Direct Detection of Gene Flow and Probable Sexual Reproduction of *Phytophthora infestans* in Northern North America

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ABSTRACT

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Gene flow into populations of *Phytophthora infestans* in the United States and Canada was detected directly in 1992 and 1993. In total, 384 isolates were analyzed for mating type and dilocus allozyme genotype. Of these, 130 isolates were analyzed for nuclear DNA fingerprint variation using probe RG57. Only 11 multilocus genotypes were identified, nine of which had never been detected previously in the United States or Canada. The A2 mating type and isolates with the *Glucose-6-phosphate isomerase 111* allele increased dramatically in frequency compared with samples in previous years. The only likely explanation for these changes is that there was massive immigration from outside the United States or Canada. Northwestern Mexico was the probable source population for these recent migrations; the two most common migrant genotypes were probably direct clonal descendants of isolates collected previously in northwestern Mexico. Populations in 118 of the 122 sites sampled appeared to be monomorphic. Thus, the genetic structure of epidemic populations in most sites was very simple and probably exclusively asexual. The two mating types were usually separated geographically. However, both mating types were detected together in three fields in British Columbia. Some isolates from these fields had unique genotypes that could have arisen by sexual recombination between the most common A1 and A2 genotypes in British Columbia. This is the first evidence for the probable occurrence of sexual reproduction of *P. infestans* in North America north of Mexico.

Late blight disease of the cultivated potato (*Solanum tuberosum* L.) probably originated in 1843 when the pathogenic oomycete *Phytophthora infestans* (Mont.) de Bary apparently was introduced into the United States from its probable center of origin in Mexico (11,18,21). Subsequently, *P. infestans* has migrated throughout the world wherever potatoes are grown (3). Recent analyses revealed that there was very little genetic diversity in most populations worldwide (11,13,20); therefore, the initial migrations out of Mexico were probably extremely limited. Only one (A1) mating type was known outside of central Mexico until very recently (16,18), so populations in most parts of the world probably were limited to asexual reproduction for more than 130 yr (both A1 and A2 mating types are required for sexual reproduction). This began to change in the late 1970s, when there were probably additional migrations from Mexico into Europe (7,20). European populations of *P. infestans* now include both A1 and A2 mating types, and are characterized by high frequencies of particular alleles at allozyme and DNA fingerprint loci (5–8, 20,22). The *Glucose-6-phosphate isomerase (Gpi) 90* and *Peptidase (Pep) 83* alleles are diagnostic for current European populations. Isolates characteristic of these populations have migrated throughout Europe (5,6,11,20,22), and have been detected recently in Africa (11), Asia (17), and South America (11), probably as a direct result of migration from Europe. Thus, a second global migration of *P. infestans* has occurred in the past decade (20). In most locations, the arrival of these immigrant genotypes has been associated with increased disease problems and a high level of insensitivity to the phenylamide fungicide metalaxyl (7).

These recent migrations did not affect populations of *P. infestans* in the United States and Canada. Among over 180 isolates analyzed from 1979 through 1991 (10), none had a genotype characteristic of the recently migrating European populations. There was evidence for a single migration event probably from northwestern Mexico into California in the late 1970s (10), but this appeared to have little impact on the genetic structure of epidemics: most populations sampled were dominated by one of only two clonal lineages. One of these lineages (US-1) may have been present since the 1840s, while the second genotype (US-6) was probably introduced in 1979 (10). Although one A2 mating type isolate was detected in Pennsylvania in 1987 and one in British Columbia in 1989 (4), as of 1991 this mating type was still extremely rare and there was no evidence for sexual reproduction in any location in North America north of Mexico. Perhaps in part due to the low level of genetic variation, late blight epidemics were infrequent in most parts of the United States and Canada during much of this period. For example, as far as we are aware there was only one late blight epidemic in New York State from 1987 through 1991.

This began to change in the summer of 1992, when severe late blight epidemics occurred simultaneously on potatoes and tomatoes throughout the United States and Canada (9). These epidemics continued into 1993. The summers of 1992 and 1993 were cool and wet over much of the northern United States and Canada, conditions that are ideal for the development of late blight disease. At least two hypotheses could explain the sudden, widespread appearance of late blight epidemics throughout the United States

and Canada. The first hypothesis is that they resulted from the migration of new (and potentially more aggressive) genotypes. This hypothesis could be tested by comparing the genotypes of isolates collected in 1992 and 1993 with those in a database of isolates collected in previous years. The identification of new genotypes beginning in 1992 would provide strong support for the migration hypothesis. The most likely origin of migrant genotypes would be recent European populations, because in the last 10 yr isolates with alleles diagnostic for these populations have been introduced into all continents where potatoes are grown except North America and Australia (8,11). However, it is also likely that migration could occur from Mexico. Although Mexican populations of *P. infestans* are separated from those in northern North America by mountain ranges and deserts, there have been persistent late blight epidemics on potatoes and tomatoes in northern Mexico in the last few years, and there is evidence for at least one migration from northwestern Mexico into California before 1980 (10). Comparisons with previously characterized populations of *P. infestans* worldwide (5,6,10,11,15,17,20,22) should allow the probable source population for any migrant genotypes to be deduced. The alternative hypothesis is that there were no new migrations, but that environmental conditions conducive to disease development increased the severity of epidemics caused by previously occurring isolates.

The purpose of this paper was to test the hypothesis that migration of new genotypes contributed to the severe late blight epidemics that began in 1992 in the United States and Canada, and to determine the probable origin of any migrant genotypes detected. A secondary goal was to determine the current geographical range and frequency of A2 mating type isolates, and to look for the products of sexual recombination. Large numbers of new genotypes in areas where both mating types occur could provide the first evidence for sexual reproduction of *P. infestans* in North America north of Mexico.

MATERIALS AND METHODS

Sources of isolates. Isolates of *Phytophthora infestans* were obtained throughout the United States and Canada from August 1992 through December 1993 (Table 1). In most cases, samples were received as infected potato or tomato tissue (foliage, stems, tubers, or fruits); initially, *P. infestans* was isolated into pure culture by surface sterilizing infected tissue pieces and allowing the oomycete to grow out onto selective media (15). An easier method was to place infected tissue directly underneath fresh potato tuber slices in sterile 9-cm petri plates. Mycelia of *P. infestans* usually grew through the tuber slices and sporulated within 4–10 days. Sporangia produced by mycelia growing through the tuber slices were transferred to fresh media aseptically on small agar blocks, and it was often possible to obtain clean transfers without the need for selective media. Transfers were also made onto selective media as a backup. Sporangia were always transferred on agar blocks without antibiotics or fungicides (these compounds appeared to inhibit germination of sporangia). Isolations were also occasionally made by transferring freshly produced sporangia (after 1–2 days incubation at 18 C) directly from infected tissue to agar media. Only one isolate was obtained per lesion or tissue piece. Isolations were usually made onto rye B (2) agar medium; pea agar and V8-juice agar were used occasionally (15). With these techniques it was often possible to obtain the fungus in pure culture within 1–2 wk after receiving infected tissue. A few isolates were received as pure cultures. Long-term culture of all isolates was on rye A (2) agar medium. Replicate cultures of most genotypes were stored cryogenically at −135 C.

Collections were made from 15 states and three provinces (Table 1). The goal was to obtain a representative sample of the total genetic diversity in populations of *P. infestans* throughout the United States and Canada. Because a previous study revealed little or no genetic variation within fields in northern North America (10), the sampling strategy adopted here was to obtain small numbers of isolates (5–10) from as many sites in different locations as possible. In total, 121 sites (commercial fields, home gardens, or greenhouses of potatoes or tomatoes) were sampled, with 1–12 isolates per site. Samples were also obtained from one population of hairy nightshade (*Solanum sarrachoides* Sendtner) growing in a potato cull pile in Wisconsin. The number of isolates obtained from all sources in both years was 384 (203 isolates in 1992, 181 in 1993).

TABLE 1. Collections (by state or province) of *Phytophthora infestans* in the United States and Canada from August 1992 to December 1993

Location (state or province)	Host	Year	No. of fields sampled	Total no. of isolates
Alberta	Potato	1992	5	11
		1993	4	7
British Columbia	Potato	1992	7	14
California	Tomato	1993	4	13
Florida	Potato	1993	9	44
	Tomato	1993	11	25
Idaho	Tomato	1993	1	1
Kentucky	Tomato	1993	1	1
Maine	Potato	1992	5	9
		1993	8	17
Michigan	Tomato	1993	1	3
Minnesota	Tomato	1993	1	1
New Jersey	Tomato	1992	1	1
		1993	1	1
New York	Potato	1992	15	67
	Tomato	1992	8	35
		1993	8	38
North Carolina	Tomato	1992	2	12
North Dakota	Potato	1992	3	12
Oregon	Potato	1992	5	21
Prince Edward Island	Potato	1992	7	9
Tennessee	Tomato	1992	1	2
Washington	Potato	1992	3	10
Wisconsin	Potato	1993	10	27
	Solanum sarrachoides	1993	1	3
Totals	Potato	1992	50	153
		1993	31	95
	Tomato	1992	12	50
		1993	28	83
	S. sarrachoides	1993	1	3

Characterization of isolates. All isolates were analyzed for mating type and for genotype at the two allozyme loci *Gpi* and *Pep* as described previously (10). A subset of isolates was analyzed for nuclear DNA fingerprint with probe RG57 (12): if all isolates within a sampling site were identical for mating type and allozymes, a small sample was tested for DNA fingerprint variation; however, most or all of the isolates within a site were assayed for DNA fingerprints when there was variability for mating type or allozymes. DNA extraction, digestion with the restriction enzyme *Eco*RI, and Southern analysis with DNA fingerprint probe RG57 were as described previously (10,12,15).

Data for all markers (mating type, two allozyme and 25 DNA fingerprint loci) were combined into a single multilocus genotype for each isolate. Although these are really multilocus phenotypes (because the exact genetic basis for mating type and some of the RG57 DNA fingerprint bands has not been determined), they will be referred to here as multilocus genotypes for simplicity. Genotypes were numbered chronologically with a two-letter prefix (US for genotypes first identified in the United States, and BC for genotypes only identified in British Columbia) followed by a dash and the number of the genotype (10). For example, US-7 was the seventh genotype identified in the United States. Genotypes that were identical except for a single change at an allozyme or DNA fingerprint locus were assumed to be members of the same clonal lineage. Different genotypes within a clonal lineage were identified by appending a period followed by a number to the genotype name, e.g., US-1.5 was the fifth member of the US-1 clonal lineage (10).

All genotypes were compared with those in a worldwide database (5,6,10,11,15,17,20,22) in an attempt to determine the probable source population for new genotypes.

RESULTS

Mating type, allozymes, and DNA fingerprints. A2 mating type isolates were common (61% of all isolates analyzed) and widely distributed in 1992 (Table 2). A2 isolates migrated into additional locations in 1993. Although A2 mating type isolates were extremely rare in previous collections (Fig. 1A), they were found in 10 states and one province during 1992–1993 (Fig. 1B).

Allozyme analysis revealed only seven dilocus genotypes among the 384 isolates (Table 2). Although individuals with the same mating type and dilocus allozyme genotype usually had the same DNA fingerprint (Table 2), fingerprint analysis did reveal some additional variation. In total, 11 distinct multilocus genotypes were detected based on the data for all markers. Isolates from the same site with the same mating type and allozyme genotype always had the same DNA fingerprint.

Among the 11 multilocus genotypes identified, only four were detected more than three times (Table 2); two of these had the A2 mating type. One of the most widely distributed genotypes, US-1 (Fig. 2, Table 2), was A1 mating type, *Gpi 86/100, Pep*

TABLE 2. Characteristics of the 11 clonal genotypes identified among 384 isolates of *Phytophthora infestans* collected in the United States and Canada from 1 August 1992 to 31 December 1993

Genotype name	Mating type	Allozyme genotype		DNA fingerprint[c]	Location (no. recovered)	Year	Total no. recovered[d]
		Gpi[a]	*Pep*[b]				
US-1[e]	A1	*86/100*	*92/100*	10111010110011010000110011	Alta. (11)	1992	114 (47)
					Alta. (7)	1993	
					FL (18)	1993	
					ME (9)	1992	
					ND (12)	1992	
					NJ (1)	1992	
					NJ (1)	1993	
					NY (4)	1992	
					OR (14)	1992	
					P.E.I. (8)	1992	
					WA (1)	1992	
					WI (28)	1993	
US-1.5	A1	*86/100*	*92/100*	10111010110011010010110011	P.E.I. (1)	1992	1 (1)
US-6[e]	A1	*100/100*	*92/100*	10111110010011000010110011	BC (8)	1992	33 (19)
					FL (9)	1993	
					OR (7)	1992	
					WA (9)	1992	
US-7	A2	*100/111*	*100/100*	10011000010011010010110011	CA (13)	1993	169 (42)
					FL (42)	1993	
					KY (1)	1993	
					ME (1)	1993	
					MI (3)	1993	
					MN (1)	1993	
					NC (12)	1992	
					NY (55)	1992	
					NY (38)	1993	
					TN (2)	1992	
					WI (1)	1993	
US-8	A2	*100/111/122*	*100/100*	10011000010011010000110111	NY (43)	1992	59 (15)
					ME (16)	1993	
US-9	A1	*100/100*	*83/100*	...	ID (1)	1993	1 (0)
US-10	A2	*111/122*	*100/100*	...	WI (1)	1993	1 (0)
BC-1	A2	*100/111*	*100/100*	10000000010011010000110011	BC (3)	1992	3 (3)
BC-2	A2	*100/100*	*100/100*	10001100000011010000110011	BC (1)	1992	1 (1)
BC-3	A2	*100/100*	*100/100*	10100010010011000010110011	BC (1)	1992	1 (1)
BC-4	A2	*100/100*	*100/100*	10000000000011000010110011	BC (1)	1992	1 (1)

[a]Glucose-6-phosphate isomerase.
[b]Peptidase.
[c]Presence (1) or absence (0) of RG57 fingerprint bands 1–25 are indicated from left to right.
[d]All isolates were tested for mating type and allozyme genotype, but not for DNA fingerprint; if the first isolates tested from a site were identical for all markers, the remainder were assumed to have the same DNA fingerprint. The number analyzed for DNA fingerprint is indicated in parentheses.
[e]Only US-1 and US-6 had been detected previously in the U.S. and Canada.

92/100, and made up 30% of the total sample. It was found in six states (WA, OR, ND, NY, ME, NJ) and two provinces (Alta., P.E.I.) in 1992, and in two of the same locations (NJ, Alta.) plus FL and WI (on potato and hairy nightshade) in 1993. Overall, this genotype was found in eight states and two provinces during the 17-mo sampling period. The US-6 genotype was also A1 mating type and *Pep 92/100,* but was *100/100* for *Gpi* compared with *86/100* for US-1 and differed from US-1 at four fingerprint loci (Table 2, Fig. 2). This genotype made up 9% of the total sample. It was found in two states (WA, OR) and one province (BC) in 1992, and in FL in 1993 (Table 2). The most frequently detected genotype, US-7 (44% of the total sample) (Fig. 2B, Table 2), was found in three eastern states (NY, NC, TN) in 1992 and in seven additional states (CA, FL, KY, ME, MI, MN, WI) in 1993. This genotype was characterized by the A2 mating type and the *100/111* genotype at the *Gpi* locus. It was *100/100* for *Pep*. The fourth genotype, US-8, was locally common in central New York in 1992 (nine potato fields in one county) and in several samples from Maine in 1993 (15% of the total sample). It was A2 mating type and had a very unusual three-allele genotype at the *Gpi* locus, *100/111/122*. Like US-7 it was *100/100* at the *Pep* locus. The remaining seven genotypes occurred rarely, each in a single location (Table 2).

Genetic change over seasons in New York State. Analyses of the large samples collected in New York revealed a change in the genetic composition and location of genotypes from 1992 to 1993 (Fig. 3). Three genotypes, US-1, US-7, and US-8, were detected in 1992 (Fig. 3A), but only the US-7 genotype was found in 1993 (Fig. 3B). The collections in 1992 came mostly from commercial fields of potatoes and tomatoes, while those in 1993 were primarily from tomatoes in home gardens (there was essentially no late blight in commercial fields in New York in 1993). Thus the predominating genotype within a region can change dramatically and unpredictably from year to year.

Comparisons with previous collections. The 11 genotypes detected in the United States and Canada in 1992 and 1993 were compared with those identified in previous analyses of over 1,200 isolates collected worldwide (5,6,10,11,15,17,22). Only two of the 11 genotypes had been identified previously in the United States or Canada; the remaining nine genotypes were new. US-1 was the most commonly detected genotype in the United States and Canada from 1979 through 1987 (10), and has been found in 16 countries on four continents (10,11,17). US-6 was the most

Fig. 1. States and provinces in which A2 mating type isolates were detected during 1979–1991 and 1992–1993. **A,** Only four A2 mating type isolates were detected among 191 isolates analyzed during 1979–1991 (4,10): one A2 mating type isolate was detected in Pennsylvania in 1987 (4), one in British Columbia in 1989 (4), and two in British Columbia in 1991 (10). **B,** A2 mating type isolates were common (61% of all isolates tested) and widely distributed among 384 isolates sampled during 1992–1993.

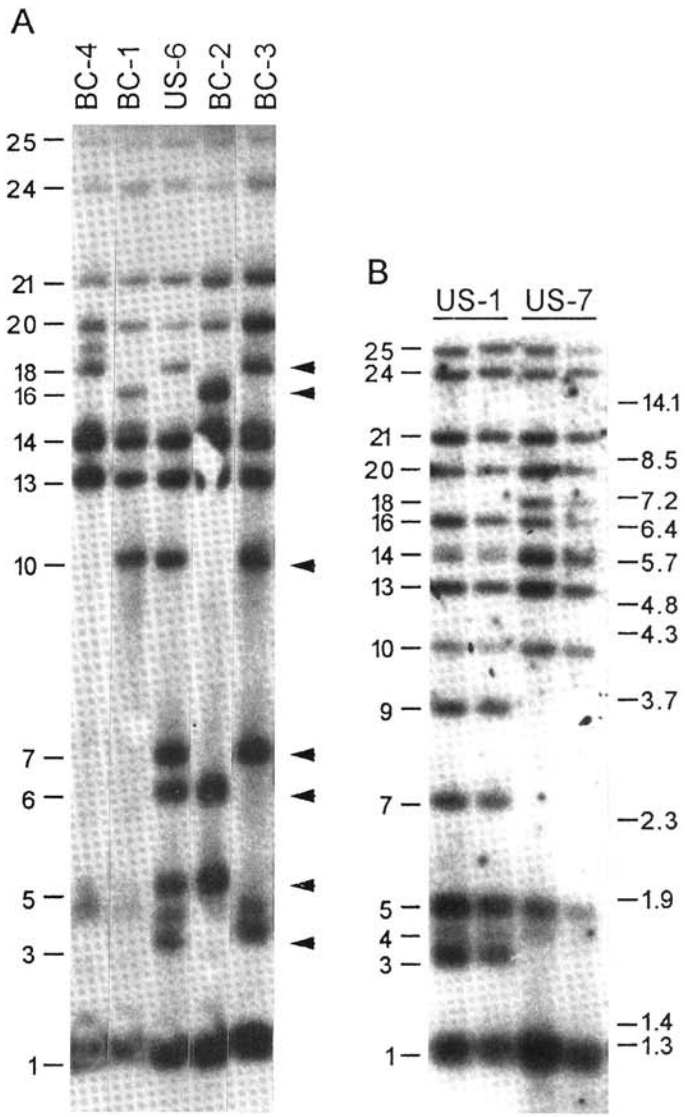

Fig. 2. DNA fingerprint patterns of seven genotypes found in the United States and Canada in 1992 and 1993. Total genomic DNA was digested with the restriction enzyme *Eco*RI and probed with ^{32}P labeled RG57 DNA in Southern analysis. Band numbers are indicated on the left, and approximate molecular weights of a size standard (phage lambda DNA digested with the restriction enzyme *Bst*EII) are indicated on the extreme right. **A,** Five genotypes that were collected in British Columbia. Arrows indicate bands that may represent genetic segregation in putative recombinants between isolates with the US-6 and BC-1 genotypes. **B,** The US-1 and US-7 genotypes that were collected in many locations in the United States and Canada.

commonly detected genotype in the United States (CA, WA, FL) during 1987–1991 and was found in British Columbia in 1991 (10). Both US-7 and US-8 had the *Gpi 111* allele, which has never been reported previously in any isolate from the United States or Canada. However, this allele was common in northern Mexico in 1988 and 1989 (15). US-7 and US-8 were identical (for mating type, dilocus allozyme genotype, and DNA fingerprint) to genotypes identified previously in populations in northwestern Mexico. US-7 was the most common genotype on tomatoes and also occurred on potatoes in northwestern Mexico in 1991, 1992, and 1993 (S. B. Goodwin et al, *unpublished*), whereas US-8 was the most common genotype on potatoes in northwestern Mexico in 1989 (15).

The US-9 genotype was unusual because it had the *Pep 83* allele, which has never been reported previously in the United States or Canada (10,11,20,22). However, this allele is common in recent European populations (6,22).

The BC-1 through BC-4 genotypes did not match those from any other location in the database. The presence of the *Gpi 111* allele in BC-1 might indicate a relationship with populations in northern Mexico, although isolates with the same DNA fingerprint pattern as BC-1 have not yet been detected in Mexico (S. B. Goodwin et al, *unpublished*).

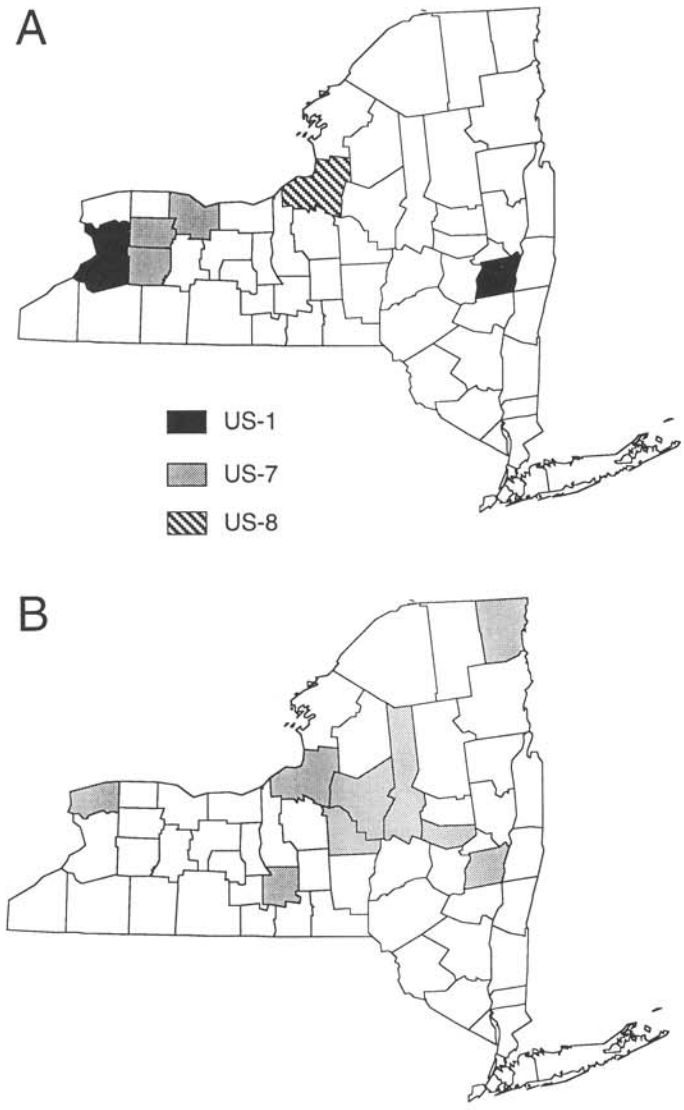

Fig. 3. Distribution of genotypes detected in New York in 1992 and 1993. **A,** The US-1, US-7, and US-8 genotypes were detected in different counties in 1992. **B,** Only US-7 was detected in 1993, when it occurred in nine counties throughout the state. No genotype was detected in the same county in both years.

Evidence for sexual reproduction. Opportunities for sexual reproduction were probably extremely limited, because only one genotype was detected in 118 of the 122 sites sampled (97%). If these samples accurately reflected the genetic composition of the populations in those fields, then reproduction must have been exclusively asexual in the vast majority of locations. However, both mating types occurred together in at least four fields. One field in Manatee County, FL, had A1 (US-6) and A2 (US-7) mating types, but no potentially recombinant genotypes were detected. Because late blight had not been detected in that location for many years, it seems most likely that both genotypes were introduced during the 1993 growing season and there had not been time for completion of the sexual cycle. The other three fields that contained both mating types were in British Columbia. US-6 and BC-1 were the most common A1 and A2 genotypes, respectively, in British Columbia (Table 2). These genotypes were found together in one field. Two other fields contained US-6 plus an A2 genotype that could have arisen by recombination between US-6 and BC-1 (Fig. 2A). US-6 has DNA fingerprint bands 3, 5, 6, 7, and 18 that are not present in BC-1, whereas BC-1 has fingerprint band 16 that is not present in US-6. The BC-2, BC-3, and BC-4 genotypes all had combinations of allozyme alleles and DNA fingerprint bands that could have arisen by hybridization between US-6 and BC-1 (Table 2, Fig. 2A). For example, BC-2 could have inherited bands 5 and 6 from the US-6 parent and band 16 from BC-1 (Fig. 2A). BC-2 is also missing band 10, which could have arisen by hybridization between isolates with the US-6 and BC-1 genotypes if both genotypes were heterozygous at this locus. Similar arguments can be made for BC-3 and BC-4.

DISCUSSION

Major changes occurred in the genetic composition of populations of *P. infestans* in the United States and Canada from 1991 to 1992. The A2 mating type had been extremely rare in collections made prior to 1992 (10), but it was widespread and common (61% of the total sample) in 1992 and 1993. Similarly, the *Gpi 111* allele had never been reported from the United States or Canada before 1992, yet 60% of all isolates analyzed in 1992 and 1993 had a genotype that contained this allele. All but two of the 11 multilocus genotypes found in the United States and Canada in 1992 and 1993 were new. Late blight in 10 states was caused primarily or exclusively by genotypes never before detected in the United States or Canada.

The most likely explanation for these changes is that they were due to massive immigration of new genotypes during or just prior to 1992. Evidently migration can occur extremely rapidly under favorable conditions; the US-7 genotype had been detected in 10 states ranging from New York and Florida to California within a year after it was first discovered. This rapid long-distance migration must have been aided by human activities, most likely by the movement of infected potato tubers (both seed potatoes and those for fresh consumption), tomato fruits, or tomato seedlings for transplants.

Weather favorable for late blight development also played a role in the severity of the 1992 epidemics. The cool, wet weather of 1992 was ideal for the establishment of new migrant genotypes. It is possible that other migrations occurred in the past but that the migrants did not become established because the weather was unfavorable for late blight. Favorable weather also allowed previously occurring genotypes such as US-1 to cause more disease than usual. For example, there were severe late blight epidemics in North Dakota in 1992 and 1993 that were probably caused exclusively by the US-1 genotype. These epidemics probably would not have occurred if the weather had been less conducive to late blight development. The two hypotheses originally proposed to explain the 1992–1993 epidemics—migration or favorable weather—are not mutually exclusive and it appears that these epidemics were caused by a combination of new migrations and favorable weather.

The primary source population for new migrant genotypes

appears to be northwestern Mexico. Genotypes in the United States and Canada were compared with those identified with the same genetic markers in previous samples of over 1,200 isolates from 24 countries on five continents (5,10,11,15,17,22). The *Gpi 111* allele (characteristic of the US-7, US-8, and BC-1 genotypes) has only been reported previously from northern Mexico (15), and the *Gpi 100/111/122* genotype (characteristic of US-8) was extremely uncommon. It was found in northeastern and northwestern Mexico (15), and with the same DNA fingerprint as US-8 in northwestern Mexico. US-8 (or isolates that were identical to US-8 at the mating type, two allozyme and 25 DNA fingerprint loci) was the most common genotype on potatoes in northwestern Mexico in 1989 (15). US-7 was the most common genotype on potatoes and tomatoes in northwestern Mexico in 1991, 1992, and 1993 (S. B. Goodwin, *unpublished*). Isolates with the same mating type and dilocus allozyme genotype as US-10 represented the third most frequently detected genotype in northwestern Mexico in 1992 and the second most common in 1993 (S. B. Goodwin, *unpublished*) (DNA fingerprint data for these isolates are not yet available). The probability of obtaining US-7 or US-8 by random recombination in northeastern Mexico was approximately 3×10^{-5} for US-7 or 2×10^{-6} for US-8 (based on published frequencies of mating types, allozyme alleles, and DNA fingerprint bands) (15). Due to the low frequency of the *Gpi 111* allele worldwide, the probability of US-7 or US-8 arising independently in any other location is close to zero. US-7, US-8, and US-10 were all common in northwestern Mexico before they were detected in the United States. Therefore, it seems extremely likely that isolates with the US-7, US-8, and US-10 genotypes in the United States are direct clonal descendants of isolates in northwestern Mexico, and were introduced into the United States by migration from Mexican potato and tomato production areas. A less likely possibility is that these genotypes were introduced into both the United States and northwestern Mexico from a third location that was not sampled. These migrations appear to have effectively ended the genetic isolation of populations of *P. infestans* in the United States and Canada that probably existed for almost 150 yr.

It is interesting that the end of genetic isolation and the introduction of A2 mating type isolates into populations of *P. infestans* in the United States and Canada was not brought about by migration from Europe. Although isolates with genotypes characteristic of recent European populations have now been detected throughout the world (7,11,17,20), only one of the isolates in this study had a genotype that could have come from the European migrating populations. This isolate had the US-9 genotype, and was obtained from a tomato plant grown in a greenhouse in Idaho. US-9 has the *Pep 83* allele (Table 2). This allele has not been reported previously in the United States, Canada, or northern Mexico (10,15,20,23), although it is common in recent European populations (6,20,22) and has also been found in central Mexico (8,15). Therefore, this genotype was most likely introduced from Europe (although the possibility of an introduction from central Mexico cannot be eliminated). If it was introduced from Europe, the introduction must have been extremely limited, because European genotypes did not play a major role in late blight epidemics in the United States and Canada in 1992 and 1993.

The allozyme genotype of US-8 isolates indicates that they are triploid at least for the chromosome containing the *Gpi* locus. Progeny containing extra chromosomes could be generated by meiotic nondisjunction. Because meiotic nondisjunction appears to occur commonly in *Phytophthora* species (14), the US-8 genotype probably originated by nondisjunction of the chromosome containing the *Gpi* locus during sexual reproduction. US-8 isolates in Mexico have DNA contents close to 2C and are fully fertile in crosses (15). Therefore, aneuploidy is not a barrier to sexual reproduction in *P. infestans* and US-8 isolates may be able to hybridize with previously occurring A1 isolates in the United States and Canada.

The biology and past history of *P. infestans* in North America provided a natural experiment for the direct detection of gene flow among populations. Gene flow among populations can be detected either directly (by monitoring the movement of marker genes or individuals) or indirectly (by analyzing the patterns of genetic variation within and among populations) (1,19). Because it is usually not possible to release and monitor marked strains, direct measures of gene flow are extremely rare for important plant pathogens. Direct detection of gene flow in this case was possible due to the availability of allozyme and DNA fingerprint markers for unambiguous identification of alleles and genotypes, and because previous characterizations of genetic variability in populations of *P. infestans* in the United States and Canada provided a baseline for identifying changes. The lack of sexual reproduction in most locations made it possible to track the movement of particular clonal genotypes.

Clonal reproduction and rapid migration caused the genetic composition of populations to change dramatically and unpredictably from year to year. This was particularly evident in New York and Florida. Three genotypes (US-1, US-7, and US-8) were detected in New York in 1992, but each genotype had an extremely localized distribution (Fig. 3A). Only one of these genotypes (US-7) was found in 1993, but it occurred in nine counties throughout the state (Fig. 3B), although not in the same counties as in 1992 (lack of detection does not necessarily mean the other genotypes were absent in 1993, only that they were not detectable with our sampling strategy). Because winter survival of *P. infestans* as vegetative (asexual) mycelium is uncertain, detection of genotypes in one year will not predict which genotypes will be present in future years. Similarly, in Florida US-1 was only detected in two southern counties in 1993. Seed for one of these fields came from North Dakota and for the other field from Maine. The growers believed that the disease came in on the seed tubers. US-1 was the only genotype detected in North Dakota and Maine in 1992, the year the seed was produced, and there was no other source of inoculum because late blight was rare in southern Florida for many years before these epidemics. Therefore, it seems extremely likely that these epidemics were the direct result of gene flow from North Dakota and Maine into Florida on infected seed potatoes. Although these migrations have dramatically altered the genetic composition of populations, they have not affected their structure: most epidemics are still caused by a single clonal genotype, although it may be a different genotype than in previous years.

The only likely evidence for sexual reproduction was in British Columbia. In general, opportunities for sexual reproduction were limited because only one genotype was detected in most fields sampled. However, both mating types were detected together in three fields in British Columbia based on very limited sampling (a maximum of three isolates per field). Some of the A2 isolates in these fields had genotypes that had not been detected anywhere else worldwide, and that could have arisen by sexual recombination between the two most common A1 and A2 genotypes in British Columbia, US-6 and BC-1, respectively. There are at least two other potential origins for these genotypes: mutation and parasexual recombination. However, neither of these possibilities seems likely. RG57 fingerprint patterns and allozyme genotypes are extremely stable (12), and if any mutations did occur they would probably only affect a single locus. Because the putative recombinant genotypes had many changes from their probable parents it is extremely unlikely that they arose by mutation. Parasexual recombination does not occur commonly in diploids, and there is no good evidence for any kind of a parasexual cycle in *P. infestans*. Therefore, the most likely explanation is that the putative recombinant genotypes arose by sexual reproduction within British Columbia. The A2 mating type was first detected in British Columbia in 1989 (4), so three or four cycles of sexual reproduction could have occurred by 1992. This is the first evidence for the probable occurrence of sexual reproduction of *P. infestans* in North America north of Mexico.

The result of these migrations as of 1993 is that genotypes of *P. infestans* are distributed in a geographic mosaic. Initial inoculum was probably limited, so that most fields were colonized by a single genotype. However, the genotypes in adjacent locations varied depending on the original source of inoculum. Further-

more, some clonal genotypes were widely distributed over large geographical areas. The simple genetic structure within epidemics and patchy distribution of genotypes has probably limited opportunities for sexual reproduction, because the two mating types have been separated spatially even when they occurred in the same region. However, as migration continues, contact between the two mating types will almost certainly increase, and it is probable that sexually reproducing populations of *P. infestans* will soon become established throughout the United States and Canada.

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