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Identity of the mtDNA haplotype(s) of *Phytophthora infestans* in historical specimens from the Irish Potato Famine

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The mtDNA haplotypes of the plant pathogen *Phytophthora infestans* present in dried potato and tomato leaves from herbarium specimens collected during the Irish potato famine and later in the 19th and early 20th century were identified. A 100 bp fragment of ribosomal DNA (rDNA) specific for *P. infestans* was amplified from 90% of the specimens ($n = 186$), confirming infection by *P. infestans*. Primers were designed that distinguish the extant mtDNA haplotypes. 86% percent of the herbarium specimens from historic epidemics were infected with the Ia mtDNA haplotype. Two mid-20th century potato leaves from Ecuador (1967) and Bolivia (1944) were infected with the Ib mtDNA haplotype of the pathogen. Both the Ia and IIb haplotypes were found in specimens collected in Nicaragua in the 1950s. The data suggest that the Ia haplotype of *P. infestans* was responsible for the historic epidemics during the 19th century in the UK, Europe, and the USA. The Ib mtDNA haplotype of the pathogen was dispersed later in the early 20th century from Bolivia and Ecuador. Multiple haplotypes were present outside Mexico in the 1940s–60s, indicating that pathogen diversity was greater than previously believed.

INTRODUCTION

Phytophthora infestans is a fungus-like, straminipilous pathogen and the causal agent of potato and tomato late blight, a devastating disease of potato and tomato worldwide (Fry & Goodwin 1997). The pathogen causes a destructive foliar blight and also infects potato tubers, tomato fruit, and a number of solanaceous hosts. The disease was first observed on potatoes in the USA in 1843 and was reported in areas around the ports of Philadelphia and New York (Stevens 1933, Peterson, Campbell & Griffith 1992). Potato blight subsequently spread to Canada, the northeastern United States and the Midwestern states by 1845 (Bourke 1993). Epidemics were observed in Belgium in June 1845 and spread to other regions in Europe that season. By mid-October 1845 the disease was observed in Britain and Ireland (Bourke 1993). Epidemics caused by *P. infestans* in the mid-19th century in Ireland led to what is known as the Irish potato famine. Approximately three million people died from starvation or were forced to migrate to other regions of the world.

Migration has played an important role in the spread of *P. infestans*. Three theories have been proposed to describe the migration of *P. infestans* to the USA and Europe and then to the rest of the world. It was proposed that the pathogen might have migrated in the mid-19th century from Mexico to the United States and then to Europe (Fry *et al.* 1993). A second theory suggests that the pathogen may have migrated to the United States and Europe from the Andean region near Peru where the disease was reported in the 19th century and earlier (Tooley, Therrien & Ritch 1989, Bourke 1993). A final theory suggests that the pathogen migrated from Mexico to Peru then to the USA and Europe (Andrivon 1996). Once introduced to Europe, *P. infestans* could have been distributed to rest the of world via international trade in seed potatoes (Fry *et al.* 1993). Another migration of *P. infestans* out of Mexico to Western Europe in the late 1970s has been well documented (Hohl & Iselin 1984, Cooke, Swan & Currie 1985, Shattock *et al.* 1990, Fry *et al.* 1992, 1993, Drenth, Turkensteen & Govers 1993, Drenth, Tas & Govers 1994). In Europe, it appears that the new populations displaced the old populations in a few years, as the new genotypes were probably more aggressive than old ones. A single genotype dominated extra-Mexican populations sampled from locations not affected by

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the 1970s migration (Goodwin, Cohen & Fry 1994b). To date, there is no evidence for any additional migrations between 1845 and the 1970s. This led to the theory that the Irish potato famine was caused by a single genotype of *P. infestans* of which US-1 is a direct clonal descendant (Goodwin *et al.* 1994b). It was further suggested that all isolates with the US-1 genotype were derived asexually from a common ancestor and that this genotype was the only genotype introduced to most locations.

While gene lineages of single copy nuclear genes can be useful for examining global phylogeographical structure in fungal populations (O'Donnell *et al.* 2000), mtDNA variation may be useful when studying migration events in *P. infestans* since it is believed to be non-recombining and reflects migration patterns of individuals (Carter *et al.* 1990). The mitochondrial genome in *P. infestans* is relatively small (38 kb) (Lang & Forget 1993, Whittaker, Assinder & Shaw 1994, Paquin *et al.* 1997). It is believed to be uniparentally inherited in *P. infestans* with no known segregation, elimination, or recombination (Whittaker *et al.* 1994) and thus can provide a useful marker for clonal progeny (Forster *et al.* 1988, Whittaker *et al.* 1994). Four mtDNA haplotypes (Ia, Ib, IIa, and IIb) have been designated in *P. infestans* using PCR approaches and RFLP analysis and the DNA sequence mutations that generate the polymorphisms have been identified (Carter *et al.* 1990, Griffith & Shaw 1998). The entire mitochondrial genome of the US-1 genotype of *P. infestans* has been sequenced and isolates with this genotype are the Ib mtDNA haplotype (Carter *et al.* 1990, Lang & Forget 1993, Paquin *et al.* 1997, Griffith & Shaw 1998, Gavino & Fry 2002). In addition, mtDNA has been used successfully in other ancient DNA studies since it is in higher copy numbers in cells than nuclear DNA (Bruns, Fogel & Taylor 1990, Fraile *et al.* 1997, Ristaino 1998, Poinar 1999).

An increasing number of interesting molecular studies have been conducted using preserved herbarium specimens (Bruns *et al.* 1990, Taylor & Swann 1994, Fraile *et al.* 1997). Herbarium specimens offer a valuable time capsule of genetic information on late blight epidemics of the past. Nineteenth and twentieth century mycologists collected and preserved potato and tomato leaves infected with the late blight pathogen in several herbarium collections around the world (Ristaino 1998, 2002, Ristaino, Groves & Parra 2001). We developed methods for analysis of nuclear and mitochondrial genes from plant pathogens preserved in dried herbarium materials (Trout *et al.* 1996, Ristaino *et al.* 2001). A 100 bp fragment from the internal transcribed spacer region 2 of the rDNA from *P. infestans* was amplified and sequenced from DNA extracted from historic Irish and British specimens collected between 1845 and 1847 (Ristaino *et al.* 2001). The Ib mtDNA haplotype was not the causal strain in late blight infections in lesions from a number of the oldest specimens obtained from the UK and the USA. Since this haplotype has been consistently associated with the US-1 genotype, our data challenged the accepted theory that the US-1 genotype was the 'old' clonal genotype that caused the epidemics of the mid-19th century (Goodwin *et al.* 1994b).

Here, we identify the mtDNA haplotype(s) of *P. infestans* responsible for historic late blight epidemics using mtDNA gene sequences from a large set of herbarium specimens from the UK, Europe, the USA, Central and South America. We examine initial migrations and colonization events and the possibility of additional migrations between the 1840s and 1970s that may have changed the population structure of the pathogen.

MATERIALS AND METHODS

Herbarium collections and specimens

Herbarium specimens were sampled from seven collections housed at the Royal Botanic Gardens Kew Mycological Herbarium (K(M)); the National Botanic Garden, Glasnevin, Dublin (DBN); the USDA National Fungus Collection, Beltsville, MD (BPI); CABI Bioscience collection, Egham (IMI); the Farlow Herbarium, Harvard University, Cambridge, MA (FH); the Museum of Evolutionary Botany, Uppsala University, Uppsala (UPS); and the National Archives of Scotland. 186 specimens were chosen for analysis based on the year collected, geographical region and host (Table 1). They represent material from six different regions of the world and date from 1845–1982. They include the oldest known specimens from late blight epidemics of potato that occurred in the USA, Ireland, the UK, and continental Europe.

DNA extractions

Small pieces (3–5 mm^2) of dried plant material were placed in sterile 1.5 ml tubes. DNA was extracted from all 186 specimens using a modified cetyltrimethylammonium bromide (CTAB) procedure (Ristaino *et al.* 2001) with the following modification: the first chloroform:isoamyl alcohol (24:1) was exchanged with a phenol:chloroform:isoamyl alcohol (25:24:1) step and each tube was centrifuged for 15 min (13 000 ***g***) at room temperature. The aqueous phase was removed to a new tube and the DNA was purified and concentrated using the QIAquick PCR purification kit (Qiagen, Valencia, CA). All DNA samples were then diluted to 1:100 for PCR reactions. All manipulations of DNA from modern isolates were performed in our laboratory in the Department of Plant Pathology, North Carolina State University (NCSU). All experimentation with the herbarium specimens was performed in the NCSU Phytotron Containment Facility Laboratory that was equipped with separate supplies, reagents and equipment and has no history of research involving *P. infestans*.

Table 1. Geographic region, year collected, number of specimens and number of positive PCR products obtained from nuclear ITS DNA and mtDNA genes.

			nDNA[b]	Mitochodrial genes[c]						
			Number of positive specimens							
										mtDNA
Geographic region[a]	Year collected	Number of specimens	ITS	*rpl*5		*cox*1		*nad*4		Haplotype (no. of specimens)
			PCR	PCR	Seq	PCR	Seq	PCR	Seq	
Central and South America	1889–1969	18	16	11	11	12	1	12	12	Ia, Ib, IIb (9), (2), (1)
North America	1855–1920	46	41	12	8	12	5	19	17	Ia (18)
Northern Europe	1866–1905	11	11	3	2	4	1	4	4	Ia (4)
Western Europe	1845–1982	52	47	12	8	9	8	18	12	Ia (15)
Eastern Europe	1892–1978	7	7	4	1	4	3	4	4	Ia (4)
UK and Ireland	1845–1974	52	46	9	7	9	5	13	11	Ia (11)
Total number		186	168	51	37	50	23	70	60	

[a] Geographic regions include: Central and South Amerca = Bolivia, Chile, Columbia, Costa Rica, Cuba, Ecuador, Guatemala, Nicaragua, Puerto Rico and Venezuela; North America = USA and Canada; Northern Europe = Denmark, Finland, Norway, Scandinavia and Sweden; Western Europe = Austria, Bavaria, Belgium, France, Germany, Italy, Malta, Netherlands and Switzerland; Eastern Europe = Czech Republic, Hungary, Latvia, Poland, Romania, Russia, Slovakia Ukraine and USSR.

[b] Primers PINF/HERB-1 were used to amplify a portion of internal transcribed spacer region 2.

[c] Primers P3F1/P3R1, P4F2/P4R3, P2F4/P2R4 were used to amplify portions of the *rpl*5, *cox*1 and *nad*4 genes in the P3, P4, and P2 region of the mitochondrial genome, respectively.

Table 2. PCR primers used to amplify nuclear and mitochondrial genes.

Target gene	Primer name	Primer sequence	Product size (bp)
IT Spacer 2	PINF	5′-CTCGCTACAATAGGAGGGTC-3′	100
	Herb 1	5′-CGGACCGCCTGCGAGTCC-3′	
*rpl*5	P3F1	5′-TTCAAAATGTCTTACAGTTTTTCG-3′	195
	P3R1	5′-GCAAGGTTATACTCTACCATTGAGC-3′	
	P3F2	5′-CAAATTCAAAATGTCTTACAGTT-3′	85
	P3R3	5′-GAATTGATACATCTATTGGAGGT-3′	
*cox*1	P4F2	5′-GGAATGCTGTAAGTAGTTTTGGTT-3′	188
	P4R3	5′-TTTAAGATCGTGGTATTAAATTAT-3′	
*nad*4	P2F4	5′-ACCAATTGTTGCGAAAACAG-3′	167
	P2R4	5′-ATTACGGCGGTTTAGCACAT-3′	
	P2F5	5′-CGTCAATAGGTTGTCCTAAAGC-3′	151
	P2R6	5′-GAACCTGATGTTGTTGGTGTTG-3′	

Identification of Phytophthora infestans *in herbarium specimens*

A 100 bp region of the internal transcribed spacer region 2 of the rDNA was amplified with primers Herb1/PINF to determine if the specimens were infected with *Phytophthora infestans* and if amplifiable DNA was present (Trout *et al*. 1996, Ristaino *et al*. 2001) (Table 2). PCR reactions were conducted in 50 μl reaction volumes consisting of 1 μl (approx. 100 μg) of template DNA [1:100 dilution of DNA in TE buffer (10 mM Tris HCl, 0.1 mM EDTA, pH 8)], 1× PCR Buffer (200 mM Tris-HCl, 500 mM KCl, pH 8.4), sterile distilled water, 0.08 mM dNTPs (Invitrogen Life Technologies, Carlsbad, CA), 0.4 mM $MgCl_2$ (Sigma Chemical, St Louis), 0.4 μM each of 10 μM primer and 2 units of Taq DNA polymerase (Invitrogen Life Technologies). Cycling parameters were initial denaturation at 94 °C for 2 min, followed by 35 cycles consisting of denaturation at 94 ° for 15 s, annealing at 55 ° for 15 s, and extension at 72 ° for 15 s. A final extension at 72 ° for 5 min followed. Appropriate positive controls were used including DNA from a modern isolate and total DNA extracted from an infected potato leaf. Negative controls included DNA from a healthy potato leaf, DNA from a healthy tomato leaf and a control without template DNA. In all manipulations control DNA was stored in a lab separate from the Phytotron lab where the herbarium specimens were manipulated using an aliquot of the same master mix reagents. Amplified products were visualized on 1.6 % agarose gels run in 1 × TBE (0.5 μg ml^{-1} of ethidium bromide). All PCR reactions were repeated twice.

PCR amplification of modern mtDNA

Three regions of the mitochondrial genome (P3, P4, and P2) that contain portions of the *rpl*5, *cox*1, and *nad*4 genes from three modern isolates of each of the known mtDNA haplotypes were amplified using primers F3/R3, F4/R4, and F2/R2 (Griffith & Shaw 1998). PCR amplifications of mtDNA were conducted in 30 μl reaction volumes consisting of 5 μl of template DNA, 1× PCR buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.4), sterile distilled water, 200 μM dNTP's (Invitrogen Life Technologies), 2.75 mM $MgCl_2$ (Invitrogen Life Technologies), 0.325 μM primer, 160 mg ml^{-1} Bovine Serum Albumin and 1 unit Taq DNA polymerase (Invitrogen Life Technologies). Positive and negative controls were used as described above. Cycling parameters were initial denaturation at 94 ° for 90 s, followed by 40 cycles consisting of denaturation at 94 ° for 40 s, annealing at 50 ° for 1 min, and extension at 72 ° for 90 s. A final extension at 72 ° for 10 min followed. Amplified products were visualized by gel electrophoresis as described above. All PCR reactions were repeated twice.

Primer design and PCR amplification of mtDNA from herbarium specimens

Additional primers were designed using Primer3 Software and NetPrimer (Premier Biosoft International, Palo Alto, CA) around polymorphic sites within each of three regions that would allow us to determine the haplotype of *Phytophthora infestans* present in the lesions. Primer pair P3F1/P3R1 amplifies a 195 bp region of DNA near the 3′ end of *rpl5* gene in the P3 region of the mitochondrial genome. The type I haplotypes possess an *Eco*RI site in this region that is absent in the type II haplotypes. The primer pair P3F2/P3R3 amplifies a smaller fragment (85 bp) that is nested within the *rpl5* site. The primer pair P4F2/P4R3 amplifies a 188 bp region near the 3′ end of the *cox*1 gene in the P4 region. These primers also amplify an *Eco*RI site that is present in type I haplotypes but absent in type II haplotypes. Use of these two sets of primers allowed separation of type I from type II haplotypes. The primers P2F4/P2R4 amplify a variable region of 167 bp near the 3′ end of the *nad*4 gene in the P2 region of the mitochondrial genome (Griffith & Shaw 1998, Ristaino *et al*. 2001). Modern isolates of the Ib mtDNA haplotype contain an *Msp*I restriction site in this region not present in the other three modern haplotypes. Use of this primer pair in conjunction with the P3 or P4 primer sets allowed separation of the Ia from Ib haplotypes. The primers P2F5/P2R6 were used to determine the specific haplotype of the pathogen in lesions that were previously identified as type II haplotypes. These primers amplify a polymorphic site in the 5′ end of the P2 region that contains an *Msp*I site that is present in IIa haplotypes but absent in IIb haplotypes. Primers sequences are shown in Table 2. All PCR reactions were conducted as described in the previous section and were repeated twice. Positive and negative controls were used as described above. The initial first round PCR products were used as template for a second round of PCR. Amplified products were visualized by gel electrophoresis as described above.

The 85 bp region of DNA amplified with primer pairs P3F2/P3R3 was digested with *Eco*RI to further confirm the type I haplotype. For restriction digest, 2 μl of a master mix consisting of 8 μl H_2O, 1 unit of restriction enzyme, and 1 μl of buffer (REact 3 buffer, Gibco BRL, 50 mM Tris-HCL, 10 mM $MgCl_2$, 100 mM NaCl, pH 8) was added to 10 μl of PCR product. The amplified DNA was digested at 37 ° for 3 h, and the enzyme was deactivated at 65 ° for 10 min. The digested DNA was then visualized by gel electrophoresis as described above.

Sequence analysis

The PCR products from the two separate PCR reactions were pooled and the products were cleaned using the QIAquick PCR Purification kit (Qiagen, Valencia, CA). The ABI Prism® BigDye Primer Cycle Sequencing kit (Applied Biosystems, Foster City, CA) was used for sequencing reactions. The DyeEx 96 kit was used for dye-terminator removal from the sequencing reactions (Qiagen). The ABI PRISM® 3700 DNA Analyzer (Applied Biosystems) was used for sequencing analysis of the amplified DNA. Computer analysis of sequence data was performed using BioEdit v5.0.9 (Tom Hall, Microbiology Department, North Carolina State University) and Vector NTI Suite 7.0 (Informax, Bethesda, MD).

RESULTS

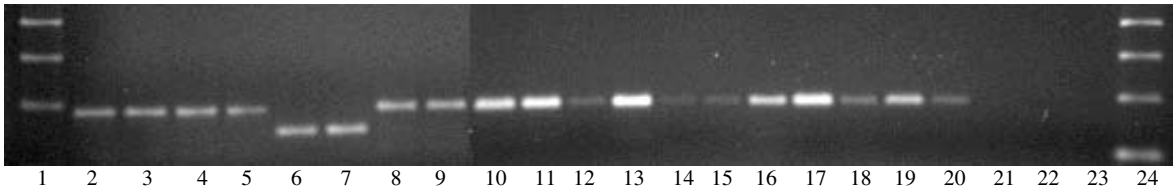
PINF and HERB-1 amplification

A 100 bp product was amplified using the PINF and HERB-1 primers from 168 of 186 herbarium specimens (Table 1). Thus, greater than 90% of the specimens were confirmed to be infected with *Phytophthora infestans*. Our success rate for amplification of rDNA from specimens with these primers was higher than in our previous report (from 31–90%) (Ristaino *et al*. 2001). The success rate of DNA amplification varied among herbarium collections.

mtDNA haplotype determination

Presence of the *Eco*RI restriction site indicated a type I haplotype (Fig. 1A). All of the specimens from which the target mtDNA genes were amplified were infected with the Type I haplotype of the pathogen except for a single specimen from Nicaragua (1956) which was infected by a type II haplotype of the pathogen (Fig. 1B, Table 3). Further PCR amplification with primer pair

(A)



(B)

Isolate	Haplotype	EcoR1
93-3	Ia	TTACAGTTTTTCGAATTAAAAACAG***AATTC***TTAAAATTTAAAGATATACCTCCAATAGA
188.1.1	Ib	TTACAGTTTTTCGAATTAAAAACAG***AATTC***TTAAAATTTAAAGATATACCTCCAATAGA
18-84	IIa	TTACAGTTTTTCGAATTAAAAGCAG*AATTT*TTAAAATTTAAAGATATACCTCCAATAGA
94-52	IIb	TTACAGTTTTTCGAATTAAAAGCAG*AATTT*TTAAAATTTAAAGATATACCTCCAATAGA

Herbarium Specimens			
Region	Number	Date	
France	K72	1845	TTACAGTTTTTCGAATTAAAAACAG***AATTC***TTAAAATTTAAAGATATACCTCCAATAGA
Germany	FH219	1873	TTACAGTTTTTCGAATTAAAAACAG***AATTC***TTAAAATTTAAAGATATACCTCCAATAGA
UK	K22	1875	TTACAGTTTTTCGAATTAAAAACAG***AATTC***TTAAAATTTAAAGATATACCTCCAATAGA
England	DBN11	1878	TTACAGTTTTTNGAATTAAAAACAG***AATTC***TTNAAATTTNAAGATATACCTCCNATAGA
US	BPI186680	1882	TTACAGTTTTTCGAATTAAAAACAG***AATTC***TTAAAATTTAAAGATATACCTCCAATAGA
Sweden	UPS2	1882	TTACAGTTTTTCGAATTAAAAACAG***AATTC***TTAAAATTTAAAGATATACCTCCAATAGA
US	BPI186896	1885	TTNCAGTTTTTCGAATTAAAAACAG***AATTC***TTAAAATTTAAAGATATACCTCCAATAGA
Chile	K79	1889	TTACAGTTTTTNGAATTAAAAACAG***AATTC***TTAAAATTTAAAGATATACCTCCAATAGA
USSR	FH227	1909	TTACAGTTTTTNGAATTAAAAACAG***AATTC***TTAAAATTTAAAGATATACCTCCAATAGA
Nicaragua	BPI186953	1956	TTACAGTTTTTCGAATTAAAAGCAG*AATTT*TTAAAATTTAAAGATATACCTCCAATAGA

Fig. 1. (A) Amplified DNA from the *rpl5* gene in the P3 region (P3F1/P3R1) of mtDNA from modern and a subset of herbarium specimens infected with *Phytophthora infestans*. Lanes 1 and 24 are 100 bp ladder; lanes 2–5 are undigested product from modern isolates of the Ia, Ib, IIa, and IIb haplotypes, respectively; lanes 6–9 are *Eco*RI digested product from modern isolates of the Ia, Ib, IIa, and IIb haplotypes, respectively; lanes 10–18 are undigested PCR product from herbarium specimens; lanes 19–23 are the controls: positive controls of DNA from mycelium of *P. infestans* (+M) and dried infected modern lesions (+L), and negative controls of DNA from noninfected potato (−PL) and tomato leaf (−TL) and template-free control (−NT). (B) mtDNA sequences around the *Eco*RI restriction site in P3 region (present in the type I haplotypes and absent in the type II haplotypes) from 4 modern and 10 herbarium specimens. Note only the specimen from Nicaragua was type II.

P2F5/P2R6, sequence analysis, and restriction digest of DNA from this specimen indicated that the lesion was caused by the IIb haplotype (Table 3). A PCR product was obtained for 70 herbarium specimens using the P3F2/P3R3 primers and *Eco*RI digestion confirmed that the specimens were infected with the type I haplotype.

PCR and sequence analysis of the *nad4* gene (P2F4/P2R4) was used to determine whether the Ib mtDNA haplotype was present in specimens previously determined to be infected with a type I haplotype (Fig. 2A). Sequence analysis of the type I haplotypes indicated that only two specimens out of 60, one from Ecuador (1967) and one from Bolivia (1944), were infected with the Ib haplotype of *P. infestans*, but the remaining specimens were infected with the Ia haplotype (Fig. 2B, Table 3). The specimen label from the sample collected in Ecuador in 1967 indicates that the pathogen was intercepted in Miami, FL (Fig. 3). The Ia, Ib and IIb mtDNA haplotypes were found in specimens collected in central and South America (Tables 1 and 3).

The Ib haplotype was not present in any of the oldest 19th and 20th century specimens tested from Western Europe, Eastern Europe, Northern Europe, the UK, and the USA and was not common among the more modern Central and South American specimens (Table 3).

DISCUSSION

Sequence data from the herbarium specimens indicate that the Ia haplotype was the predominant haplotype present during historical late blight epidemics. The oldest specimens infected with *Phytophthora infestans* from the 1840s in England and France were infected with the Ia haplotype (Table 3). Specimens from a wide geographical area were infected with the Ia mtDNA haplotype of *P. infestans* (Table 3). The Ia mtDNA haplotype of *P. infestans* was therefore responsible for the epidemics of the Irish potato famine.

It is interesting that the Ib mtDNA haplotype of the pathogen was only identified in two South American

Table 3. Identity of the mitochondrial DNA haplotype(s) in the oldest herbarium specimens known to be infected with *Phytophthora infestans* from the UK, Ireland, Europe, Central and South America.

Herbarium and specimen no.[a]	Year	Collector	Country	PCR products obtained				
				nDNA[b]	mtDNA[c]			
				ITS	*rpl5*	*cox*1	*nad*4	mtDNA Haplotype
K 72	1845	*M. Desmazieres*	France	+	+	+	+	Ia
FH 302	1846	*M. Desmazieres*	France	+	+	+	+	Ia
K 6	1847	*C. L. Broome*	England	+	+	+	+	Ia
K 116	1851	*J. B. Ellis*	US, NY	+	−	+	+	Ia
K 89	1855	*R. Caspray*	Germany	+	+	+	+	Ia
K 83	1866	*P. A. Karsten*	Finland	+	+	+	+	Ia
BPI US0186683	1874	*P. A. Saccardo*	Italy	+	+	−	+	Ia
K 22	1875	*J. E. Vize*	England	+	+	+	+	Ia
UPS 1	1876	*E. Rostrup*	Denmark	+	−	+	+	Ia
DBN 5	1877	*C. E. J. Oudemans*	Netherlands	+	+	−	+	Ia
FH 222	1877	*P. Magnus*	Germany	+	+	−	+	Ia
FH 224	1878	*S. Gartner*	Switzerland	+	+	+	+	Ia
DBN 11	1878		England	+	+	+	+	Ia
K 42	1879	*M. J. Berkeley*	England	+	+	+	+	Ia
K 44	1879	*M. J. Berkeley*	England	+	+	+	+	Ia
BPI US0186680	1880	*W. Trelease*	US, WI	+	+	−	+	Ia
DBN 13[d]	1882	*Thompson*	Ireland	+	+	+	+	Ia
UPS 2	1882	*J. Eriksson*	Sweden	+	+	+	+	Ia
FH 263	1882	*Lindhardt*	Hungary	+	+	+	+	Ia
K 31	1884	*W. B. Grove*	England	+	−	+	+	Ia
K 33	1886	*J. E. Vize*	England	+	−	+	+	Ia
BPI US0186896	1886	*W. R. Dudley*	US, NY	+	+	+	+	Ia
BPI US0186899	1889	*W. R. Dudley*	US, NY	+	+	+	+	Ia
BPI US0186668	1895	*F. L. Scribner*	US, TN	+	+	+	+	Ia
FH 232	1897	*S. Rostowzew*	USSR	+	+	+	+	Ia
K 79	1889	*P. Hennings*	Chile	+	+	+	+	Ia
BPI US0186968	1913	*J. M. Vargas Vergara*	Colombia	+	+	+	+	Ia
FH 292	1929	*W. H. Weston*	Colombia	+	+	+	+	Ia
BPI US0186816[d]	1941	*A. S. Muller*	Guatemala	+	−	+	+	Ia
BPI US0186943	1942	*A. S. Muller*	Guatemala	+	+	+	+	Ia
BPI US0186832[d]	1942	*J. A. Stevenson*	Guatemala	+	+	+	+	Ia
BPI US0187022	1942	*J. A. Stevenson*	Costa Rica	+	+	+	+	Ia
BPI US0186941	1944	*M. Cardenas*	Bolivia	+	+	+	+	Ib
BPI US0186817[d]	1947	*J. A. Stevenson*	Costa Rica	+	+	+	+	Ia
BPI US0186956	1954	*M. O'Brien*	Nicaragua	+	+	+	+	Ia
BPI US0186953	1956	*J. A. Stevenson*	Nicaragua	+	+	+	+	IIb
BPI US0186908	1967	*F. D. Matthews*	Ecuador	+	+	+	+	Ib

[a] 186 specimens were sampled from the collections housed at the Royal Botanic Gardens Kew Mycological Herbarium (K); the National Botanic Garden, Glasnevin, Dublin (DBN); the USDA National Fungus Collection, Beltsville, MD (BPI); CABI Bioscience, Egham (IMI); the Farlow Herbarium, Harvard University, Cambridge, MA (FH); the Museum of Evolutionary Botany, Uppsala University, Uppsala (UPS); and the National Archives of Scotland, Edinburgh (NAS). Data shown are from the oldest samples from which the haplotype was identified. A supplementary table of the complete data can be found at http://www.cals.ncsu.edu/plantpath/faculty/ristaino/index.html).

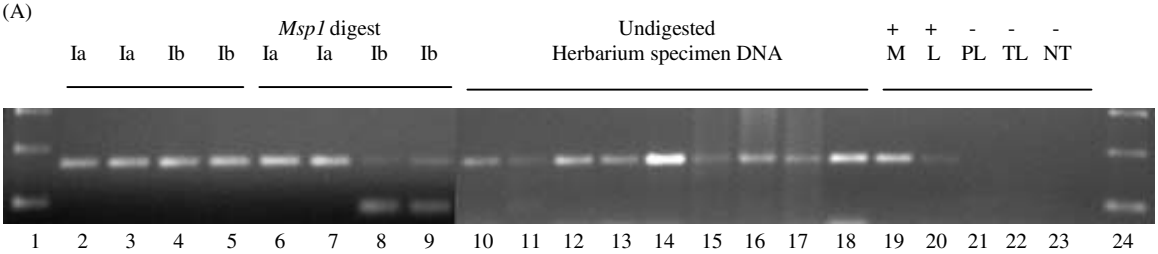
[b,c] See Table 1.

[d] Samples DBN 13, BPI US0186816 and BPI US0186817 were sampled from *Lycopersicon esculentum* and BPI US0186832 was sampled from *Petunia hybrida*.

Note: GenBank accession nos AY323419–AY323452.

specimens from Ecuador and Bolivia in the mid-20th century. A consistent association has been observed between the Ib haplotype and the US-1 genotype and its derivatives (Carter *et al*. 1990, Gavino & Fry 2002). As it is clear that the Ib mtDNA haplotype was not the predominant haplotype during the 19th century in the US and Europe as suggested by others (Goodwin *et al*. 1994b, Fry & Goodwin 1997), it is not likely that the US-1 genotype caused the earliest epidemics during the 1840s. However, the populations of modern day isolates collected between the 1950s and the 1970s in Europe and before 1980 in other regions of the world, including the USA, were dominated by the Ib haplotype (Goodwin *et al*. 1994b). This would suggest that another migration of the Ib mtDNA haplotype might have occurred from Bolivia, Ecuador or Peru, displacing the original Ia mtDNA haplotype in many areas of the world (Forbes *et al*. 1997, Perez *et al*. 2001). This could have occurred with the movement of potato germplasm in the first half of the 20th century. Shipment of infected potatoes through ports of entry is likely to be the route by which the Ib entered the

(A)



(B)

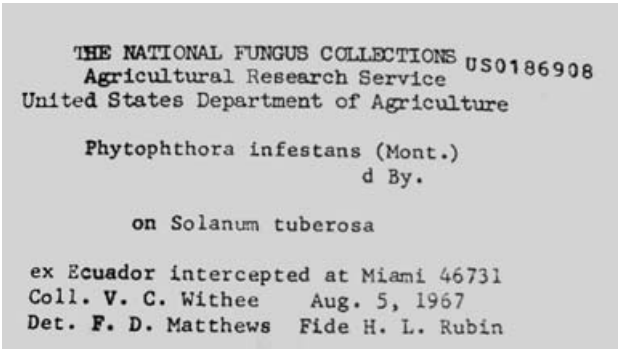
Modern Isolates

Isolate	Haplotype	*Msp*1
93-3	Ia	CTCCAACAAAACTACTTGAA*CCTG*GAATAGACATATTTGCTAATACATAAATAAATAATGCAAT
188.1.1	Ib	NTCCAACAAANCTACTTGAA***CCCG***GAATAGACATATTTGCGTAAGCATAAATAAATAATGCAAT
18-94	IIa	CTCCAACAAAACTACTTGAA*CCTG*GAATAGACATATTTGCTAATACATAAATAAATAATGCAAT
94-52	Ib	CTCCAACAAAACTACTTGAA*CCTG*GAATAGACATATTTGCTAATACATAAATAAATAATGCAAT

Herbarium Specimens

Region	Number	Date	
UK	K6	1847	CTCCAACAAAANTNCTTNAA*CCTG*GAATAGACATATTTGCTAATACATAAATAAATAATGCAAT
US	K116	1851	CTCCAACAAAACTACTTGAA*CCTG*GAATAGACCTGTTNGCTANNNCATAAATAAATAATGCANN
Germany	K136	1863	CTCCAACAAAACTACTTGAA*CCTG*GAATAGACATATTTGCTAATACATAAATAAATAATGCAAT
Germany	H219	1873	CTCCAACAAANCTACTTGAA*CCTG*GAATAGACATATTTGCTAATACATAAATAAATAATGCAAT
UK	K22	1875	CTCCAACAAAACTACTTGAA*CCTG*GAATAGACATATTTGCTAATACATAAATAAATAATGCAAT
US	BPI186680	1882	CTCCAACAAAACTACTTGAA*CCTG*GAATAGACATATTTGCTAATACATAAATAAATAATGCAAT
Sweden	UPS2	1882	CTCCAACAAAACTACTTGAA*CCTG*GAATAGACATATTTGCTAATACATAAATAAATAATGCAAT
Hungary	H263	1882	CTCCAACAAAACTACTTGAA*CCTG*GAATAGACATATTTGCTANTNCATAAATAAA---------
Chile	K72	1889	CTCCAACAAAACTACTTGAA*CCTG*GAATAGACATATTTGCTANTNCATANNTAAATNATGNNNT
Bolivia	BPI186941	1944	CTCCANCANAACTACTTGAA***CCCG***GAATAGACATATTNGNTAATACATAAATAAATAATGCANT
Ecuador	BPI186908	1967	CTCCAACAAAACTACTTGAA***CCCG***GAATAGACATATTTGCTAATACATAAATAAATAATGCAAT

Fig. 2. (A) Amplified DNA from the *nad4* gene in the P2 region (P2F4/P2R4) of mtDNA from modern and a subset of the herbarium specimens infected with *Phytophthora infestans*. Lanes 1 and 24 are 100 bp ladder; lanes 2–5 are undigested product from two modern isolates each of the Ia and Ib haplotypes, respectively; lanes 6–9 are *Msp*I digested product from modern isolates of the Ia and Ib, respectively; lanes 10–18 are undigested PCR product from herbarium specimens; lanes 19–23 are the controls: positive controls of DNA from mycelium of *P. infestans* (+M) and dried infected modern lesion (+L), and negative controls are DNA from noninfected potato (–PL) and tomato leaf (–TL) and template-free control (–NT). (B) mtDNA sequences around the *Msp*I restriction site in P2 region (present in the Ib haplotype and absent in other haplotypes) from 4 modern and 11 herbarium specimens. Note only the specimens from Ecuador and Bolivia were Ib haplotypes.


THE NATIONAL FUNGUS COLLECTIONS US0186908
Agricultural Research Service
United States Department of Agriculture

Phytophthora infestans (Mont.)
d By.

on Solanum tuberosa

ex Ecuador intercepted at Miami 46731
Coll. V. C. Withee Aug. 5, 1967
Det. F. D. Matthews Fide H. L. Rubin

Fig. 3. Herbarium specimen label from the USDA National Fungus Collection (BPI US0186908). The sample collected in Ecuador in 1967 was imported into the US *via* Miami and was identified as the Ib haplotype.

USA. The Ib mtDNA haplotype has not been found in Mexico, so this country is not the likely source of the early 20th century migrations of the Ib mtDNA haplotype (Gavino & Fry 2002). The Ib mtDNA haplotype probably evolved from the Ia mtDNA haplotype in the Andean region of South America and was subsequently dispersed to the US and Europe and then to the rest of the world in the 20th century. Wild *Solanum* species in the Andean region in Ecuador have recently been documented as hosts for novel mtDNA haplotypes of *P. infestans*, and Ecuador is a center of mtDNA diversity for the pathogen (Olivia *et al*. 2002, Adler *et al*. 2004). Further studies of Andean populations of the pathogen should lead to a clearer understanding of both the source of 19th century epidemics and the center of origin of *P. infestans*.

A single specimen collected in 1956 from Nicaragua was infected with the IIb mtDNA haplotype of the pathogen. This was surprising since the US-6 genotype, which is the only modern genotype presently known to possess the IIb mtDNA haplotype, had only previously been reported in northwestern Mexico, parts of North America and Canada (Goodwin *et al*. 1994a, Gavino & Fry 2002). It is possible that this genotype may have entered Nicaragua in the 1950s from movement of Mexican tomatoes or potatoes. The Ia mtDNA haplotype was also present in Nicaragua in 1954 (Table 3). Studies of present-day populations have shown that Ia mtDNA haplotypes are associated with a diverse range of phenotypes, including both A1 and A2 mating types, and are observed in many locations (Carter *et al*.

1990, Griffith & Shaw 1998, Gavino & Fry 2002). Our data indicate that multiple haplotypes of the pathogen were present outside of Mexico in the 1940s–60s and that the pathogen population outside Mexico may have been more diverse than previously believed (Goodwin *et al*. 1994b). This opens up the possibility that both mating types may have existed in other countries outside Mexico prior to the discovery there of the A2 mating type in the 1958 by Gallegly & Galindo (1958).

Our data does not rule out Mexico as a possible source of inoculum for 19th century potato late blight epidemics since the Ia mtDNA haplotype is quite common there in present day populations (Gavino & Fry 2002). However cultivated potatoes were not grown in Mexico in the 1840s, so movement of the pathogen in wild *Solanum* species has been speculated (Goodwin *et al*. 1994b). The Ia mtDNA haplotype is also present in the Andean region of South America (Perez *et al*. 2001). Peru and other Andean countries exported guano for fertilizer and potato seed on steamships to the USA and Europe in the 1840s, so a South American source of inoculum for 19th century potato late blight epidemics is a more likely scenario (Bourke 1993). We are currently analyzing modern and historic samples using both nuclear and mitochondrial gene genealogies to address this question.

Historic collections can be valuable to epidemiologists and population geneticists who study plant diseases. Many important plant disease herbaria and natural history collections have recently been critically under-funded and are in jeopardy of being lost. Misleading data can be derived from studies that examine only extant phylogeographic patterns of genotypes of plant pathogens (Goodwin *et al*. 1994b). Our work demonstrates the importance of herbarium collections for clarifying important questions regarding the population biology of this devastating plant pathogen.

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