DNA Sequence Analysis of the Late-Blight Pathogen Gives Clues to the World-wide Migration.

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Keywords: *Phytophthora infestans*, oomycete, phytopathology, population genetics

Abstract

Previous work with modern isolates of *Phytophthora infestans* had shown that a single clonal lineage (US-1), characterized by mtDNA haplotype Ib, was present across the globe in the 1970s leading to the hypothesis that one strain of the pathogen had spread from a single source, probably Mexico where it was known to be more variable. But mitochondrial DNA sequences from archival materials have identified the Ia mtDNA haplotype of *P. infestans* in 19th century samples from Europe, the US and Ireland and not the Ib haplotype. Multilocus sequence data from nuclear and mitochondrial loci support an Andean origin of *P. infestans* and also suggest that the source for the potato famine epidemics in Ireland was the Andean region rather than the Mexican highlands. Closely related, novel species including *Phytophthora andina*, have evolved in the Andean region of South America and share a common ancestor with *P. infestans*. More recently, we studied historic samples *of P. infestans* from Asia. DNA was extracted from 41 archival samples from 5 herbarium collections. Early samples from Asia, Russia and Australia were all the Ia mtDNA haplotype (China, 1938, 1940; Japan, 1901, 1930, 1931; India, 1913; Peninsular Malaysia, 1950; Nepal, 1954, The Philippines 1910; Australia, 1917; Russia, 1917; Latvia, 1935; and the Ukraine). In contrast, only later samples included the Ib mtDNA haplotype (China, 1952, 1954, 1956, 1982; India, 1968, 1974; Thailand, 1981). Modern Chinese populations of *P. infestans* contain all four mitochondrial haplotypes suggesting more recent origins of the IIa and IIb mtDNA haplotypes. All the samples analyzed for mitochondrial haplotypes so far indicate that colonization of North America, Europe and Asia was first, by type Ia and subsequently by type Ib and that this haplotype migrated from the Andes rather than from Mexico.

INTRODUCTION

Phytophthora infestans is considered the most important biotic constraint to production of potatoes and is a major threat to food security (http://gilb.cip.cgiar.org/). Worldwide losses exceed 5 billion dollars annually (Anonymous, 1996). *P. infestans* is a major problem in developing countries where fungicide use is less common on potato and certified seed programs are not well established. Potatoes are one of the four major global food crops in addition to wheat, rice and corn. The United Nations named 2008 as the "International Year of the Potato" (www.potato2008.org). The US now ranks 5th in production and China ranks first. Late blight is considered an emerging infectious disease of potato (Anderson et al., 2004). Emerging infectious diseases are caused by pathogens that: 1) have increased in incidence, geography or host range; 2) have changed pathogenesis; 3) have newly evolved; or 4) have been discovered or newly recognized. Evolution of fungicide resistant strains and novel pathotypes of *P. infestans* continue to challenge the sustainable production of potatoes. The development of rapid detection and diagnostic assays for the late blight pathogen coupled with population genetic studies using statistical analysis and gene genealogies can help track migrations of plant pathogens, determine centers of origin of novel strains, and enable studies of gene flow between regions (Price and Carbone, 2005; Gomez et al., 2007). Understanding both historical and current global population

structures of *P. infestans* is important to improve management of this disease and safeguard world potato supplies.

POPULATION BIOLOGY

P. infestans belongs to the oomycetes, a group of organisms that is more closely related to brown algae than to true fungi (Gunderson et al., 1987). Phylogenetic analysis of species in the genus *Phytophthora* indicates that *P. infestans* is a member of the 1c clade (Cooke et al., 2000). This clade includes other species such as *P. ipomoeae*, *P. mirabilis*, *P. andina* and *P. phaseoli* (Galindo and Hohl, 1985; Cooke et al., 2000; Flier et al., 2002; Gomez et al., 2008).

P. infestans reproduces predominately by asexual means and forms sporangia on infected host tissue that either germinate directly to form infection hyphae or release zoospores that are responsible for infections. Sporangia can be dispersed by wind and rain at local and national scales. The pathogen typically survives from season to season as mycelium in infected potato tubers, volunteer potato plants or infected potato cull piles that contribute to epidemic development on subsequent potato crops. The pathogen can form sexual oospores via outcrossing of two mating types called A1 and A2 (Judelson, 2007). The oospore is a resistant structure that can survive for many years in soil or plant debris. Oospores were first described in Mexico in 1958 (Gallegly and Galindo, 1958).

Phenotypic markers including mating type, specific virulence phenotypes on plants and fungicide sensitivity have been used to characterize populations of *P. infestans* (Fry et al., 1993). However, since phenotypic markers are under selection pressure and are thus not neutral markers, their use for analysis of migration of pathogen populations is limited. Allozyme alleles at glucose-6-phosphate isomerase (Gpi-1) and peptidase (Pep-1) loci have been useful co-dominant genetic markers for characterizing *P. infestans* populations (Tooley et al., 1989; Fry et al., 1993). A multilocus DNA probe (RG57) has been used extensively for identifying clonal genotypes of *P. infestans* (Goodwin et al., 1992; Goodwin et al., 1994a). The most common genotype on potato in the US from 1994 to 1996 was US-8 (Goodwin and Drenth, 1997; Goodwin et al., 1998; Frasier et al., 1999). Populations in the US are considered to be mostly asexual although nineteen clonal genotypes of the pathogen have been identified in US regional populations and there is some evidence of sexual recombination in some local populations (Goodwin et al., 1994a; Goodwin et al., 1998; Gavino et al., 2000; Wangsomboondee et al., 2002). In contrast, in Europe, where some populations are known to be reproducing sexually, first RFLP markers and subsequently microsatellite markers have been used to document predominant genotypes within countries (Knapova and Gisi, 2002; Lees et al., 2006; Cooke, et al., 2006; Cooke et al., 2007). Over 170 genotypes have been observed in the Netherlands where the pathogen population is undergoing sexual reproduction (Drenth et al., 1994; Zwankhuizen et al., 1998, Zwankhuizen et al., 2000). If sexual reproduction in US populations of the pathogen becomes more common, this could have devastating effects for potato growers since pathogen strains will be more variable and potentially more difficult to control. There are clearly different genotypes on tomato and potato (Wangsomboondee et al., 2002). We have preliminary evidence of host specific adaptation to tomato (Ristaino and Ivors, unpublished).

There are four known mtDNA haplotypes (Ia, Ib, IIa, IIb) of *P. infestans* (Carter et al., 1990; Griffith and Shaw, 1998; Lang and Forget, 1993). It was previously believed that worldwide populations of *P. infestans* prior to the 1980s originated in Mexico, were the A1 mating type, asexual and derived from a single clonal lineage called the US-1 genotype or Ib mitochondrial DNA (mtDNA) haplotype (Goodwin et al., 1994b, Fry, 2008). However, much of these data were based on studies of modern populations of the pathogen that occurred after 1950 and little was actually known about the original strain(s) of the pathogen that existed in the 19th century.

There have clearly been multiple migrations of the pathogen out of Mexico in the latter half of the 20th century and sexual reproduction and the occurrence of diverse populations have been well documented there (Fry, 2008). In the mid 1970s new populations, comprising both the A1 and A2 mating types, were introduced independently into Europe and the US, probably from Mexico, and displaced the initial A1 lineage (Fry et al., 1992; Goodwin et al., 1994b; Fry, 2008). Oospores now play a role in the disease cycle in some areas of Europe and Canada (Drenth et al., 1994;

Gavino et al., 2000). Understanding variation in pathogen populations of *P. infestans* is clearly a significant factor in deploying effective and durable control strategies. *P. infestans* populations continue to change and late blight management remains a significant challenge to the potato and tomato industry.

MITOCHONDRIAL HAPLOTYPES IN *P. INFESTANS* FROM 19th CENTURY EPIDEMICS

The first records of late-blight disease of potato in the US were from Philadelphia and New York in 1843. The disease was then noted in Belgium in 1845 from where it spread rapidly across a large part of Europe. In Ireland it caused widespread crop failure and the subsequent, widely reported, economic and socio-political impacts (Bourke, 1964; Berkeley, 1846). The early populations are considered to have been largely clonal.

Nineteenth and early twentieth century scientists collected and preserved potato and tomato leaves infected with *P. infestans* and specimens exist from the Irish potato famine (Fig. 1). We used these historical specimens to answer questions about the epidemiology, evolution and population biology of the pathogen (Ristaino, 1998; Ristaino et al., 2001; Ristaino, 2002; May and Ristaino, 2004). Historical collections of late blight infected potatoes were obtained from several locations including the Mycological Herbarium of the Royal Botanic Gardens, Kew, England (Ristaino, 1998; Ristaino et al., 2001; Ristaino, 2002; May and Ristaino, 2004). Disputes about nomenclature, phylogenetics, function and evolution of genes, and origins of populations can be addressed with herbarium specimens. Genomes of specimens are preserved in herbarium collections making them a valuable resource for sampling populations over time when coupled with the use of molecular techniques (Ristaino, 2006).

We used the polymerase chain reaction (PCR) to amplify minute amounts of DNA from *P. infestans* infected leaves from historic epidemics. We identified the mtDNA haplotype(s) present in specimens collected during the Irish potato famine and later in the 19th and early 20th century (Ristaino et al., 2001; May and Ristaino, 2004). First, a 100 bp fragment of DNA from internal transcribed spacer region 2 specific for *P. infestans* was amplified from 90% of the samples tested, confirming infection by *P. infestans* (Trout et al., 1997; Ristaino et al., 1998; Ristaino et al., 2001). Then primers were designed that amplify short segments of mtDNA around variable restriction sites that separate mtDNA haplotypes and the DNA was sequenced. Surprisingly, 86% of the leaf lesions from historic epidemics were infected with the Ia mtDNA haplotype of *P. infestans* (May and Ristaino, 2004).

Both the Ia and IIb haplotypes were found in specimens collected in 1954 and 1956 in Nicaragua (Table 1). Our data challenge the hypothesis that a single clonal lineage of *P. infestans* existed outside of Mexico in the 19th century since 2 haplotypes were present in samples from Nicaragua (Table 1). Thus, pathogen diversity was greater than previously believed (Goodwin et al., 1994b). These data also open the possibility that both mating types were present outside of Mexico before the first reports of oospores in 1958 by Gallegy and Galindo (1958), since the Ia haplotype can be either an A1 or A2 mating type. Evidence of the presence of infectious oospores and thus both mating types in Minnesota in the 1940s can be found in a Master's thesis (Kotilla, 1946).

We have evidence from two samples of blight (Bolivia, 1944 and Ecuador, 1967) that haplotype Ib was present in the Andean region in the mid 20th century (Table 1). A sample of blight from 1967, originating in Ecuador but intercepted in Miami in the US, was the Ib haplotype (Fig. 1C). We therefore conclude that Ib was dispersed from the Andean region later in the 20th century whereas earlier introductions, back to the mid 19th century, to US and Europe, no doubt on breeding material, were probably of the Ia haplotype.

GLOBAL MIGRATIONS AND EVOLUTIONARY HISTORY

We have sequenced the mitochondrial genomes of three haplotypes of *P. infestans* (Avila-Adame et al., 2006). Phylogenetic and coalescent analysis revealed that although the type I and II haplotypes shared a common ancestor, they clearly formed two lineages that evolved

independently. Type II haplotypes diverged earlier than the type I haplotypes. Our data do not support a previous hypothesis that the type II lineages evolved from type I lineages (Gavino and Fry, 2002). Our data support the hypothesis that all the extant mitochondrial lineages of *P. infestans* evolved from a common ancestor in South America (Gomez, 2007).

We assessed the genealogical history of *P. infestans* using sequences from portions of two nuclear genes (β-*tubulin* and *Ras*) and several mitochondrial loci P3, (*rpl*14, *rpl*5, tRNA) and P4 *(Cox1*) from 94 modern isolates from South America, Central America, North America, and Ireland (Gomez et al., 2007). Summary statistics, migration analyses and the genealogy of populations of *P. infestans* for both nuclear and mitochondrial loci are consistent with an "out of South America" origin for *P. infestans*. rather than an "out of Mexico" origin. Mexican populations of *P. infestans* from the putative center of origin in Toluca Mexico harbored less nucleotide and haplotype diversity than Andean populations. Coalescent-based genealogies of mitochondrial loci were congruent and demonstrate the existence of two lineages leading to present day haplotypes of *P. infestans* on potatoes (Fig. 2). Mitochondrial haplotypes found in Toluca, Mexico were from the type I haplotype lineage, whereas those from Peru and Ecuador were derived from both type I and type II lineages. Haplotypes found in populations from the US and Ireland were derived from both ancestral lineages that occur in South America suggesting a common ancestry among these populations. The oldest lineage was associated with isolates from the section Anarrhichomenum including S*olanum tetrapetalum* from Ecuador (Gomez et al., 2008). This lineage evolved from a common ancestor of *P. infestans* and has been named *P. andina* (Fig. 2). The geographic distribution of mutations on the rooted coalescent tree demonstrates that the oldest mutations in *P. infestans* originated in South America; this is consistent with an Andean origin (Gomez et al., 2007).

P. infestans continues to evolve on *Solanum* hosts in the Andean region and Mexico and there is evidence that hybrid strains have developed that infect hosts that are of potential interest for import to the United States (Flier et al., 2002; Adler et al., 2004; Oliva, 2007; Gomez et al., 2008). We have examined phylogenetic relationships of isolates of *P. infestans* sensu lato from the Andean Highlands of South America (Gomez et al., 2008). Three clonal lineages (US-1, EC-1, EC-3) and one heterogeneous group (EC-2.1c, EC-2.1a) were found in association with different host species in the genus *Solanum* (Adler et al., 2004). The EC-1 clonal lineage of *P. infestans* sensu lato was confirmed to be *P. infestans* based on sequences of the mitochondrial cytochrome oxidase I (*cox* I) gene and intron 1 of the *ras* gene (Gomez et al., 2008). However, the EC-2.1c isolates from the section Anarrhichomenum including S*olanum tetrapetalum* formed a distinct branch in the 1c clade (*P. infestans, P. mirabilis*, *P. phaseoli* and *P. ipomoeae)* for both *cox* I and *ras* intron 1 sequence phylogenies. These have been assigned to a new species, *P. andina* (Kroon et al., 2004; Gomez et al., 2008) (Fig. 3). The *Ras* intron 1 sequence suggests that *P. andina* may have arisen from hybridization between *P. infestans* and *P. mirabilis* (Goodwin and Fry, 1994; Gomez et al., 2008). *P. mirabilis* has not been found in South America and was first reported in Mexico (Galindo and Hohl, 1985) so further exploration in the Andean region is warranted. We are currently sequencing the mitochondrial genomes of all the isolates in the Ic clade to clarify their evolutionary relationships.

Both the EC-2 (A2) and US-1(A1) genotypes have been found on pear melon (Adler et al., 2004). Thus, pear melon could provide a bridge species in the Andes, allowing sexual reproduction of *P. infestans* with *P. andina* to form hybrids. Other new species of *Phytophthora* have also been described from hybrid origins on a number of different hosts (Brasier et al., 1999). As fruits of pear melon are imported into the US, tracking genotypes of *P. infestans* south of our border is of importance to protect US potato growers from invasive new strains of the pathogen.

HISTORIC EVIDENCE OF MIGRATIONS OF *P. INFESTANS* TO ASIA

We have examined potato leaves infected with *P. infestans* from herbarium collections from Asia and Australia (Fig. 1B). Twelve samples from China containing late blight lesions collected between 1938 and 1982 from Beijing, Hebei, Sichuan, Shanxi, Chongqing, and Yunnan were examined. Twenty-nine samples containing potato or tomato leaves with lesions caused by *P. infestans* were examined from 5 herbarium collections including samples from Australia (1911-1917), India (1913-1974), Japan (1901-1931), Peninsular Malaysia (1950-1987), Nepal (1954-1965), The Phillipines (1910-1916), Taiwan (1908-1913), Thailand (1981), Russia (1917), Latvia (1931-1935) and The Ukraine (Table 2).

We developed a Taq-Man real-time quantitative PCR assay to detect *P. infestans* in dried potato leaves from herbarium specimens. Ribosomal DNA was successfully amplified from 41 diseased leaves from samples collected between 1901 and 1987 and *P. infestans* was confirmed in 24 and 39 of the 41 samples by conventional and real time PCR, respectively.

The Ia mtDNA haplotype of *P. infestans* was found earlier in China than other mtDNA haplotypes. Samples collected by different researchers in China from tomato in 1938 in Kunming and from potato in 1940 in Chengjiang and Chongqing were the Ia mtDNA haplotype (Table 2). In contrast, the earliest record of the Ib haplotype of *P. infestans* in China was in 1952 on potato in the Sichuan region, in 1954 on potato in Hebei and in 1956 on tomato in Beijing. The Ib haplotype still occurs in the Beijing area on tomato (Guo et al., 2008).

In other areas in Asia, the earliest documentation of the Ia mtDNA haplotype was in Japan in 1901. The Ia mtDNA haplotype was also found in India in 1913, in the Philippines in 1910, and in Russia and Australia in 1917. In contrast, the Ib mtDNA haplotype was only found in 3 samples in India on potato in 1968 and 1974 and in Thailand on tomato in 1981. All the rest of the herbarium plant samples from other countries and regions were infected with the Ia mtDNA haplotype of *P. infestans* (Table 2). Modern Chinese populations contain all four mitochondrial haplotypes of *P. infestans* suggesting more recent introductions of the IIa and IIb haplotypes (Guo et al., 2008) although small sample size (n=12) may have limited detection of these haplotypes earlier.

CONCLUSIONS

We used historical specimens to clarify present day questions about the evolution and population biology of *P. infestans*. Our work with historic herbarium collections has shed new light on the population biology of this devastating plant pathogen. We refute the hypothesis that a strain of Ib haplotype originating in Mexico was responsible for historic outbreaks around the world. We also refute the hypothesis that the Type II haplotype lineage evolved from the Type I lineage (Ristaino et al., 2001; May and Ristaino, 2004; Avila-Adame et al., 2006; Gomez et al., 2007). Further work with microsatellite markers may be possible on historic specimens to provide yet more evidence for the comparison of the genetic structure in old populations and present day populations of the pathogen. An important message from our work is that sample selection is highly important in population genetic studies. Both temporal and spatial scales need to be considered when documenting population structure. Archival materials can be used to enhance the resolution of migration experiments. Unfortunately, archival collections are continually threatened as resources dwindle for maintaining herbaria. Plant pathologists need to join forces with mycologists by contributing to collections and helping preserve and utilize herbarium collections for population genetics research.

Phytophthora species are responsible for devastating diseases on a wide range of host crops, natural vegetation and forestry worldwide. They represent a significant and emerging biosecurity threat, in large part due to increases in plant movement via international trade (Brasier, 2008). *P. infestans* exemplifies this threat; it was the first species in the genus to be described and left a path of devastation on potato in its wake in the US and Europe in the nineteenth century (Berkeley, 1846; Bourke, 1964; Fry, 2008). The potential for the emergence of new pathogens through hybridization, global migration, and accidental release due to expanding agricultural activities and

trade, as well as increased concerns about agroterrorism, underscore the importance of studies on the structure of these pathogens (Brasier, 2008). Clearly, migration has played a major role in the population biology of *P. infestans*. Species hybridization within the clade and development of novel species such a *P. andina* in the tropics demonstrate the need for further research on biodiversity in the genus on tropical crops.

ACKNOWLEDGEMENTS

The authors would like to thank many individuals that have contributed to the research discussed in this paper including former postdoctoral associates Cruz Avila–Adame (Dow AgroSciences, Indianapolis, IN), Carol Trout Groves, and Kimberley May (Melbourne University), visiting scholar Liyun Guo (China Agricultural University), former graduate assistant Luis Gomez-Alpizar (Universidade de Costa Rica), former technician Greg Parra (USDA APHIS) and collaborators Robin Buell (formerly TIGR, now Michigan State Univ), Ignazio Carbone (NC State University), Greg Forbes (CIP), Ricardo Oliva (John Innes Institute, UK), and Tom Randall (UNC Bioinformatics Center).

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Tables

Table 1. Identity of the mitochondrial DNA haplotype(s) of *Phytophthora infestans* in the oldest herbarium specimens known to be infected from Central and South America.

					PCR products obtained				
				rDNA[2]	mtDNA[3]				
Year	Collector	Country	Host	Specimen number and collection[1]	ITS	*Rpl5*	*Cox*1	*Nad* 4	mtDNA[5] Haplotype
1889	P. Hennings	Chile	*S. tuberosum*	Kew 79	+	+	+	+	Ia
1913	J. M. Vargas Vergara	Colombia	*S. tuberosum*	BPI US0186968	+	+	+	+	Ia
1929	W. H. Weston	Colombia	*S. tuberosum*	FH 292	+	+	+	+	Ia
1941	A. S. Muller	Guatemala	*L. esculentum*[4]	BPI US0186816	+	-	+	+	Ia
1942	A. S. Muller	Guatemala	*S. tuberosum*	BPI US0186943	+	+	+	+	Ia
1942	J. A. Stevenson	Guatemala	*Petunia hybrida*	BPI US0186832	+	+	+	+	Ia
1942	J. A. Stevenson	Costa Rica	*S. tuberosum*	BPI US0187022	+	+	+	+	Ia
1944	M. Cardenas	Bolivia	*S. tuberosum*	BPI US0186941	+	+	+	+	Ib
1947	J. A. Stevenson	Costa Rica	*L. esculentum*	BPI US0186817	+	+	+	+	Ia
1954	M. O'Brien	Nicaragua	*S. tuberosum*	BPI US0186956	+	+	+	+	Ia
1956	J. A. Stevenson	Nicaragua	*S. tuberosum*	BPI US0186953	+	+	+	+	IIb
1967	F. D. Matthews	Ecuador	*S. tuberosum*	BPI US0186908	+	+	+	+	Ib

[1] Specimens were sampled from the collections housed at the Royal Botanic Gardens Mycological Herbarium, Kew, England (K); the USDA National Fungus Collection, Beltsville, MD (BPI); and the Farlow Herbarium, Harvard University, Cambridge, MA (FH).

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

[3] Primers P3F1/P3R1, P4F2/P4R3, P2F4/P2R4 were used to amplify portions of the *Rpl5*, *Cox*1 and *Nad* 4 genes in the P3, P4, and P2, region of the mitochondrial genome, respectively.

[4] *Lycopersicon esculentum* is now called *Solanum esculentum*

[5] Table reproduced from May and Ristaino, 2004

Table 2. Identity of the mitochondrial DNA haplotype(s) of *P. infestans* in herbarium samples from Australia, China, Southeast Asia, and Russia.

Year	Collector	Country	Host	Collection[1]	mtDNA Haplotype
1901	Fukahashi	Japan, Sapporo	*S. tuberosum*	BPI	Ia
1902	Unknown	Russia	*S. tuberosum*	BPI	Ia
1910	H. S. Yates	Philippines-Luzon	*S. tuberosum*	BPI	Ia
1913	J. F. Dastur	India, Bagalphur	*S. tuberosum*	BPI	Ia
1917	W. A. Birmingham	Australia, Hurston Park, NSW	*S. tuberosum*	BPI	Ia
1917	F. Buchoitz	Russia	*S. tuberosum*	BPI	Ia
1930	Unknown	Japan	*S. tuberosum*	HMAS	Ia
		Ukraine	*S. tuberosum*	BPI	Ia
		Ukrania	*S. tuberosum*	BPI	Ia
1931	K. Togashi	Japan	*S. tuberosum*	FH	Ia
1935	K. Starcs	Latvia	*S. tuberosum*	BPI	Ia
1938	C.C. Cheo	China-Yunnan-Kunming	*S. tuberosum*	HMAS	Ia
1938	F. L. Tai	China-Yunnan-Kunming	*L. esculentum*[2]	HMAS	Ia
1940	Heng Zhang-xun	China-Yunnan-Chengjiang	*S. tuberosum*	HMAS	Ia
1940	Qio Yuan	China-Chongqing	*S. tuberosum*	HMAS	Ia
1950	A. Johnston	Peninsular Malaysia	*S. tuberosum*	Kew	Ia
1952	Yang Zuo-min	China-Sichuan	*S. tuberosum*	HMAS	Ib
1954	Huang He	China-Hebei-Shalingzi	*S. tuberosum*	HMAS	Ib
1954	Stauton	Nepal	*S. tuberosum*	Kew	Ia
1956	Huang He	China-Beijing	*L. esculentum*	HMAS	Ib
1968	J.A. Russell	India	*S. tuberosum*	IMI	Ib
1974	D. N. Bardoloi	India	*S. tuberosum*	IMI	Ib
1981	R. Black	Thailand	*L. esculentum*	Kew	Ib
1982	Qin Yun	China-Sichuan-Yaan	*S. lyratum*	HMAS	Ib
1987	B.C. Sutton	Pennisular Malayasia	*L. esculentum*	Kew	Ia

[1] Specimens were sampled from the Mycological Herbaria housed at the Royal Botanic Gardens, Kew, England (Kew); the USDA National Fungus Collection, Beltsville, MD, USA (BPI); the International Mycological Institute Collection, CABI, Egham, England (IMI); the Farlow Herbarium, Harvard University, Cambridge, MA, USA (FH); and the Institute of Microbiology Academia Sinica, People's Republic of China, Beijing (HMAS).

[2] *Lycopersicon esculentum* is now called *Solanum esculentum*

Figures

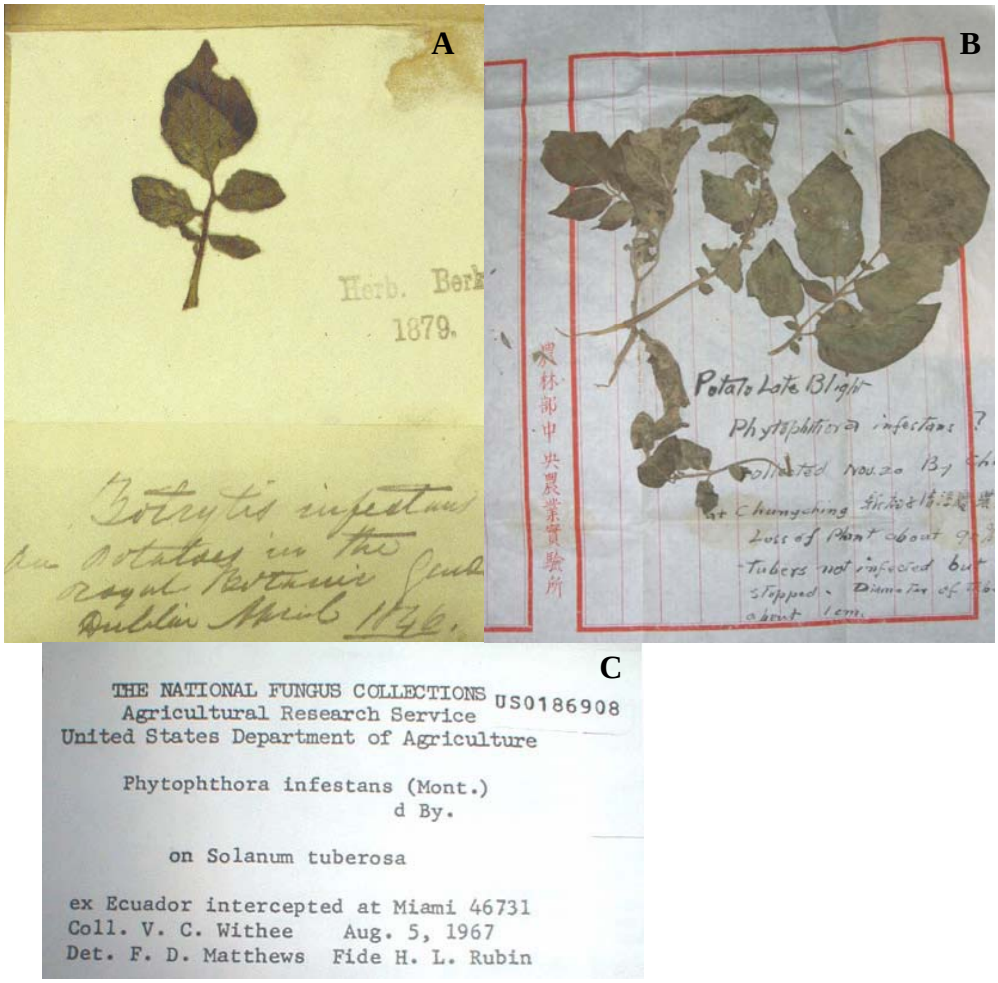

Fig. 1. Historic specimens of potato infected with *Phytophthora infestans*. A) Herbarium sample collected by Dr. John Lindley in 1846 in the Royal Botanic Gardens, Glasnevin, Dublin, Ireland. This is one of several of the oldest known specimens of potato that still exist from the potato famine epidemics. B) Herbarium samples of potato late blight from 1938 from the Institute of Microbiology (HMAS), Academia Sinica, Beijing, People's Republic of China.; C) Specimen label from Ecuador from potato intercepted in Miami in 1967 that was infected with the Ib mtDNA haplotype of *Phytophthora infestans*. Image 1A is reproduced from Ristaino et al., 2001 and image 1C from Ristaino et al., 2006.

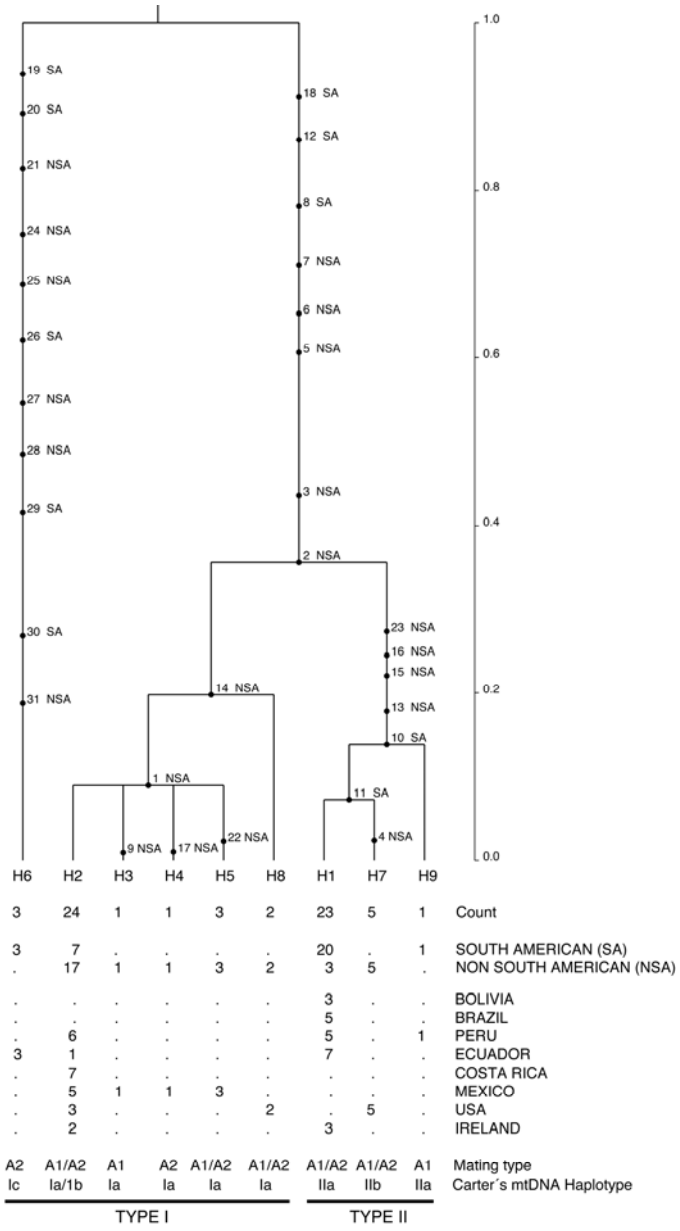

Fig. 2 The rooted coalescent-based gene genealogy showing the distribution of mutations for South American (SA: Peru, Ecuador, Bolivia, Brazil) and non South American (NSA: Costa Rica, Mexico, US, Ireland) populations for the mitochondrial (P3+P4) loci of *Phytophthora infestans* generated using GENETREE. The time scale is in coalescent units of effective population size and the direction of divergence is from the top (past-oldest) to the bottom (present-youngest). Numbers below the tree from top to bottom designate each distinct haplotype and its count (i.e., the number of occurrences of the haplotype in the sample), the count of each haplotype in each population, the mating type of the isolates, and the mtDNA haplotype. Image reproduced from Gomez et al., 2007.

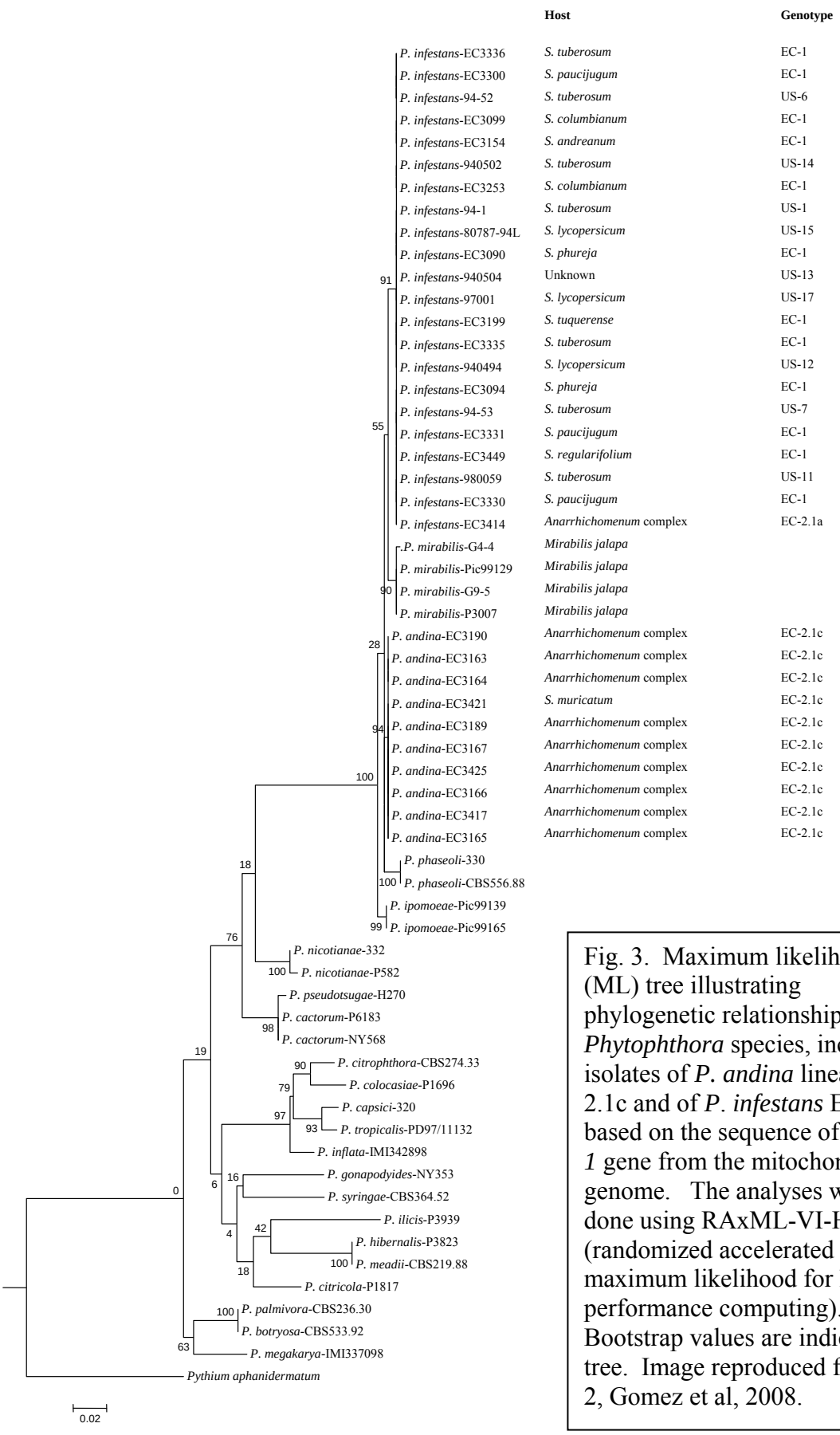

Fig. 3. Maximum likelihood (ML) tree illustrating phylogenetic relationship among *Phytophthora* species, including isolates of *P. andina* lineage EC-2.1c and of *P. infestans* EC-2.1a based on the sequence of the *cox 1* gene from the mitochondrial genome. The analyses were done using RAxML-VI-HPC (randomized accelerated maximum likelihood for high performance computing). Bootstrap values are indicated on tree. Image reproduced from Fig. 2, Gomez et al, 2008.