit was eliminated.
[c] Not known.

was due to variation within lineages, whereas 63% was due to differentiation among lineages.

DISCUSSION

Pathogenicity, both to particular resistance genes within a species and to host genus, evidently can evolve rapidly within clonal lineages of *Phytophthora infestans*. Both types of change occurred within the US-1 clonal lineage. Although most US-1 isolates had very simple pathotypes and did not infect tomato, pathogenicity to all of the potato and tomato differentials except potato differentials R8 and R9 did occur in at least some US-1 isolates in the United States and Canada. Selection for increased pathogenicity within clonal lineages is sufficient to explain the rapid breakdown of introduced resistance genes (e.g., 21).

In spite of the variability, pathotypes among isolates in the same clonal lineage were more similar to each other than to those in other clonal lineages, and most of the overall pathogenic variation was due to differentiation among (63%) rather than within (37%) genotypes. This is most likely the result of asexual reproduction and the rapid spread of genotypes in infected host tissue. For example, US-1 isolates that were race 7,10 were found in four states (Florida, Maine, North Dakota, and Oregon) and two provinces (Prince Edward Island and Alberta). There is good evidence that US-1 isolates from two of these states (North Dakota and Maine) in 1992 were introduced to Florida in infected seed tubers in 1993 (15). The Florida isolates were probably recent, direct clonal descendants of the isolates in Maine and North Dakota and it is not surprising that they also had the same pathotype. Because *P. infestans* can be spread so widely in infected seed tubers, there is no relationship between geographical distance and genetic distance among isolates.

Knowing when each clonal lineage was first introduced into the United States delimits the probable time frame in which the observed pathogenicity changes occurred. There was more variation in older clonal lineages than in those that were recent introductions. Assuming founder populations were small for all introductions, there was probably little pathogenic variation in the initial populations of each clonal lineage. In support of this assumption, there was essentially no pathogenic diversity in the small samples (3 to 5 isolates each) of the US-7 and US-8 clonal lineages from 1992, when they were first detected in the United States (15). The seven pathotypes in the US-1 lineage, including three that infected tomato, probably evolved in the 150 years or less that US-1 has been in the United States. Evolution of nine pathotypes in the US-6 lineage appeared to be even more rapid; US-6 was probably first introduced into the United States in 1979 (9), only 14 years before the most recent samples were obtained. Interestingly, the earliest US-6 isolates assayed (collected from 1989 through 1991) had more complex pathotypes, including the pathotype that infected all of the potato and tomato resistance genes tested, than those that were collected later (in 1992 and 1993). Similarly, three US-8 isolates collected in 1992 infected all nine potato differential cultivars, whereas isolates with the same genotype collected 2 years later were nonpathogenic to resistance genes R5, R8, and/or R9. Although the sample sizes are too small for firm conclusions, this may indicate that pathogenicity to many resistance genes is being lost rapidly within the US-6 and US-8 clonal lineages. Because pathogenicity evolves so rapidly and independently of molecular markers within clonal lineages, it seems unlikely that analyses with molecular markers alone can ever be a substitute for pathogenicity analyses.

Mutation is the source of all new genetic variation within lineages. The occurrence of many different pathotypes within lineages may indicate that mutation rates at pathogenicity loci are higher than they are at those for the molecular markers that define each genotype. It is also possible that the observed rate of change of a phenotype within a lineage may depend on the dominance relationships at each locus. For example, if avirulence is dominant, a single mutation would be sufficient for a change in phenotype from virulence to avirulence. However, two mutations (or one mutation followed by mitotic crossing over) would be necessary to change the phenotype from avirulence (assuming homozygosity) to virulence. Thus, the observed rate of evolution within asexual lineages may be higher for changes from virulence to avirulence than from avirulence to virulence, even if the mutation rates are identical. This may explain the rapid loss of virulence that appears to be occurring in the US-6 lineage.

Current dogma assumes that avirulence occurs when the host recognizes a particular pathogen gene product, and that virulence results from the loss of the recognition factor by the pathogen. Many different mutations could result in the loss of the recognition factor, but only one specific change can reverse the mutation. Therefore, the apparent mutation rate from avirulence to virulence should be higher than that from virulence to avirulence. However,

TABLE 5. Mean number of differential cultivars infected by each clonal genotype of *Phytophthora infestans* (data from Tables 2 and 4)

Genotype	Mean number of potato differentials infected	Mean number of tomato differentials infected	Sample size
US-1	1.5	0.3	53[a]
US-1.1	0	...[b]	1
US-1.2	2.0	...	2
US-1.3	2.0	...	2
US-1.6	0	...	1
US-5	6	...	1
US-6	7.1	2.9	19[c]
US-7	3.2	3.0	10
US-7 (Mexico)	1.0	3.0	2
US-8	7.4	0.1	16
US-12	4	3	1
BC-2	4	3	1
BC-3	4	3	1
ND[d] (Mexico)	4.0	1.5	2

[a] Sample size was 25 for potato differential R9 and for all three tomato cultivars.
[b] Not tested.
[c] Sample size was 17 for potato differential R9 and for tomato differential Ph2.
[d] Not determined.

TABLE 6. Mean pathogenic diversity in old (US-1, US-6) and more recently immigrated (US-7, US-8) clonal lineages of *Phytophthora infestans* in the United States and Canada, and in genetically heterogeneous sexually reproducing populations in central Mexico, as measured by the Shannon information statistic

Genotype[a]	Mean pathogenic diversity	Normalized pathogenic diversity[b]	Number of isolates
US-1	0.32	0.46	59[c]
US-6	0.30	0.43	19
US-7	0.10	0.14	10
US-8	0.13	0.18	16
Central Mexico			
1983–1984[d]	0.44	0.63	16
1985–1986[e]	0.60	0.87	78

[a] The US-1 and US-6 lineages have been in the U.S. and Canada for more than 12 years (9). The US-7 and US-8 genotypes are recent migrants, first detected in 1992 (15).
[b] The mean values were divided by the maximum possible diversity, $ln2$, to obtain the normalized diversity values.
[c] Includes 25 US-1 isolates collected during 1992–1994, 28 US-1 isolates collected from 1979 to 1985 (30,31), and six isolates in the US-1 clonal lineage (US-1.1–US-1.6) collected from 1979 to 1985 (30,31).
[d] Data from Tooley et al. (30) for potato differentials R1–R5, R7, R8, and R10.
[e] Data from Rivera-Peña (24) for potato differentials R1–R10 (excluding R6). Data for R11 were available but were not analyzed.

if mutations are due to the movement of transposable elements, they would be easily reversible and could occur at high frequency. This could explain all of the observed phenomena, and is a hypothesis that could be tested in future investigations.

Two other potential sources of new pathotypes are mitotic recombination and somatic fusion. Mitotic recombination could reveal recessive phenotypes that were hidden by dominant alleles. Such an event should also generate homozygosity for markers at all loci distal to the recombination site on the same chromosome arm. This could be detected by analyses with additional markers. However, the markers used in this study were not sufficient to address the question of whether or not mitotic recombination contributed to the observed variation. Somatic fusion has generated new pathotypes in the lettuce downy mildew pathogen (17), but there is no evidence that it occurs in *P. infestans*. Somatic fusion among lineages would generate new genotypes for molecular markers and can be eliminated as a potential source of variation within *P. infestans* clonal lineages. However, somatic fusion within lineages could not be detected with the molecular markers used in this study and thus remains a possibility.

The high level of pathogenic variability within established clonal lineages provides the raw material for rapid evolutionary changes due to selection. Most United States potato cultivars do not contain *P. infestans* resistance genes, so selection probably did not play much of a role in generating the observed pattern of pathogenic variation in this study. However, it may be important in other locations. For example, Tooley et al. (31) believed that selection caused a high frequency of virulence to R1 in Peru. The present study revealed that those changes occurred within the US-1 clonal lineage. Selection may also explain the apparent high frequency of tomato pathogenicity in *P. infestans* clones from northwestern Mexico, where both potatoes and tomatoes are cultivated extensively. Sufficient variation already occurs within clonal lineages to allow a rapid response to introduced resistance genes.

There are at least two potential causes of genetic drift within *P. infestans* clonal lineages, both of which have probably contributed to the observed pattern of pathogenic variation in the United States and Canada. Genetic drift in colonizing populations (founder effects) probably explains the low level of pathogenic variation in the recently immigrated US-7 and US-8 genotypes compared with US-1 and US-6. The other cause of genetic drift within lineages is the limited overwintering of *P. infestans* in infected host tissue. Population sizes during the winter are many orders of magnitude smaller than they are during summer epidemics. The most likely effect of overwintering genetic drift would be the elimination of rare variants. This may explain the greater similarity of pathotypes within than among lineages.

In contrast to *Magnaporthe grisea*, where pathogenic variation within lineages was limited (18,19), *P. infestans* clonal lineages seem capable of evolving pathogenicity to most or all of the resistance genes tested. Some US-8 isolates that were obtained from naturally infected tomato tissue were rated as nonpathogens of tomato in the greenhouse studies. Although these isolates would probably not cause severe epidemics on tomato in the field, they did cause limited disease on some cultivars. Such infections might increase inoculum in tomato fields where rare mutants with full pathogenicity to tomato would have a selective advantage. This could facilitate the evolution of pathogenicity within *P. infestans*.

Much of the pathogenic variation in current populations of *P. infestans* in the United States and Canada is the result of migration of additional clonal lineages from northwestern Mexico. Some of these isolates defeated all of the potato and tomato resistances tested, so currently available sources of major-gene resistance are not likely to provide effective control against the populations of *P. infestans* that presently exist in the United States and Canada. Another consequence of these migrations is that genotypes capable of infecting tomato are now widely distributed. Most US-1 isolates did not infect tomato, so prior to the migration of US-6 into the United States, late blight on potato was unlikely to spread to tomato. With the widespread distribution of the US-6 and US-7 genotypes, however, late blight epidemics on potato could serve as a source of inoculum for tomato fields. Ability to infect tomato also may have assisted the migration of these genotypes by increasing the area of host tissue available for colonization, including home gardens that are not usually sprayed with fungicides. Movement in infected tomato fruits or transplants may also increase the rate of migration. The result of these migrations may be an increased incidence of late blight disease on potatoes and tomatoes in the United States and Canada.

LITERATURE CITED

1. Black, W., Mastenbroek, C. Mills, W. R., and Peterson, L. C. 1953. A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivatives. Euphytica 2:173-179.
2. Burdon, J. J., and Roelfs, A. P. 1985. Isozyme and virulence variation in asexually reproducing populations of *Puccinia graminis* and *P. recondita* on wheat. Phytopathology 75:907-913.
3. Burdon, J. J., and Roelfs, A. P. 1985. The effect of sexual and asexual reproduction on the isozyme structure of populations of *Puccinia graminis*. Phytopathology 75:1068-1073.
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. de Bruyn, H. L. G. 1952. Pathogenic differentiation in *Phytophthora infestans* (Mont.) DeBary. Phytopathol. Z. 18:339-359.
6. Gilmour, J. 1973. Octal notation for designating physiologic races of plant pathogens. Nature 242:620.
7. Goodwin, S. B., Allard, R. W., Hardy, S. A., and Webster, R. K. 1992. Hierarchical structure of pathogenic variation among *Rhynchosporium secalis* populations in Idaho and Oregon. Can. J. Bot. 70:810-817.
8. Goodwin, S. B., Allard, R. W., and Webster, R. K. 1990. A nomenclature for *Rhynchosporium secalis* pathotypes. Phytopathology 80:1330-1336.
9. Goodwin, S. B., Cohen, B. A., Deahl, K. L., and Fry, W. E. 1994. Migration from northern Mexico as the probable cause of recent genetic changes in populations of *Phytophthora infestans* in the United States and Canada. Phytopathology 84:553-558.
10. Goodwin, S. B., Cohen, B. A., and Fry, W. E. 1994. Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. Proc. Natl. Acad. Sci. United States 91:11591-11595.
11. 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.
12. Goodwin, S. B., and Fry, W. E. 1991. Global migration of *Phytophthora infestans*. (Abstr.) Phytopathology 81:1191.
13. Goodwin, S. B., and Fry, W. E. 1994. Genetic analyses of interspecific hybrids between *Phytophthora infestans* and *Phytophthora mirabilis*. Exp. Mycol. 18:20-32.
14. Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N., and Fry, W. E. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82:955-961.
15. Goodwin, S. B., Sujkowski, L. S., Dyer, A. T., Fry, B. A., and Fry, W. E. 1995. Direct detection of gene flow and probable sexual reproduction of *Phytophthora infestans* in northern North America. Phytopathology 85:473-479.
16. Graham, K. M., Dionne, L. A., and Hodgson, W. A. 1961. Mutability of *Phytophthora infestans* on blight-resistant selections of potato and tomato. Phytopathology 51:264-265.
17. 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.
18. Levy, M., Correa-Victoria, F. J., Zeigler, R. S., Xu, S., and Hamer, J. E. 1993. Genetic diversity of the rice blast fungus in a disease nursery in Colombia. Phytopathology 83:1427-1433.
19. Levy, M., Romao, J., Marchetti, M. A., and Hamer, J. E. 1991. DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. Plant Cell 3:95-102.
20. Lewontin, R. C. 1972. The apportionment of human diversity. Evol. Biol. 6:381-398.
21. Malcolmson, J. F. 1969. Races of *Phytophthora infestans* occurring in Great Britain. Trans. Br. Mycol. Soc. 53:417-423.

22. Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. United States 70:3321-3323.
23. Neiderhauser, 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.
24. Rivera-Peña, A. 1990. Wild tuber-bearing species of Solanum and incidence of *Phytophthora infestans* (Mont.) de Bary on the western slopes of the volcano de Toluca. 3. Physiological races of *Phytophthora infestans*. Potato Research 33:349-355.
25. Roelfs, A. P., and Groth, J. V. 1980. A comparison of virulence phenotypes in wheat stem rust populations reproducing sexually and asexually. Phytopathology 70:855-862.
26. Spielman, L. J., McMaster, B. J., and Fry, W. E. 1989. Dominance and recessiveness at loci for virulence against potato and tomato in *Phytophthora infestans*. Theor. Appl. Genet. 77:832-838.
27. Spielman, L. J., Sweigard, J. A., Shattock, R. C., and Fry, W. E. 1990. The genetics of *Phytophthora infestans:* Segregation of allozyme markers in F2 and backcross progeny and the inheritance of virulence against potato resistance genes *R2* and *R4* in F1 progeny. Exp. Mycol. 14:57-69.
28. Stevens, N. E. 1933. The dark ages in plant pathology in America: 1830-1870. J. Wash. Acad. Sci. 23:435-446.
29. Tooley, P. W., Fry, W. E., and Villarreal Gonzalez, M. J. 1985. Isozyme characterization of sexual and asexual *Phytophthora infestans* populations. J. Hered. 76:431-435.
30. 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.
31. Tooley, P. W., Therrien, C. D., and Ritch, D. L. 1989. Mating type, race composition, nuclear DNA content, and isozyme analysis of Peruvian isolates of *Phytophthora infestans*. Phytopathology 79:478-481.
32. Toxopeus, H. J. 1956. Reflections on the origin of new physiologic races of *Phytophthora infestans* and the breeding of resistance in potatoes. Euphytica 5:221-237.
33. Wilson, J. B., and Gallegly, M. E. 1955. The interrelationship of potato and tomato races of *Phytophthora infestans*. Phytopathology 45:473-476.
34. Xia, J. Q., Correll, J. C., Lee, F. N., Marchetti, M. A., and 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.
35. Zhang, Q., Webster, R. K., and Allard, R. W. 1987. Geographical distribution and associations between resistance to four races of *Rhynchosporium secalis*. Phytopathology 77:352-357.