

Review

Tracking historic migrations of the Irish potato famine pathogen, *Phytophthora infestans*

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Abstract

The plant pathogen *Phytophthora infestans* causes late blight, a devastating disease on potato that led to the Irish potato famine during 1845–1847. The disease is considered a reemerging problem and still causes major epidemics on both potato and tomato crops worldwide. Theories on the origin of the disease based on an examination of the genetic diversity and structure of *P. infestans* populations and use of historic specimens to understand modern day epidemics are discussed.

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1. Introduction

Late blight caused by the plant pathogen *Phytophthora infestans* is a devastating disease of potato and tomato in the U.S. and worldwide (Fig. 1) [1]. The pathogen causes a destructive foliar blight and also infects potato tubers and tomato fruit under cool, moist conditions (Fig. 1). The pathogen can be transported long distances in infected plant materials.

Epidemics caused by *P. infestans* in 1845 led to the Irish potato famine and the mass emigration and death of millions of people in Ireland. The first record of the occurrence of late blight in the United States was in 1843 around the port of Philadelphia [2]. Epidemics of late blight subsequently spread to a five-state area and Canada over the next 2 years. In 1845, late blight epidemics also occurred in Belgium, Holland, Germany, Switzerland, France, Italy, England, Ireland and Scotland. For the people in Ireland, who subsisted on the potato as a main food source, the late blight epidemics were devastating [1]. An average male consumed 12 pounds of potatoes per day. In a 5-year period, the population in Ireland decreased dramatically as over 1.5 million people died from starvation and disease and an equal number emigrated from Ireland [3].

Epidemics of potato late blight occurred before the germ theory had been clearly elucidated. Some believed weather was responsible for the malady on potato [2]. Others blamed the devil or bad soil. In a pamphlet written on late blight in Scotland, it was said, “It is certain that a fungus appears in the leaves, stems, and tubers of the plants which have been attacked, but it is uncertain how far the fungus is the cause or the consequence of the disease — how far it is to be considered as a parasite upon the living potato, or as a mere devourer of its dying parts” [4]. The painstaking work of J. Tschermacher 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 the disease [5,6]. Late blight epidemics appeared in Europe before Louis Pasteur’s pioneering work on the germ theory of disease. 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 discipline of plant pathology [5,6].

Late blight has become a reemerging disease worldwide in recent years, more than 150 years after the great famine. The disease has reached epidemic proportions in North America, Russia, and Europe due to the development of resistance to phenylamide fungicides in populations of the pathogen and the widespread occurrence of new genotypes [7–9]. The disease has been responsible for the extensive use of fungicides on potato, and in many areas of the world the crop cannot be grown without their frequent application.

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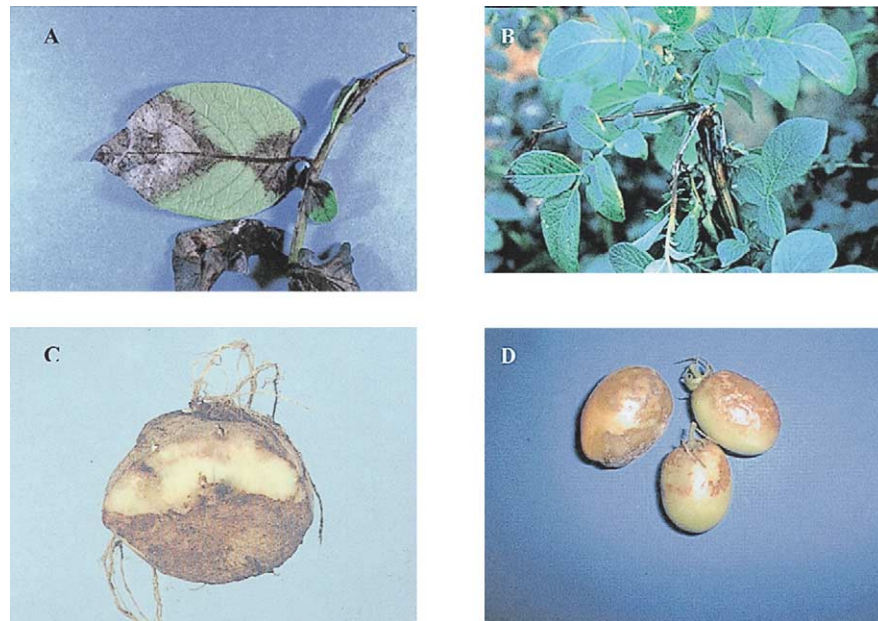


Fig. 1. Symptoms of late blight caused by *P. infestans* on infected (A) potato leaf; (B) potato stem; (C) potato tubers, and (D) tomato fruits.

P. infestans is an oomycete pathogen and, unlike true fungi, contains cellulose rather than chitin in its cell walls and produces motile zoospores. The organism is more closely related to brown algae than to true fungi [10]. *P. infestans* reproduces predominately by asexual (clonal) means and forms sporangia (Fig. 2A) on infected host tissue that either germinate directly to form infection hyphae or release zoospores (Fig. 2B) that are responsible for addi-

tional infections. The asexual stage of the pathogen was thought to be the primary mode of reproduction occurring in most fields in the US before 1990, and in Europe before 1980 [9]. The pathogen typically survives from season to season as mycelium (Fig. 2C) in infected potato tubers and debris when the asexual cycle is predominant. Infected tubers are an important source of inoculum and contribute to epidemic development on subsequent potato crops (Fig. 1C).

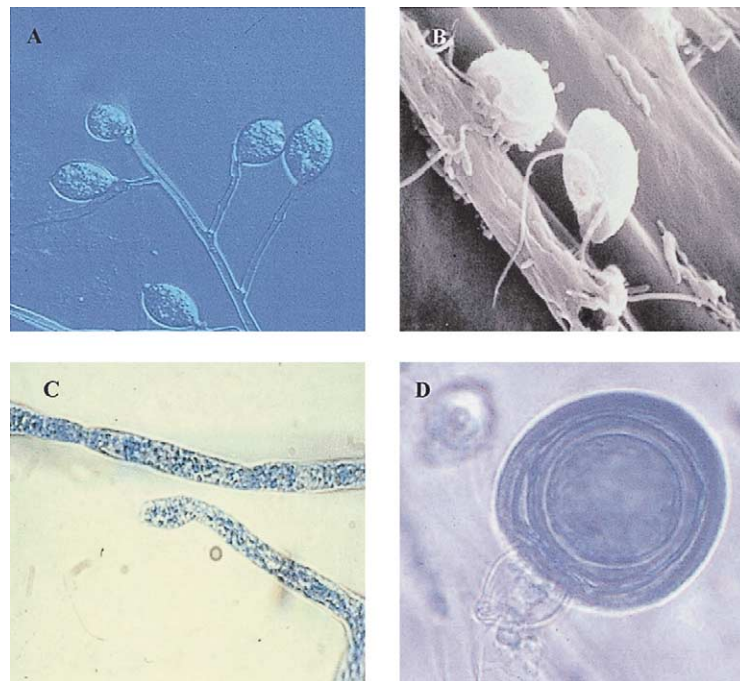


Fig. 2. *P. infestans* spreads long distances aerially (A) by producing asexual sporangia on infected tissue (photo courtesy of William Fry, Cornell University). Sporangia can germinate directly or release (B) motile zoospores that infect tissue (*Phytophthora* sp. zoospore photo courtesy of David Shew,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

P. infestans is a heterothallic pathogen and reproduces sexually by outcrossing of two mating types termed the A1 and A2 [11]. These mating types are actually compatibility types and do not correspond to dimorphic forms of the organism [12]. Mating types are distinguished by the production of specific hormones that induce the formation of gametangia in the opposite mating type [13]. Diploid vegetative mycelia differentiate to form either antheridia (male gametangia) or oogonia (female gametangia) in which meiosis occurs. Fusion of the gametangia results in the formation of diploid oospores that can survive for long periods in soil (Fig. 2D). During sexual reproduction (outcrossing), genetic recombination occurs as the oospore is formed and leads to genetic diversity in subsequent generations of the pathogen [12].

2. The unsolved mysteries of potato late blight: sexual reproduction, centers of origins and migrations

It has been proposed that the center of origin and diversity of the late blight pathogen is in Mexico [9,14]. Isolates of *P. infestans* of the A2 mating type and oospores in infected plant material were first discovered in central Mexico in 1956 [11]. Mexican populations of the pathogen are highly diverse for neutral DNA markers, and pathotype [14]. Prior to 1980 both the A1 and A2 mating types of the fungus were reported only in Mexico, while the A1 mating type was reported elsewhere in the world [9,11]. This situation changed in the 1980s when the A2 mating type was observed by Hohl in Switzerland [15]. The A2 mating type has now been reported in Europe, Asia, South America, US and Canada. Sexual reproduction is also more common and has been reported to occur in Europe and Canada [7,16–18]. The presence of both mating types within the same field and the occurrence of diverse new genotypes of the pathogen has exacerbated disease problems, since some genotypes are resistant to phenylamide fungicides and at the same time are more aggressive to potato [7–9,14].

Anton De Bary elucidated the life cycle of *P. infestans* in 1876, but the role of the sexual oospore in the epidemiology of the disease has been debated for many years [5,19–21]. It is widely assumed that because the A2 mating type of *P. infestans* was not found in the US or Europe until the 1980s, that pathogen reproduction was strictly asexual [7–9,11,15–17]. Morphological evidence of oospores was found in dried herbarium materials and archival samples from specimens dating from 1876 through 1946 [19,21]. Evidence of oospores in field samples from Minnesota in 1946 suggests that pathogen reproduction was not exclusively asexual prior to 1950 in the US [19,22]. Evidence of oospores in plant material from field samples over 100 years old, challenges the current theories that “the late blight pathogen has been strictly asexual for more than 150 years” [9]. These results also demonstrate the importance and utility of examining preserved historic herbarium materials in clarifi-

cation of basic questions concerning pathogen reproductive biology [21]. Observation of the type of spore is not predictive of the genetic structure in the pathogen, since clonal or recombining reproduction is clearly uncoupled from reproductive morphology in *P. infestans* [23,24]. Although reproductive mode in *P. infestans* can vary in space and time, nucleotide sequence variation and statistical methods can be used to detect clonality and recombination in nature [23].

If Mexico is the center of origin for the late blight pathogen, was Mexico also the source of inoculum for the 19th century epidemics that led to the Irish potato famine? One theory proposes that Mexico represents the center of origin and diversity of the late blight pathogen and that Mexico provided the source inoculum for the late blight epidemics of the 1840s [9,25]. These hypotheses are based on the fact that (1) both mating types occur in Mexico; (2) host resistance genes are present in wild *Solanum* populations in Mexico; and (3) pathogen populations are highly diverse in Mexico. Genetic analysis of worldwide populations of *P. infestans* with a repetitive DNA probe demonstrated that they were dominated by a single clonal lineage known as the US-1 “old” genotype [9,25]. Mexican populations of *P. infestans* are highly diverse for genotypic and phenotypic markers and thus, Mexico clearly represents a present-day center of diversity of the pathogen [14]. However, since Mexican populations of the pathogen are highly diverse, Goodwin et al. proposed that the pathogen population must have undergone a genetic bottleneck during dispersal, thus greatly reducing genetic diversity, in order to explain the dominance of a single “old” clonal genotype (US-1 genotype) in modern worldwide populations [9,25]. These workers have provided clear evidence for recent migrations of the pathogen from Mexico after the 1970s [14], but provided no definitive evidence for migrations of *P. infestans* during the interval between 1840 and 1970. Recently, the ‘bottleneck’ theory of Goodwin et al. [25] has been challenged, thus causing renewed debate [21,26–28]. Domesticated potatoes were not grown for export in Mexico in the 1840s and tuber blight was not common, so the actual source of inoculum for late blight epidemics has remained unclear [3,25].

A second theory proposes that Peru represents the center of origin of *P. infestans* and that source inoculum for the 19th century late blight epidemics originated there [26,29]. This theory is based on historical writings that indicate the disease may have been endemic in the Andean region for centuries [5,6,26,29]. In addition, until relatively recently, only the US-1 clonal genotype has been found in Peru and Ecuador, and this genotype was common in worldwide populations in the 1980s and 1990s [25,30,31]. Interestingly, the US-1 clonal genotype of *P. infestans* has not been found in Mexican populations of *P. infestans* [25]. Surprisingly, few studies have examined the genetic structure of *P. infestans* populations in South America [28,30,31].

A third theory suggests that Mexico represents the center of origin of the late blight pathogen, but that source inoculum for 19th century epidemics in Europe and the US originated

from Peru. The disease was reported in the 19th century and earlier [3,27,28] in Peru, where only the US-1 genotype has been reported until relatively recently [31,28]. A two-stage migration of the US-1 genotype of *P. infestans* first from Mexico to Peru, and then subsequently to the US and Europe was hypothesized [27]. In the 19th century, Peruvian bat guano was used as fertilizer, and the development of steamships increased trade between Peru, the US, and Europe and may have facilitated movement of the pathogen [5]. It is interesting that the first three potato varieties to succumb to late blight in Europe in 1845 were named “Lima”, “Peruviennes” and “Cordilleres” [3]. Human activities undoubtedly were an important mechanism in the dispersal of this pathogen over long distances [1]. A satisfactory answer to the center of origin and source inoculum questions is yet to be resolved. We are currently examining multilocus gene sequence variation from both nuclear and mitochondrial genes from diverse populations of the pathogen to better understand the phylogeography of the pathogen.

3. Studies of DNA sequence variation with historic and modern specimens can help us identify ancestral strains and track migrations of the pathogen

Herbarium specimens can provide information on the changing genetic structure and diversity of populations. Disputes about taxonomy, nomenclature, phylogenetics, function and evolution of genes, and origins of populations can be addressed by examining herbarium specimens. Genomes of pathogens are preserved in herbaria collections, making them a valuable genetic repository that can be used to sample populations when coupled with molecular technology.

Advances in polymerase chain reaction (PCR) methodology and DNA sequencing have facilitated the examination of preserved specimens that were not feasible with earlier technology. Intriguing epidemiological and evolutionary questions are now being addressed using preserved specimens. As an example, ancient DNA from the hypervariable region 1 and 2 of mitochondrial DNA (mtDNA) of a Neanderthal bone was amplified and sequenced and indicated that the Neanderthal mtDNA sequence variation fell outside the variation found in modern humans, and thus Neanderthals went extinct without contributing mtDNA to modern humans [32]. The diet of an extinct ground sloth was analyzed from fossilized feces using PCR of the ribulose biphosphate carboxalase gene (*rbcL*) in the chloroplast [33]. The DNA from the bacterium *Mycobacterium tuberculosis* recovered from the lung tissue of a 1000-year-old Peruvian mummy was used to identify the causal agent and confirm that *M. tuberculosis* arose independently in the New World prior to Old World contact [34]. RNA from the Spanish influenza virus from the 1918 epidemic was recently sequenced and the subgroup of the virus was determined [35]. A forensic study using PCR analysis of human tissue samples from a 1979 Russian anthrax epidemic revealed the presence of multiple

Bacillus anthracis strains in different victims, suggesting inoculum sources were from a nearby biological weapons facility that propagated all the strains [36].

Nineteenth and early 20th century scientists collected and preserved potato leaves and tubers infected with *P. infestans*, and specimens exist from the Irish potato famine (Fig. 3). We are using these specimens to track migration patterns of the pathogen [21,37]. We have developed methods for analysis of DNA from plant pathogens preserved in dried herbarium materials [37–39]. One hundred and eighty-seven herbarium specimens from seven different collections were chosen for analysis based on the date collected, country of origin and the host plant. These samples represent material from six different regions of the world including North America, Central and South America, Western, Eastern, and Northern Europe, Ireland and the UK.

To better identify what genetic individual of *P. infestans* caused the Irish potato famine, we amplified and sequenced 100-bp fragments of ribosomal DNA (rDNA) from the internal transcribed spacer region 2 from samples from the 19th and 20th centuries. DNA was extracted from herbarium samples according to a modification of a cetyltrimethylammonium bromide (CTAB) procedure. Small pieces of dried lesions were placed in sterile 1.5 ml microcentrifuge tubes, and DNA extractions were performed as previously reported [37]. Either dilutions of ethanol precipitated DNA (1:100) or DNA concentrated from QIAquick PCR Purification Kits (QIAGEN, Inc., Valencia, CA) after extraction were suitable for subsequent PCR amplification [37]. A PCR primer set (PINF/Herb) was developed that amplifies the 100-bp region of the rDNA that is specific to *P. infestans* [37,38]. We successfully amplified pathogen rDNA from 88% of the samples tested and confirmed that *P. infestans* caused the disease lesions in the specimens. We have amplified *P. infestans* DNA from four herbarium samples dating back to 1845 collected in Britain and France, three samples from 1846 collected in Ireland and Britain, and one sample from 1847 collected in Britain (Fig. 4A, lanes 2–7) [37]. We also 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 (Fig. 3D). The plant became diseased in Ireland in 1846. David Moore of Ireland sent a specimen to M. J. Berkeley in England for diagnosis [5]. This is the earliest known definitive diagnosis of an alternative solanaceous host for *P. infestans* [37]. The genus was subsequently reported as a host for the pathogen by Julius Kuhn in 1859 [40].

Mitochondrial DNA has been studied extensively to identify mutational changes that contribute to disease traits and the evolution of a wide range of organisms including microorganisms, plants, animals and humans [23,41–44]. The mitochondrial genome of fungi are smaller in size than plant or animal mitochondrial genomes (17–176 kb) and can undergo structural rearrangements as well as length mutations and base substitutions [23]. Oomycetes such as *P. infestans* have

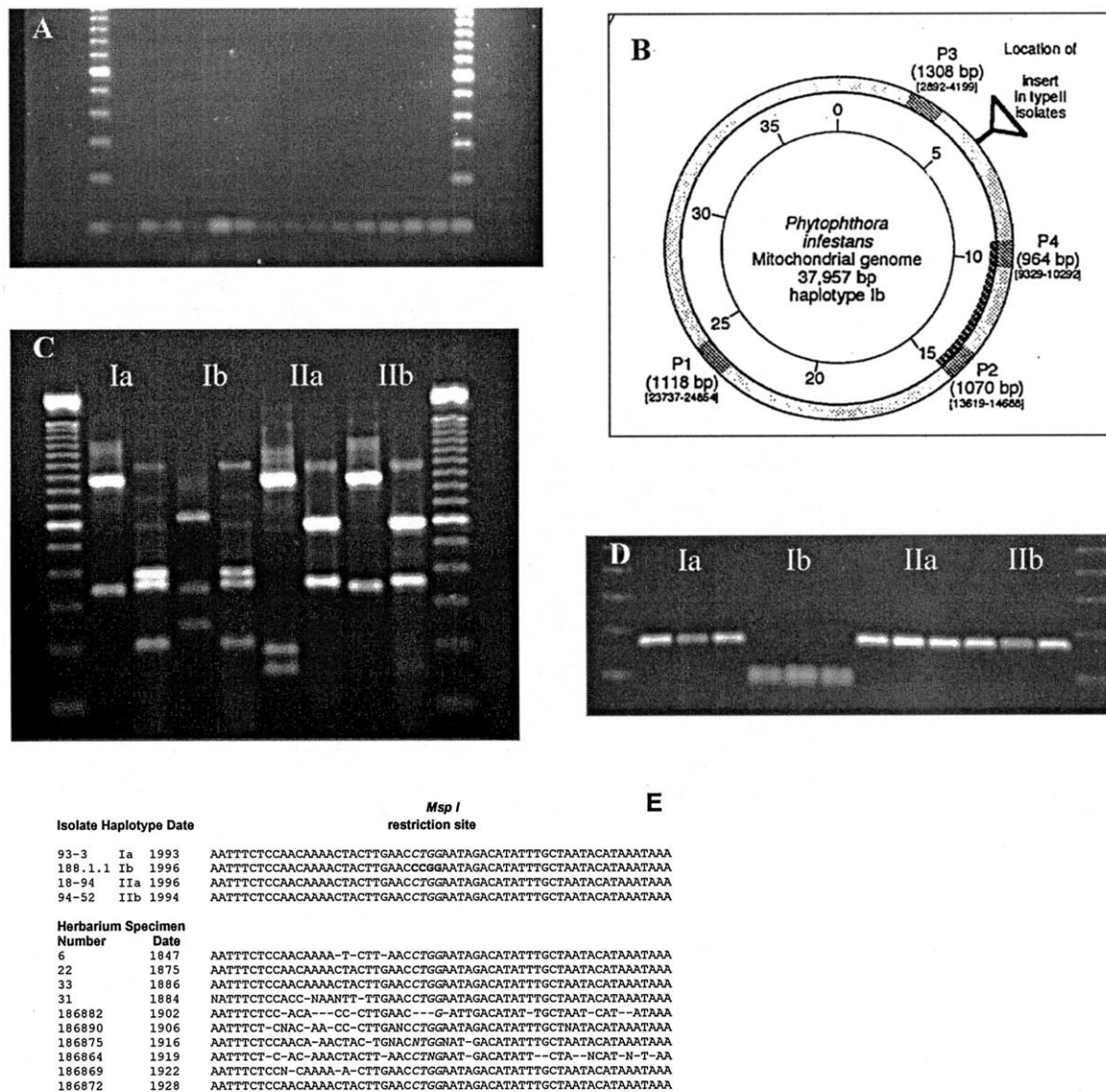


Fig. 4. Amplified PCR products from (A) *P. infestans*-infected herbarium samples collected between 1845 and 1886. We have amplified *P. infestans* ribosomal DNA from four herbarium samples dating back to 1845 collected in Britain and France, three samples from 1846 collected in Ireland and Britain, and one sample from 1847 collected in Britain (lanes 2–7). (B) The mitochondrial genome of *P. infestans* illustrating the location of various regions (shaded) and genes. The position of the 1.6-kb insert present in haplotype II isolates is shown. Photo is courtesy of Griffith and Shaw [50]. (C) Mitochondrial DNA haplotypes of *P. infestans*. DNA in every other lane amplified with the P2 or P4 primer pairs. P2 products digested with *MspI* and P4 products digested with *EcoRI*. One hundred bp ladder (lanes 1 and 10). (D) *MspI*-digested PCR products from amplification of mitochondrial DNA (mtDNA) from four haplotypes of *P. infestans* with primers pair P2 F4/R4. The *MspI* site is found in Ib haplotypes but absent in the other haplotypes. (E) Mitochondrial DNA sequences around the *MspI* restriction site in four modern and ten historic samples of potato infected with *P. infestans*.

Modern Ib haplotypes contain an *MspI* restriction site in the P2 region, while the other three haplotypes did not contain this restriction site (Fig. 4C). Amplification of the P2 and P4 regions of mtDNA and restriction digestion are used to distinguish the four extant haplotypes (Fig. 4B, C). We used primers derived from modern sequences to amplify a 167-bp region of mtDNA around the *MspI* restriction site found in the P2 region (Fig. 4D). The *P. infestans* mtDNA derived from 10 historic herbarium samples lacked the variable mtDNA region found in modern Ib haplotypes (Fig. 4E) [37].

Thus, present theories that assume the Ib haplotype is the ancestral strain responsible for the Irish famine are incorrect and need to be reevaluated [9,25]. Our data emphasize the importance of using historic specimens when making inferences about historic populations. We are currently using mtDNA sequence data obtained from over 70 additional herbarium samples from two other variable regions in the mitochondrial genome (P3 and P4 regions) to identify the haplotype responsible for 19th and early 20th century epidemics [39].

4. *P. infestans* as a re-emerging pathogen in the 20th century

P. infestans continues to cause disease on modern day potato and tomato crops worldwide. In the Columbia basin potato-growing region of Washington and Oregon, the cost of fungicides to manage the disease was estimated in excess of 30 million dollars in 1995 [53]. The occurrence of isolates resistant to the fungicide metalaxyl in populations of *P. infestans* has played a major role in the increased severity of late blight epidemics observed in recent years [7,9]. It is believed that resistance in US and Canadian isolates to metalaxyl originated by migration, rather than by mutation and selection after migration. In contrast, resistance to metalaxyl in Europe may have evolved from selection of metalaxyl-resistant mutants within clonal lineages [54]. New fungicides are currently available with different modes of action that show promise for disease control, particularly in areas where resistant strains have been reported.

Occurrence of more aggressive strains of the pathogen on potato has also exacerbated disease control strategies [9,15–17]. Nineteen genotypes have been reported in the US using DNA fingerprinting with the RG57 probe [9,14]. The US-7, -8, -18 and -19 genotypes are all haplotypes of the Ia lineage, and this haplotype is common in the US. New exotic strains have resulted from migration of inoculum on potato seed tubers around the world and from sexual recombination in fields. The A2 mating type is now common in many areas, and novel genotypes have been reported in the Netherlands, British Columbia, England, the US and elsewhere [8,14,16–18]. In North Carolina, novel genotypes and greater genetic diversity has occurred on tomato than potato [55,56].

A Global Initiative on Late blight (GILB) was begun several years ago by the International Potato Center in Lima, Peru. Meetings and research collaborations have occurred among GILB researchers. A global marker database on genetic characteristics of strains from different countries has been developed and is maintained on the CIP website (<http://www.cipotato.org>) that will aid in tracking the occurrence of genotypes in the field [57]. Allozyme genotypes, RFLP markers, and mtDNA haplotyping have been used most extensively for genotyping strains [8,9]. Extensive potato and tomato breeding programs are in place around the world to develop resistant varieties to combat the disease. Germplasm from wild species is being incorporated into cultivated potato to increase host resistance. Knowledge of the spatial occurrence of different strains of the pathogen can aid breeding efforts. An ambitious effort to sequence the genome of *P. infestans* has also begun (<http://www.ncgr.org/pgi/index.html>). An AFLP linkage map of *P. infestans* has been published, and expressed sequence tags (ESTs) have been developed to study gene diversity in the pathogen [58,59]. Work is also under way to develop ESTs in both the potato host in response to

P. infestans infection and in the pathogen in response to mycelial growth under various environmental conditions, and formation and germination of sporangia and oospores.

5. Conclusions

The migration of the late blight pathogen from its ancestral home to Ireland led to one of the largest human migrations in recent history. Plant pathogens can prove to be invasive on susceptible crop hosts and have changed our food crops, landscapes, and history many times in the past [9]. New molecular technologies are providing powerful tools for both elucidating present epidemics and opening a window to historic epidemics of the past [37]. Clear knowledge of the genetic diversity and structure of current populations of *P. infestans* and historic populations will allow us to address questions on the rate of genetic evolution in pathogen populations and allow us to design more effective diagnostic assays and control measures. To reduce the potential deleterious effects of deliberately released plant pathogens on crop production, we must be able to accurately fingerprint pathogens and discriminate between those that occur naturally and those that are deliberately introduced as agents of bioterror. Molecular epidemiology has much promise in the future for clarifying sources of inoculum and understanding spread of some of the world's most devastating plant pathogens. These tools will allow us to safeguard our food supply in a world of infectious agents.

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