TRACKING THE EVOLUTIONARY HISTORY OF THE POTATO LATE BLIGHT PATHOGEN WITH HISTORICAL COLLECTIONS

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Introduction

Phytophthora infestans (Mont.) de Bary causes late blight, a devastating disease of potato and tomato (Smart & Fry, 2001). The pathogen can infect and cause foliar blight and also infects potato tubers, tomato fruit, and a number of other Solanaceous hosts (Fig. 1). Epidemics caused by *P. infestans* in the mid 19th century in Ireland led to what is known as the Irish potato famine. Approximately 1.5 million people died from starvation and another 1.5 million were displaced and forced to emigrate from Ireland to other regions of the world. The disease was first observed on potatoes in the United States in 1843 in areas around the ports of Philadelphia and New York (Peterson, Campbell, & Griffith, 1992), and subsequently spread to Europe, the UK and Ireland (Bourke, 1993). The pathogen is still wreaking havoc for potato growers in many areas of the world.

Epidemics of potato late blight occurred before Louis Pasteur's pioneering work on the germ theory of disease. Some believed weather was responsible for the malady on potato while others blamed the devil or bad soil (Berkeley, 1846; Peterson, Campbell, & Griffith, 1992). The painstaking work of J. Teschemacher in the United States, M.J. Berkeley in Great Britain, Montagne in France, and later DeBary in Germany, clearly elucidated that a fungus-like organism was responsible for late blight (Berkeley, 1846, DeBary, 1876; Peterson, Campbell, & Griffith, 1992). Research by early mycologists who studied the late blight pathogen was some of the first to document that fungi were capable of causing plant disease and laid the groundwork for the disciplines of microbiology and plant pathology (Berkeley, 1846, DeBary, 1876).

Late blight has become an increasingly important disease worldwide in recent years, more than 150 years after the great famine (Smart and Fry, 2001). Since susceptible potatoes are widely planted in many areas of the world, the crop cannot be grown without frequent fungicide application. The disease has reached epidemic proportions in North America, Russia, and Europe due to the development of resistance to the phenylamide fungicide metalaxyl in populations of the pathogen and the widespread occurrence of new and more virulent genotypes (Drenth et al, 1994; Kadish and Cohen, 1995). Some strains including the Ib or US-1 clonal lineage are sensitive to the fungicide metalaxyl and mefenoxam, while many other strains have developed resistance to these fungicides. More fungicides are used on potato worldwide, than any other food crop, largely because of late blight. The cost of fungicide use by US potato growers exceeds 3 billion dollars annually. The disease can be devastating in the developing world where fungicides are often not affordable or available.

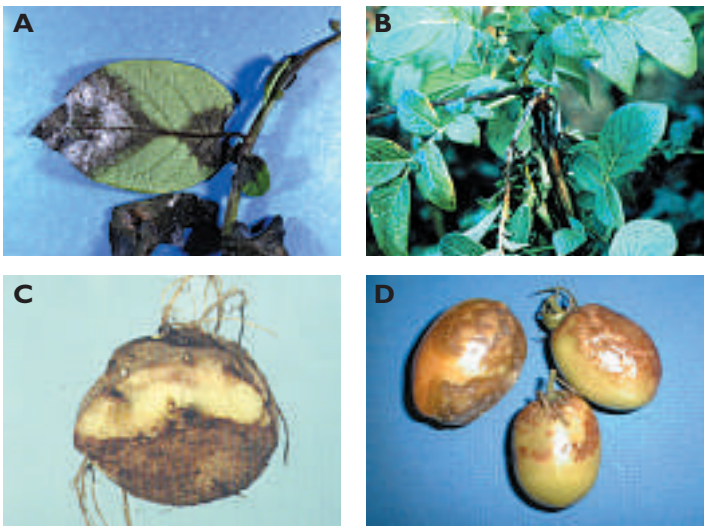

Figure 1. **Symptoms of late blight caused by *Phytophthora infestans* on an infected: A) potato leaf; B) potato stem; C) potato tubers, and D) tomato fruits.**

Pathogen Biology

P. infestans is an oomycete pathogen and more closely related to brown algae than to true fungi (Gunderson et al, 1987). *P. infestans* reproduces predominately by asexual means and forms sporangia on infected host tissue (Fig. 2A) that either germinate directly to form infection hyphae or release zoospores (Fig. 2B) that are responsible for additional infections. Sporangia can be dispersed by wind and rain over hundreds of meters. 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 (Fig. 1C). The pathogen can form sexual oospores via outcrossing of two mating types called the A1 and A2 (Fig. 2C)(Judelson, 1996). 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).

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Figure 2. ***Phytophthora infestans*** **spreads long distances aerially by: A) producing asexual sporangia on infected tissue (photo courtesy of William Fry, Cornell University); Sporangia can germinate directly or release B) motile zoospores that infect tissue (photo courtesy of David Shew, N. C. State University). C) The sexual oospore of the pathogen is produced if both mating types are present in the infected tissue.**

P. infestans can infect and kill a field of potatoes in a matter of days. To reduce the potential deleterious effects of plant pathogens on crop production, it helps to be able to accurately "fingerprint" plant pathogen variants and discriminate between those that are locally endemic and those that have been relatively recently introduced from geographically remote locations. A clear understanding of changes in pathogen population structure over the long-term is important. 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, further underscore the importance of research on the genetic structure of oomycete pathogens.

What strain or haplotype was responsible for disease during 19th century epidemics?

It was previously believed that worldwide populations of the pathogen prior to the 1980's originated in Mexico, were the A-1 mating type, asexual and derived from a single clonal lineage called the US-1 genotype or Ib mitochondrial DNA (mtDNA) haplotype (Goodwin et al, 1994). However, much of this data was based on studies of modern populations of the pathogen and little was actually known about the original strain(s) of the pathogen that existed during famine-era epidemics. There have clearly been multiple migrations of the pathogen in the latter half of the 20th century out of Mexico and in recent years sexual reproduction and the occurrence of multiple clonal lineages have been well documented (Smart & Fry, 2001).

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. 3). We used these historical specimens to clarify present day questions about the epidemiology, evolution and population biology of the late blight pathogen (Ristaino, 1998; Ristaino 2002; Ristaino et al, 2001; May et al, 2004). Historical collections of late blight infected potatoes were obtained from the Mycological Herbarium of the Royal Botanic Gardens in Kew England, among other collections (Ristaino, 1998; Ristaino 2002; Ristaino et al, 2001; May et al, 2004). Disputes about nomenclature, phylogenetics, function and evolution of genes, and origins of populations can be addressed with herbarium specimens (Hermann and Hummel, 1994). Genomes of specimens are preserved in herbarium collections making them a valuable scientific

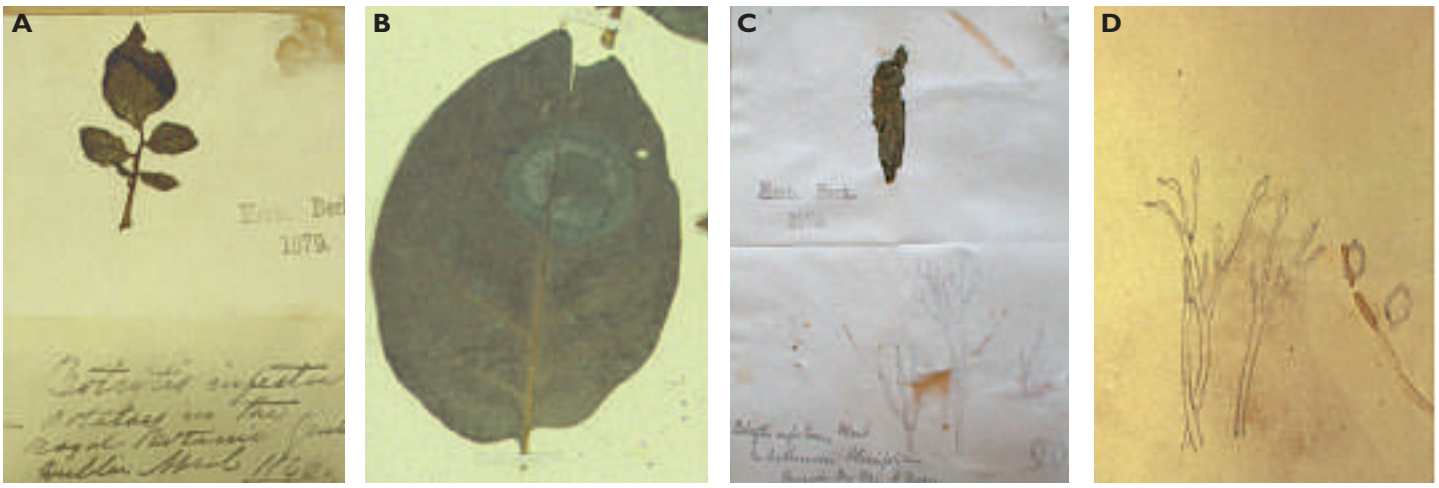

Figure 3. **Historic specimens of potato infected with the plant pathogen *Phytophthora infestans*, causal agent of the Irish potato famine epidemics. A) This sample from 1846 in the Royal Botanic Gardens, Dublin Ireland and is one of several of the oldest known specimens of potato that still exist from the potato famine epidemics. B) This specimen was collected by Krieger in Germany in 1888; C) Sample of *Anthocercis ilicifolia*, an evergreen Solanaceous shrub from Australia, infected with *P. infestans* in 1846; D) Sample herbarium label from petunia collected By C. E. Broome in England in 1856 and infected with *P. infestans* showing an illustration of the sporangia and sporangiophores of *P. infestans*.**

database for sampling populations when coupled with the use of molecular techniques.

The polymerase chain reaction (PCR) can be used to detect small quantities of DNA and is used widely in medical diagnostics for pathogen identification or fingerprinting individual strains. We used PCR to amplify minute amounts of DNA from pathogen infected potato leaves from historic epidemics (Fig. 3). We identified the mitochondrial DNA (mtDNA) haplotype(s) present in specimens collected during the Irish potato famine and later in the 19th and early 20th century using genetic analysis of mitochondrial DNA (May et al, Ristaino et al) (Fig. 4). A 100bp fragment of ribosomal DNA (rDNA) specific for *P. infestans* was amplified from 90% of the samples tested, confirming infection by *P. infestans* (Trout et al, 1996; Ristaino et al, 2001). Primers were designed that amplify short segments of mtDNA around variable restriction sites that separate strains, and the DNA was sequenced. Eighty six percent of the herbarium specimens from historic epidemics were infected with the Ia mtDNA haplotype (Fig. 4). 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 1950's. Our 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 US and not the Ib haplotype as was previously believed (Goodwin and Fry, 1994, May et al, 2004). The Ib mtDNA haplotype of the pathogen was most likely dispersed later in the early 20th century from the Andean region of Bolivia and Ecuador. In fact, labels from herbarium collections implicated Ecuador as one source for the introduction of the Ib haplotype into the US in the early 20th century (Fig. 4C). Our data challenge the hypothesis that a single clonal lineage of *P. infestans* existed outside of Mexico in the 19th century since multiple haplotypes were present in herbaria collections in the 1950's and pathogen diversity was greater than previously believed (Goodwin et al, 1994). These data also open the possibility that both mating types were present outside of Mexico before the first reports of oospores in 1958 since the Ia haplotype can be either an A1 or A2 mating type (Gallegy and Gallindo, 1958; Kotilla, 1946).

Figure 4. **A) Amplified DNA from the Nad4 gene in the P2 region (P2F4/P2R4) of mtDNA from modern and herbarium specimens infected with *P. infestans*. Lanes 1 and 24 are 100bp ladder; lanes 2-5 are undigested product from two modern isolates each of the Ia and Ib haplotypes, respectively; lanes 6-9 are MspI digested product from two modern isolates of the Ia and Ib, respectively; lanes 10 –18 are nondigested PCR product from herbarium specimens; lanes 19-23 are the controls: positive controls of DNA from mycelium of *P. infestans* and dried infected modern lesion, and negative controls are DNA from noninfected potato and tomato leaf and nontemplate control. 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 10 herbarium specimens. Note the last two samples are the Ib haplotype and have the MspI restriction site. C) Specimen from Ecuador from potato infected with the Ib haplotype of *Phytophthora infestans* intercepted in Miami in 1967.**

Where did the source of inoculum for 19th century epidemics in the US and Europe originate?

Movement of plant materials is a common means of introduction of plant pathogens into previously pathogen-free areas. We amplified rDNA of *P. infestans* from a sample of *Anthocercis ilicifolia,* a Solanaceous evergreen shrub native to Western Australia that was introduced into the National Botanic Gardens at Glasnevin in Dublin in 1842 (Figure 3C). The plant was reported diseased in Ireland in 1846. David Moore of Ireland sent a specimen to M. J. Berkeley in England for diagnosis (Berkeley, 1846). This is the earliest known definitive diagnosis of an alternative *Solanaceous* host for *P. infestans* (Ristaino et al, 2001). It is most likely that *A. illicifolia* became diseased in Ireland rather than being introduced with *P. infestans* from Australia, since late blight was not known in Australia until the 20th century. Petunia was also known to harbor the pathogen in the 19th century in England (3D).

Potatoes were widely used as ship stores for hungry sailors and were often discarded at the dockside with the booming maritime trade between continents in the 19th century. Potatoes were shipped into Europe and the UK from the Andes of South America and the US during the famine-era to replenish depleted stocks that had succumbed to another disease – dry rot- of potato. In fact the first three cultivars to succumb to the disease in Europe were named "Lima", "Peruviennes" and "Cordilieres" (Bourke, 1964). Some suggest that the late blight pathogen was introduced into the US and subsequently into Ireland from wild potatoes from Mexico (Goodwin and Fry, 1994; Smart and Fry, 2001). We believe a South American origin of the disease is more likely (Gomez-Alpizar, 2004; Ristaino et al, 2001).

We are currently using both mitochondrial and nuclear gene sequence data from extant strains of the pathogen from

Central and South America to track the migration pattern of the pathogen and determine its ancestral home (Gomez-Alpizar, 2004). There are four known mtDNA haplotypes (Ia, Ib, IIa, IIb) of the pathogen. 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. Our data support the idea that all the extant mitochondrial lineages of *P. infestans* evolved from a common ancestor in South America (Gomez-Alpizar, 2004).

From population biology to disease management

Most potato varieties currently planted in the US and elsewhere, are highly susceptible to late blight. Growers choose to plant potatoes based on agronomic characters rather than host resistance, so fungicides are still the main source of disease control in many developed countries. Our genetic data from historical strains has helped to pinpoint the center of origin of this devastating pathogen. A clear understanding of the center of origin of the late blight pathogen is important since breeders continue to collect new germplasm and develop new varieties with enhanced resistance. Vertical or R gene-based resistance in potato derived from *S. demissum* in Mexico has lacked durability. Mutations in the pathogen able to overcome R gene-based resistance are readily selected. More durable, quantitative resistance is being sought with germplasm that originated in both Central and South America.

The entire genome of *P. infestans* is being sequenced at the Broad Institute at Massachusetts Institute of Technology in Cambridge. The full genome sequence of the pathogen, coupled with mapping of host resistance genes in potato and tomato will enable a more informed approach to the development of more durable resistance. Understanding avirulence genes in the pathogen that elicit defense responses in the host may one day lead to potato varieties that are both resistant to the pathogen and agronomically acceptable to the grower (Jiangs et al, 2005).

Clearly, multifaceted approaches that include understanding both historic and present day populations of the pathogen using molecular approaches, screening pathogen populations for fungicide sensitivity, understanding host resistance mechanisms and how pathogen populations evolve to overcome those mechanisms and comparative genomic approaches should lead to enhanced management of this important potato disease.

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