Migration from Northern Mexico as the Probable Cause of Recent Genetic Changes in Populations of Phytophthora infestans in the United States and Canada

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ABSTRACT


Isolates of Phytophthora infestans from five U.S. states (California, Florida, New York, Washington, and Wisconsin) and one Canadian province (British Columbia) collected between October 1987 and October 1991 were analyzed for mating type and for genetic variation at two allozyme loci. A subset of these 155 isolates, which included all of the allozyme genotypes plus 26 isolates from our culture collection that had been collected in the United States and Canada during 1979-1987, were analyzed for DNA fingerprint variation. These analyses revealed 18 genotypes among the 120 isolates that were analyzed for all markers. One genotype (US-1), which occurred in New York and Wisconsin in 1987 and 1991, respectively, and also in older isolates dated back to 1979, has been found throughout the world and may have persisted asexually in the United States and Canada since the first reports of late blight disease in the 1840s. Four other genotypes that appeared to be members of the same clonal lineage were variants of US-1. The most common genotype in the collections from 1987 to 1991, US-6, was identical to previously characterized isolates from northern Mexico, so it was probably a recent migrant. Five additional genotypes were variants of US-6 and differed from it by a single allozyme or DNA fingerprint allele; thus, they were probably members of a single clonal lineage. Overall, 13 of the 18 genotypes identified in isolates collected during 1979-1991 could have resulted from two probable migration events. Only two isolates (1.3% of the total sample) from a single field in British Columbia in 1991 were A2, and these also were probably introduced recently from Mexico. Each field sampled appeared to have been colonized by a single clonal lineage. Therefore, future surveys in the United States and Canada can be accomplished most efficiently by sampling only a low number of isolates per field but increasing the number of fields sampled. These data provide a baseline for monitoring future genetic changes in populations of P. infestans in the United States and Canada.

Phytophthora infestans (Mont.) de Bary, the cause of late blight disease of potato and tomato, is a diploid oomycete that probably evolved in the highlands of central Mexico (6,19); populations in all other locations originated by migration during historical times. Relative to those in other locations, Mexican populations have greater genetic diversity for virulence (11,16,20,21,33), allozymes (10,32), and DNA fingerprints (10). Populations in other locations contain a subset of the variation in central Mexico (4,10,26).

Migration plays a pivotal role in the population biology of P. infestans. Previous migrations out of Mexico appear to have been extremely limited, so that populations in most areas of the world were dominated by a single genotype (8). In the 1980s, different genotypes, which were probably the result of a second migration from Mexico, appeared in Europe (27). These migrant populations were characterized by the A2 mating type (12,14, 25,31), previously undetected allozyme genotypes (5,24,27,30), and a much greater diversity of DNA fingerprints (4,30). The number of DNA fingerprint genotypes in Europe increased from the two or three members of a single clonal lineage before 1980 (8) to hundreds of genotypes by the early 1990s (4,30; S. B. Goodwin et al., unpublished), and there is now evidence that at least some of this increase in genetic diversity may have been the result of sexual reproduction (30). In contrast, there was only one mating type (A1) and little genetic variation in populations of P. infestans in the United States and Canada on the basis of analyses of two allozyme loci and virulence to particular potato resistance genes (27,32,33).

Additional migration could increase the level of genetic variation in populations of P. infestans in the United States and Canada and could also lead to the establishment of the A2 mating type. Recent migrations have already brought increased genetic variability, including the A2 mating type, to Asia (13,17), South America (1), and northern Mexico (10). Single A2 isolates have been found recently in British Columbia and Pennsylvania (3). Therefore, there is an increased probability that additional genetic variants, including the A2 mating type, could become established in America north of Mexico. Sexual reproduction between A1 and A2 mating types would not only lead to increased genotypic diversity, but the sexual oospores could provide a long-lived source of inoculum that could complicate disease control measures (19).

Analyses of genetic variation in P. infestans populations in the past were hindered by a lack of well-characterized markers. Allozymes and recently developed DNA fingerprinting probes provide ideal markers for genetic analysis of P. infestans populations. One DNA fingerprinting probe, RGF5, has been particularly useful because it provides data at more than 25 genetic loci (7,9,10). The banding patterns produced by this probe are stable mitotically (through single-oospore reproduction), so that clones have identical fingerprints (7). These bands also segregate in progeny as single dominant markers (7). Because heterozygotes can be distinguished from homozygotes (at most loci) by visual inspection of the autoradiograms and most of the loci are unlinked.

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(7), it is possible to make a genetic, as well as a phenotypic, interpretation of the data.

The purpose of this study was fourfold. The first goal was to assess the impact of possible recent migrations on genetic variability in U.S. and Canadian populations of *P. infestans* and to identify the probable routes of migration, if any. The three most likely outcomes were that 1) there were no recent migrations, in which case the variation would be the same as in previous studies; 2) there were migrations from Mexico, which could be identified by comparisons with previously described Mexican collections (10); or 3) there was migration from Europe (not limited exclusively to Europe but including any of the genotypes in the recently migrating populations worldwide). Identifying the same clonal genotype in more than one location would provide strong evidence for migration. The second goal was to quantify the levels of genetic variation within fields to serve as a guide for future sampling in northern North America. The third goal was to determine the distribution of the A2 mating type in recent populations of *P. infestans* in the United States and Canada. Finally, because previous studies had rather limited sample sizes, the fourth goal was to develop a database containing information on genetic variation in U.S. and Canadian populations of *P. infestans* to serve as a baseline against which future genetic changes could be monitored.

**MATERIALS AND METHODS**

**Sources of isolates.** Isolates were obtained from naturally infected commercial fields in five U.S. states and the Canadian province of British Columbia between October 1987 and October 1991 (Table 1). Infected potato and tomato tissue pieces (from leaves, stems, fruits, or tubers) were surface sterilized and plated onto V8-juice agar medium containing antibiotics and fungicides (10). Because some genotypes do not grow well on V8-juice agar, some isolates were made with pea agar (filtrate from 120 g of autoclaved frozen peas per liter) similarly amended with antibiotics and fungicides. Sample sizes within most fields ranged from one to six, but three fields were sampled more intensively (Table 1) to obtain better estimates of intraspecific diversity. Long-term culture of isolates was on rye A agar medium (2) at 18°C; replicate cultures of most isolates were stored permanently at −15°C. To determine historical trends, 26 additional isolates from our initial collection, for which allozyme and mating type data have been reported previously (26), were analyzed for DNA fingerprint variation. These isolates had been collected throughout the United States and Canada during 1979–1987.

**Characterization of isolates.** Mating types were determined by pairing each isolate with known A1 and A2 tester strains on 10% clarified V8-juice agar medium (15) or on rye B agar medium (2). Oospores usually formed in the zone of contact between compatible isolates within 7–10 days. Isolates that formed oospores with the A1 tester but not with the A2 were scored A2, and isolates that formed oospores with the A2 tester but not with the A1 were scored A1. Isolates that produced oospores with both testers or that formed oospores before coming into contact with a tester isolate were scored self-fertile (homothalic).

Mycelia for allozyme and DNA analyses were grown in 10% V8 broth, pea broth (10), or rye B broth (2). Isolates were cultured exclusively in pea broth after the initial test. Crude protein extracts for allozyme analysis for most isolates were prepared by grinding fresh tissue as described in Goodwin and Fry (9). For a few isolates, allozymes were assayed from lyophilized, powdered tissue (10) that had been stored at −80°C. A small amount (approximately 20 mg) of powdered tissue was rehydrated with 300–400 μl of water, mixed, and centrifuged for 1–2 min at 13,000 g. Paper wicks were dipped into the supernatant and loaded onto 12% starch gels. Gels for the enzyme glucose-6-phosphate isomerase (GPI, EC 5.3.1.9) were run on a discontinuous histidine-Tris citrate buffer system (28) (gel: 0.01 M histidine-HCl, pH 6.0; electrode: 0.135 M Tris and 0.04 M citric acid, pH 6.0) at 150 V (approximately 45–50 mA) for 14–18 h. Gel slices were stained with an agar overlay made by combining 10 mL of 1% melted agar solution with 10 mL of staining solution (10 mL of 0.1 M Tris, 0.1% MgCl₂, pH 7.0, 50 mg of fructose-6-phosphate, 20 mg of Na₂EDTA, 10 mg of MTM, 1 mg of phenazine methosulfate, and 30 U of glucose-6-phosphate dehydrogenase). Gels for peptidease (PEP, EC 3.4.1.5) were run on a Tris-citrate buffer system (29; gel: 0.025 M Tris and 0.055 M citric acid, pH 8.0; electrode: 0.687 M Tris and 0.157 M citric acid, pH 8.0) at 60 V (approximately 25 mA) for 12–18 h. This system was first used for PEP gels of *P. infestans* in Japan (18). Gel slices were stained with an agar overlay made as described above except with 10 mL of peptidease staining solution (10 mL of 0.045 M Tris, 0.25 M boric acid, 2.5 × 10⁻⁴ M EDTA, pH 8.7, 50 mg of glycyl-leucine, 15 mg of o-dianisidine, 4,000 U of peroxidase, and 1.5 U of α-aminocarboxylic acid oxidase). Allozyme alleles were designated by numbers representing their percent mobility with regard to a standard as described in Tooley et al. (32).

DNA extraction, digestion with the restriction enzyme EcoRI, and Southern analysis with the DNA fingerprint probe RG57 were as described previously (7,9,10).

**Data analysis.** A multilocus genotype was constructed for each isolate by combining data on mating type, dioecious allozyme genotype, and presence or absence of a band at each of the DNA fingerprinting loci. Because the exact genetic basis for mating type and some of the DNA fingerprint phenotypes are not known, these are really multilocus characteristics for simplicity. Genotypes were named with a prefix of US or CDA, depending on whether they were first identified in the United States or Canada, and

<table>
<thead>
<tr>
<th>State or province</th>
<th>Location</th>
<th>Year</th>
<th>Host</th>
<th>Sample size</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>British Columbia</td>
<td></td>
<td>1991</td>
<td>Potato</td>
<td>30</td>
<td>G. McCollum and D. Ormrod</td>
</tr>
<tr>
<td>California</td>
<td>San Benito County</td>
<td>1989</td>
<td>Tomato</td>
<td>2</td>
<td>R. Smith</td>
</tr>
<tr>
<td>Florida</td>
<td>Naples</td>
<td>1991</td>
<td>Tomato, field 1</td>
<td>15</td>
<td>Glades Crop Care</td>
</tr>
<tr>
<td>Washington</td>
<td>Bellingham</td>
<td>1990</td>
<td>Potato, cv. Commercial Russet</td>
<td>6</td>
<td>D. Ormrod</td>
</tr>
<tr>
<td>Skagit County</td>
<td></td>
<td>1990</td>
<td>Potato, cv. Norchip</td>
<td>2</td>
<td>D. Ormrod</td>
</tr>
<tr>
<td>Whatcom County</td>
<td></td>
<td>1990</td>
<td>Potato, cv. Red LaSoda</td>
<td>4</td>
<td>D. Ormrod</td>
</tr>
<tr>
<td>Skagit County</td>
<td></td>
<td>1991</td>
<td>Potato, cv. unknown</td>
<td>5</td>
<td>D. Ormrod</td>
</tr>
<tr>
<td>Grant and Adams counties</td>
<td></td>
<td>1991</td>
<td>Tomato, cv. Roma</td>
<td>2</td>
<td>D. Ormrod</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>Oconto County</td>
<td>1991</td>
<td>Potato</td>
<td>7</td>
<td>D. A. Inglis</td>
</tr>
</tbody>
</table>

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numbered chronologically; e.g., US-2 was the second genotype identified in the United States. Members of a clonal lineage were identified by the name of the progenitor genotype followed by a period and the number of that genotype within that clonal lineage; e.g., US-6.3 was the third variant of the US-6 genotype. A drawback of this system is that some variants in a clonal lineage may have the prefix US, for example, even though they occurred in Canada. These multilocus genotypes were compared for matches to genotypes identified previously in Mexico (10) and worldwide (8). Cluster analysis was performed on Dice coefficients calculated among all 18 multilocus genotypes in the United States and Canada as

\[ F = 2n_{xy}/(n_x + n_y) \]

where \( n_{xy} \) is the number of characters shared between two genotypes and \( n_x \) and \( n_y \) are the number of characters in genotypes x and y, respectively. A dendrogram was constructed from the matrix of Dice coefficients by using the UPGMA algorithm of the NTSYS statistical package (22).

**RESULTS**

**Mating type, allozymes, and DNA fingerprints.** The A2 mating type was very rare during 1987–1991 (Table 2); only two of 155 isolates tested (1.3%) were A2. All of the isolates collected between 1987 and 1991 and tested for allozymes (n = 145) had one of four diolose allozyme genotypes (Table 2). These were (with frequencies indicated in parentheses) Gpi 86/100, Pep 92/100 (19%); Gpi 100/100, Pep 92/100 (78%); Gpi 86/100, Pep 100/100 (1%); or Gpi 100/100, Pep 100/100 (2%). DNA fingerprint analyses of 95 isolates revealed four additional genotypes among isolates that were identical for mating type and allozyme genotype. There were eight multilocus genotypes for mating type, allozyme, and DNA fingerprints (Fig. 1) among 94 isolates that were scored for all markers between 1987 and 1991 (Table 2).

DNA fingerprint analyses of 26 additional isolates collected between 1979 and 1987 combined with previously published allozyme and mating type data (26) revealed 12 multilocus genotypes (Table 3), only two of which were found in the samples between 1987 and 1991. Thus, in total there were 18 multilocus genotypes among 120 isolates that were scored for all markers between 1979 and 1991. Thirteen of these 18 genotypes were identified only once, and only two genotypes (US-1 and US-6) occurred more than three times.

When these 18 multilocus genotypes were compared with those in a database of Mexican (10) and worldwide (8; S. B. Goodwin, unpublished) isolates, the US-1 genotype (Fig. 1, Tables 2 and 3) was identical to a genotype that has been found panaglobally (except in Mexico) and that is usually the predominant genotype in the oldest collections obtained from each region (S. B. Goodwin et al., unpublished). The US-6 genotype, found in California, Washington, British Columbia, and Florida in this study, was the most common genotype (68% of the 88 isolates sampled) in northwestern Mexico (near Los Mochis, Sinaloa) in 1989, but it was not identified from any other location in the database. This genotype had restriction fragment length polymorphism pattern A in research reported by Goodwin et al (10).

Other genotypes that were also detected outside the United States and Canada were US-1.1 and US-1.2, which both occurred in Poland. US-1.1 was identical to Polish genotype PO-2, and US-1.2 was identical to Polish genotype PO-3 (30).

**Cluster analysis.** There were 38 characters included in the cluster analysis: mating type (A1 or A2), the presence or absence of seven Gpi and five Pep alleles, and the presence or absence of a band at each of 25 DNA fingerprint loci. RG57 fingerprint band 4 was excluded from all analyses because data for this band are not always repeatable. However, inclusion of a previously unreported band, 11a (between bands 11 and 12), kept the number of DNA fingerprint loci scored at 25. Many allozyme alleles were not detected in these populations, and many of the DNA fingerprint loci were monomorphic, leaving 17 informative characters in the cluster analysis. To be on the conservative side, only those DNA fingerprint changes that resulted in the loss of a band at a locus were scored; changes that appeared to result in a doubling in the intensity of some bands were not included in the analysis.

There were two major clusters that included the US-1 and US-6 genotypes (Fig. 2). Within the cluster containing US-1, genotypes US-1.1, US-1.2, and US-1.3 were identical to US-1 except for a change from heterozygosity to homozygosity at a single allozyme or DNA fingerprint locus. The US-1.4 genotype differed from US-1 at one allozyme and one DNA fingerprint locus. Because all the changes from US-1 in the US-1.1–1.4 genotypes were at loci that appeared to be heterozygous in US-1, these five genotypes were assumed to be part of a single clonal lineage derived from US-1. US-1 was assumed to be the progenitor genotype for this clonal lineage because it was the most common genotype in that cluster and it was found in a large number of isolates over many years (Tables 2 and 3). The US-3 and CDA-1 genotypes each had three differences from US-1. They were assumed to represent closely related genotypes that may have been introduced from the same source population and at the same time as US-1 (Fig. 2).

The second major cluster (Fig. 2) contained genotype US-6 and five other genotypes (US-6.1–6.5) that were identical to US-6 except for a change at a single allozyme or DNA fingerprint locus. Most of these changes involved heterozygous loci in US-6 that became homozygous, resulting in the loss of an allozyme allele or DNA fingerprint band. The only exception was genotype US-6.3, which had gained a DNA fingerprint band in addition to those characteristic of US-6. Although the US-6 genotype was very common, the variants (US-6.1–6.5) were each identified only once. The similarity among these six genotypes was 94% or greater.

**TABLE 2. Multilocus genotypes of Phytophthora infestans in the United States and Canada, 1987–1991, on the basis of mating type, allozyme, and DNA fingerprint analyses**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mating type</th>
<th>Gpi</th>
<th>Pep</th>
<th>RG57 fingerprint*</th>
<th>Location</th>
<th>Year</th>
<th>Sample size for allozymes</th>
<th>Sample size for DNA fingerprints</th>
</tr>
</thead>
<tbody>
<tr>
<td>US-1</td>
<td>A1</td>
<td>86/100</td>
<td>92/100</td>
<td>10111010101101000100111011111111111111111111</td>
<td>New York</td>
<td>1987</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>US-6</td>
<td>A1</td>
<td>100/100</td>
<td>92/100</td>
<td>101111111110100010011001111111111111111111</td>
<td>Wisconsin</td>
<td>1991</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>US-6.2</td>
<td>A1</td>
<td>100/100</td>
<td>92/100</td>
<td>1011101010110100010011101111111111111111111</td>
<td>California</td>
<td>1989</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>US-6.3</td>
<td>A1</td>
<td>100/100</td>
<td>100/100</td>
<td>101111110010100010011101111111111111111111</td>
<td>Washington</td>
<td>1990</td>
<td>39</td>
<td>34</td>
</tr>
<tr>
<td>US-6.4</td>
<td>A1</td>
<td>100/100</td>
<td>100/100</td>
<td>101111110010100010011101111111111111111111</td>
<td>British Columbia</td>
<td>1991</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>US-6.5</td>
<td>A1</td>
<td>100/100</td>
<td>100/100</td>
<td>101111110010100010011101111111111111111111</td>
<td>Florida</td>
<td>1991</td>
<td>19</td>
<td>18</td>
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<tr>
<td>CDA-2</td>
<td>A1</td>
<td>100/100</td>
<td>92/100</td>
<td>1011101010110100010011101111111111111111111</td>
<td>Washington</td>
<td>1990</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CDA-3</td>
<td>A2</td>
<td>86/100</td>
<td>100/100</td>
<td>1011101010110100010011101111111111111111111</td>
<td>British Columbia</td>
<td>1991</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>British Columbia</td>
<td>1991</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>British Columbia</td>
<td>1991</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

*Presence (1) or absence (0) of RG57 fingerprint bands 1–25 are indicated from left to right.
(Fig. 2). Consequently, they were assumed to be members of a single clonal lineage derived from the US-6 genotype.

The remaining five genotypes fell into four different clusters (Fig. 2). There were no obvious relationships among these clusters. They could have resulted from four separate migration events, or they could be unrelated genotypes that were introduced at the same time as were other genotypes. The cluster containing US-2 and US-5 (Fig. 2) was closely related to the US-6 cluster. However, because US-5 was found in Maine whereas US-6 has not been detected, they were not assumed to be part of the same migration event. Many of the rare genotypes occurred in British Columbia (Table 2). The A2 genotype from British Columbia (CDA-3) was the outlier among all 18 genotypes (Fig. 2).

![DNA fingerprint patterns of isolates of Phytophthora infestans collected in the United States (US) and Canada (CDA) during 1987-1991 and an isolate that was collected in northern Mexico (near Los Mochis, Sinaloa [NWMEX]) in 1989 (10) for comparison. Band numbers are indicated on the left and are the same as described previously (7,10). The approximate locations of size markers (in kilobases) for phage lambda DNA digested with the restriction enzyme BstEII are indicated on the right.](image)

**DISCUSSION**

At least two major migration events appear to have had major impacts on the amount of genetic variation in populations of *P. infestans* in the United States and Canada. The first migration probably brought in the US-1 genotype. This genotype has been present in the United States and Canada since at least 1979, and it has been found throughout the world (8). Because US-1 was present in the oldest collections obtained worldwide, it was hypothesized to have been the genotype that caused the Irish potato famine in the 1840s (8, S. B. Goodwin et al., unpublished). Genotypes US-3 and CDA-1 appear to be closely related to US-1 and thus may have been introduced together from the same source population in Mexico. These genotypes may have persisted asexually in the United States and Canada since the original introduction of *P. infestans* (29) in 1843. Genotypes US-1.1-1.4 each had very limited distribution, and all could have been derived from US-1 by the loss of one or two alleles at loci that were heterozygous in the US-1 genotype. These variants could have been generated easily by mitotic crossing over or the original heterozygous diploid and thus are probably members of a single clonal lineage.

The second major migration probably introduced the US-6 genotype in the late 1970s. US-6 was the most common and widespread genotype in the United States and Canada during 1987-1991. In the worldwide database, the only other location where this genotype was identified was northwestern Mexico (near Los Mochis, Sinaloa) in 1989, where it was the most common genotype on tomatoes and also infected potatoes (10). Although northwestern Mexico was not sampled prior to 1989, the high frequency and wide distribution of this genotype in that location implies that it may have been there for many years. Therefore, it seems likely that this genotype was introduced recently into the United States and Canada from northwestern Mexico. A potential alternative explanation is that the US-6 genotype existed at a low frequency in the United States and Canada and remained undetected for many years. However, this seems very unlikely. Because there was essentially no genetic variation within fields, even the relatively limited sample sizes in this study should have been sufficient to detect US-6 if it occurred at any appreciable frequency. Furthermore, during the 1980s in the United States and Canada, late blight epidemics were relatively rare, and this argues against the survival of many different genotypes. It is equally unlikely that the direction of migration was from the United States to Mexico: the US-6 genotype was the most common one in northwestern Mexico in 1989 (10) before it became common in the United States and Canada.

The most likely mechanism for migration of the US-6 genotype is the transport of infected tomato fruits, although transport could also occur in potato tubers, tomato transplants, or possibly seeds. *P. infestans* can survive for months in tomato fruits and seeds (34), and there is commerce in tomato fruits across the U.S.-Mexico border. Late blight was absent from tomatoes in California for 32 yr until 1979 (34). The US-6 genotype was isolated from tomatoes in California as early as 1982, so it was probably the cause of the 1979 epidemic. This genotype may have a survival advantage over the US-1 clonal lineage because it infects both tomatoes and potatoes (S. B. Goodwin and L. S. Sujkowski, unpublished), and it now appears to be established in Washington, where it was isolated in 1992 (S. B. Goodwin et al., unpublished, 1990, and 1991. The US-4 genotype, although not closely related to US-6 (Fig. 2), was isolated from tomatoes in California in 1980 and therefore was probably also a cause of the 1979 epidemic. However, in contrast to US-6, the US-4 genotype may not have persisted after 1980, and it does not appear to have migrated to other areas.

We found no evidence for migration from Europe into the United States or Canada. This was somewhat surprising because genotypes that were first detected in Europe in the early 1980s have not been found in many areas of the world. Recent European populations are characterized by high frequencies of the Gpi 90 and Pep 83 alleles (5,27,30), neither of which was detected in
TABLE 3. Multilocus genotypes of *Phytophthora infestans* identified in isolates collected during 1979–1987

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mating type</th>
<th>Gpi</th>
<th>Pep</th>
<th>RG57 fingerprint*</th>
<th>Location</th>
<th>Year</th>
<th>Sample size</th>
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<td>US-1</td>
<td>A1</td>
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<td>100/100</td>
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<td>US-3</td>
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</table>

* Presence (1) or absence (0) of RG57 fingerprint bands 1–25 are indicated from left to right.

* Not known.

* In addition to the fingerprint bands listed, this genotype had a previously unreported band, 11a, located between bands 11 and 12.

Fig. 2. Cluster analysis of Dice coefficients among 18 multilocus genotypes of *Phytophthora infestans* identified in the United States (US) and Canada (CDA) during 1979–1991. The number of times each genotype occurred among the 120 isolates scored for all markers is indicated in parentheses. Genotypes that were closely related and thus probably the result of a common migration event are indicated by brackets.

the United States or Canada. The Gpi 100/100, Pep 100/100 dilocus allozyme genotype (characteristic of the US-6.4 and CDA-2 genotypes) does occur in Europe but not with the same DNA fingerprints as the US-6.4 and CDA-2 genotypes in Canada.

There was essentially no genetic variation among isolates collected from the same field. Each epidemic appeared to have been initiated by a single clonal lineage, and some clonal lineages were widely distributed. With this type of simple genetic structure, it is not necessary to sample high numbers of isolates from each field; however, it would be useful to know the geographical range of particular genotypes. Therefore, in future studies, the number of fields sampled should be increased, and there should be only several isolates (five or six should be sufficient) per field. The only within-field variants were genotypes that were part of the US-6 clonal lineage. These variants could have been generated during asexual (clonal) reproduction through mutation or mitotic recombination. Mutation is the most likely source of the US-6.4 genotype because it had gained a band, and this could not have occurred as a result of mitotic recombination. Interestingly, the band it gained (RG57 band 12) has been found previously only in a few isolates from central Mexico (10).

Although the A2 mating type was first detected in the United States and Canada in 1987 (3), it was not widely distributed as of 1991, and there was no evidence for sexual reproduction in any location. The A2 isolates in this study had the Gpi 86/100 genotype, which has not been reported in association with the A2 mating type in isolates outside Mexico, so they were most likely of recent Mexican origin. They could not have originated by a mating type change from one of the A1 isolates with the same allozyme genotype analyzed in this study, because the DNA fingerprints were very different.

On the basis of this analysis of 181 isolates collected during 1979–1991, it appears that *P. infestans* populations in the United States and Canada are very different from those in most parts of Europe and Mexico. This could change if there are additional migrations from Mexico or Europe. If the A2 mating type becomes established, sexual recombination could generate new genotypes. The data presented here provide a baseline for monitoring future genetic changes in populations of *P. infestans* in the United States and Canada.

LITERATURE CITED

genetics and isoenzyme patterns of Brazilian isolates of *Phytophthora infestans* (Mont.) de Bary. M.S. thesis. Universidade Federal de Viçosa, Viçosa, Brazil.


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