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RESEARCH ARTICLE

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Mitochondrial genome sequences and molecular evolution of the Irish potato famine pathogen, *Phytophthora infestans*

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Abstract The mitochondrial genomes of haplotypes of the Irish potato famine pathogen, *Phytophthora infestans*, were sequenced. The genome sizes were 37,922, 39,870 and 39,840 bp for the type Ia, IIa and IIb mitochondrial DNA (mtDNA) haplotypes, respectively. The mitochondrial genome size for the type Ib haplotype, previously sequenced by others, was 37,957 bp. More than 90% of the genome contained coding regions. The GC content was 22.3%. A total of 18 genes involved in electron transport, 2 RNA-encoding genes, 16 ribosomal protein genes and 25 transfer RNA genes were coded on both strands with a conserved arrangement among the haplotypes. The type I haplotypes contained six unique open reading frames (ORFs) of unknown function while the type II haplotypes contained 13 ORFs of unknown function. Polymorphisms were observed in both coding and non-coding regions although the highest variation was in non-coding regions. The type I haplotypes (Ia and Ib) differed by only 14 polymorphic sites, whereas the type II haplotypes (IIa and IIb) differed by 50 polymorphic sites. The largest number (152) of polymorphic sites was found between the type IIb and Ia haplotypes. A large spacer flanked by the genes coding for tRNA-Tyr (trnY) and the small subunit RNA (rns) contained the largest number of polymorphic sites and corresponds to the region where a large indel that differentiates type II from type I haplotypes is located. The size of this region was 785, 2,666 and 2,670 bp in type Ia, IIa and IIb haplotypes, respectively. Among the four haplotypes, 81 mutations were identified. Phylogenetic and coalescent analysis revealed that although the type I and II haplotypes shared a common ancestor, they clearly formed two independent lineages that evolved independently. The type II haplotypes diverged earlier than the type I haplotypes. Thus our data do not support the previous hypothesis that the type II lineages evolved from the type I lineages. The type I haplotypes diverged more recently and the mutations associated with the evolution of the Ia and Ib types were identified.

Keywords *Phytophthora infestans* · Late blight · Mitochondria · Potato

Introduction

The mitochondrial genome is considered an attractive model to study organismal evolutionary biology because of its mutation rate, uniparental inheritance with rare or no recombination and uniform genetic background due to homoplasmy (Chen and Hebert 1999; Chesnick et al. 2000). More than 150 mitochondrial genomes have been sequenced from animals, plants, fungi and protists (Chesnick et al. 2000); however, few mitochondrial genomes from plant pathogens have been sequenced. *Phytophthora infestans* (Montagne) de Bary is an oomycete pathogen that was responsible for Ireland's potato famine of the nineteenth century. *P. infestans* is a widespread and destructive pathogen on modern-day potato and tomato. This pathogen was originally placed in the kingdom fungi based on its fungal-like thallus. Analysis of genes contained in the genome of mito-

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chondria of *P. infestans* permitted a better understanding of the phylogenetic position of this organism. Analysis of the *nad*4L polypeptide subunit of the NADH dehydrogenase complex (Chesnick et al. 1996) and the nuclear small subunit rRNA (Gunderson et al. 1987; Föster et al. 1990) showed that oomyceteous organisms, including *P. infestans*, are more related to members of the kingdom Stramenopila than to brown algae.

Polymorphisms at various regions of the mitochondrial genome of *P. infestans* have been successfully employed to study origin, migration and population diversity in this plant pathogen (Carter et al. 1990; Griffith and Shaw 1998; Ristaino et al. 2001; Gavino and Fry 2002; May and Ristaino 2004; Wattier et al. 2003). Mitochondrial restriction fragment length polymorphisms (RFLP) employing different restriction enzyme combinations distinguished four haplotypes of *P. infestans* named Ia, Ib, IIa, and IIb (Carter et al. 1990). The entire mitochondrial genome sequence of the Ib haplotype was sequenced and further characterized (Lang and Forget 1993; Paquin et al. 1997). The type Ib sequence was used to design an RFLP-based method to identify the four haplotypes by amplifying a 964 bp region (P4) and a 1,070 bp region (P2) of mtDNA. *Eco*RI restriction enzyme digestion of the P4 region distinguishes haplotypes Is from haplotypes IIs, whereas *Msp*I restriction enzyme digestion of the P2 region allows the distinction of haplotype Ib from the rest (Griffith and Shaw 1998).

It is unclear which mitochondrial haplotype evolved first in this pathogen with only the genome sequence of the Ib haplotype available at the present time. Limited mutational change analysis at specific mitochondrial regions, as well as a presumed 2 kbp indel present in haplotypes II but absent in type I isolates, led to the suggestion that type I haplotypes may have given rise to the type II haplotypes (Carter et al. 1990; Gavino and Fry 2002). Recently, reports using historic herbarium specimens have shown that the Ia mtDNA haplotype predominated in most of the nineteenth century populations of *P. infestans*, refuting previous hypotheses that the Ib caused nineteenth century epidemics (Ristaino 1998; Ristaino et al. 2001; May and Ristaino 2004). In the present report, the full mitochondrial genome sequences of the other three extant haplotypes are reported and compared with the complete mitochondrial genome sequence of the Ib haplotype in order to delineate the evolution and genomic diversity in the mitochondrion of this important plant pathogen.

Materials and methods

Origin of isolates

Isolates of *P. infestans* used in this study originated from potato plants. They were provided by Dr Louise R. Cooke (Queen's University of Belfast, UK) isolate 15/99; Dr William E. Fry (Cornell University, USA) isolate 94-52 and Dr Francine Govers (Wageningen University, The Netherlands) isolate 80029 (Table 1). All isolates were maintained on rye V8 medium at 18°C. Single zoospore cultures from isolates 15/99 and 80029 were prepared from sporangia produced by 3-week-old cultures grown on rye V8 medium. Zoospores produced by isolate 94-52 germinated but failed to form colonies; therefore, single sporangium cultures were prepared following a procedure similar to that employed for generating single zoospore cultures.

DNA preparation

Cultures derived from either a single zoospore or a single sporangium were grown at 18°C without shaking in pea-broth containing 0.5 g/l of sucrose, 35 mg/l ampicillin and 20 mg/l vancomycin. Cultures of isolates 15/99 and 80029 were grown for 8–12 days and cultures of isolate 94-52 for 15–18 days. Mycelia were harvested by filtration through four layers of cheesecloth and thoroughly rinsed with distilled water. Mycelial mats were frozen at −70°C, and then ground with a mortar and pestle in liquid nitrogen. Total DNA was extracted by a modified cetyltrimethylammonium bromide method (Klimczak and Prell 1984) and mtDNA was separated in a cesium chloride (CsCl) equilibrium density gradient (1.1 g CsCl for each ml of TE buffer) in the presence of bisbezimide (120 μg per ml of CsCl–DNA solution; Klimczak and Prell 1984; Carter et al. 1990; Garber and Yoder 1983). Mitochondrial DNA was purified by three consecutive centrifugations at 200,000*g* at 20°C for 36–40 h using a Beckman ultracentrifuge with 70.1 Ti rotor. CsCl was removed by dialysis against TE buffer at 4°C for 16 h including three changes of buffer. The mtDNA was concentrated by ethanol precipitation in the presence of 1/10 3.0 M sodium acetate, pH 5.2 and DNA was resuspended by dissolving in 10 mM tris–HCL, pH 8.0.

Table 1 Isolates of *Phytophthora infestans* used to extract mtDNA for sequencing

Isolate	Origin	Haplotype[a]	Mating type	GenBank accession no.
80029	The Netherlands	Ia	A1	AY894835
ATCC 16981	ATCC	Ib	A1	NC002387
15/99	United Kingdom	IIa	A1	AY898627
94-52	USA	IIb	A1	AY898628

[a]Haplotypes were defined according to Carter et al. (1990). The sequence of the haplotype Ib was obtained from the GenBank (Paquin et al. 1997)

Cloning, sequencing and assembly

Mitochondrial DNA was nebulized and a small insert library was constructed as described previously (Tettelin et al. 2001) Templates were prepared using a standard alkaline lysis method. Sequencing reactions were performed using BigDye Terminators (Perkin-Elmer, Applied Biosystems, Foster City, CA, USA) with high-throughput reaction conditions and run on ABI 3730×l sequencers (Perkin-Elmer, Applied Biosystems). Shotgun clones were sequenced to generate ~8-10-fold coverage of each *P. infestans* mitochondrial genome. Sequences were trimmed for low-quality bases and vector using an in-house trimming program (Chou and Holmes 2001). The sequences were assembled using TIGR Assembler (Sutton et al. 1995). Sequence gaps and low coverage regions were addressed using re-sequencing, primer walking and sequencing of PCR products.

Annotation

Identification of genes, open reading frames (ORFs) and other biological features in the mitochondrial genome was facilitated using the previously published sequence of the mitochondrial genome of the Ib mtDNA haplotype available in GenBank (accession number NC002387). Sequence analysis among haplotypes was performed using the software Vector NTI suit 9.0. Accession numbers for the mitochondrial genome sequence data are AY894835, AY898627 and AY898628 for the Ia, IIa and IIb genomes, respectively (Table 1).

Phylogenetic analysis

Sequence alignments of mtDNAs and phylogenetic analysis were done with MEGA 2.1 (Kumar et al. 2001). Relationships among haplotypes were inferred by maximum parsimony (MP) analysis with a heuristic tree search and neighbor joining (NJ) tree reconstruction using the Kimura 2-K parameter correction method. Complete or pairwise deletions were used for treating gaps in NJ. Bootstrapping (1,000 replicates) was conducted to test the support for the nodes in the resulting trees. Coalescence-based gene genealogies were constructed using Genetree from SNAP WorkBench (Carbone et al. 2004; Griffiths and Tavaré 1994; Price and Carbone 2005). The genealogy with the highest root probability, the ages of mutations and the time since the most recent common ancestor were estimated from coalescent simulations (Carbone et al. 2004).

Results

We completed the mitochondrial genome sequencing of the Ia, IIa and IIb haplotypes of *P. infestans* and the genome sizes are presented in Table 2. The full mitochondrial genome sequence of the Ib mtDNA haplotype of *P. infestans* was published previously by B. Franz Lang at the University of Montreal as part of the Fungal Mitochondrial Genome Project (Lang and Forget 1993; Paquin et al. 1997). Haplotype Ia had the smallest genome. The Ib haplotype was 35 bp larger than the Ia haplotypes. The genome sizes were 37,922, 37,957, 39,870 and 39,840 bp for the types Ia, Ib, IIa and IIb, respectively. The genome of Ib haplotype was 1,913 bp smaller than the genome of IIa haplotype and 1,883 bp smaller than the genome of IIb haplotype. The genome of the IIa haplotype was 30 bp longer than the genome of the IIb haplotype (Table 2).

Sequence identity between Ia and Ib and between IIa and IIb genomes was 99.99%. All four genomes were rich in adenine and thymine with less than 23% G–C content (Table 2). The coding regions in all four haplotypes accounted for more than 90% of the genome (Table 2). Sixty-one genes with known function were identified including 18 genes involved in electron transport, 2 genes encoding RNA, 16 ribosomal protein genes and 25 transfer RNA genes (Table S1). Six and 13 ORFs whose function is unknown were identified in haplotypes I and II, respectively. Genes were coded in both strands of DNA and their arrangement was identical in all four haplotypes. Genes were separated by intergenic spacers that varied in size from 1 to 785 bp (Fig. 1, Table S1). The pair of genes coding for the ribosomal proteins rps12 and rps7 overlapped and shared 3 bp in common (Fig. 1a). Likewise, an overlap of 70 bp was observed in the pair of genes coding for the *NADH* dehydrogenase subunit 1 (*Nad1*) and subunit 11 (*Nad11*). Twenty-five genes coding for tRNAs were present in all the haplotypes with the exception of the genes coding for arginine and threonine tRNAs (Fig. 1, Table S1).

Polymorphisms among haplotypes were observed in both coding and non-coding regions (Table S2). A large spacer over 2 kbp in length was found in the type II but

Table 2 Genome size and characteristics of the mitochondrial genomes of four haplotypes of *Phytophthora infestans*

Haplotype	Genome size (bp)	Coding		Non-coding		G + C content (%)
		bp	%	bp	%	
Ia	37,922	34,208	90.2	3,714	9.8	22.3
Ib	37,957	34,209	90.1	3,748	9.9	22.3
IIa	39,870	36,016	90.3	3,854	9.7	22.3
IIb	39,840	35,961	90.3	3,879	9.7	22.3

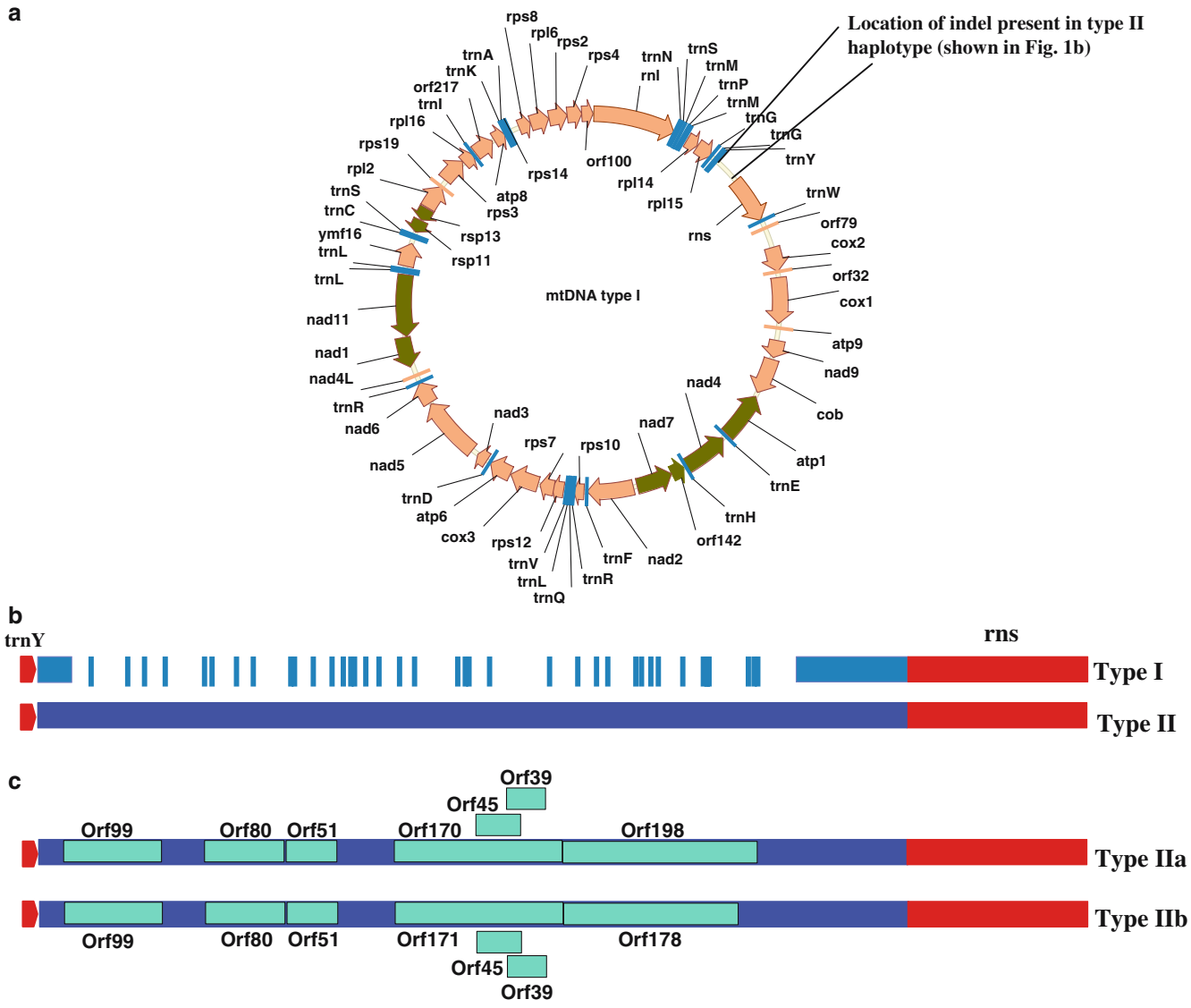

Fig. 1 Schematic diagram of the mitochondrial genome of *Phytophthora infestans*. **a** Genes are indicated with *arrows* and *filled boxes*. The direction of transcription is indicated by *arrows*. The indel that separates the type I and II haplotypes is located between the genes coding for trnY and the small subunit ribosomal RNA (rns). **b** Schematic diagram of the 2 kbp indel that separates the type I from type II haplotypes. The sequence in this indel was identical at the 5′ end (108 bp) and the 3′ end (343 bp) in both type I and II haplotypes. **c** Organization of the ORFs in the IIa and IIb haplotypes. Seven ORFs were found in the type II and absent in the type I haplotypes. ORFs 39 and 45 were nested in ORFs 170 (IIa) and 171 (IIb). ORF 198 was 20 amino acids longer than ORF178

not in the type I haplotypes and was located between the genes trnY and rns (Fig. 1b). Differences between type I and type II haplotypes were observed in the spacer between the genes coding for trnY and the small subunit ribosomal RNA (rns) (4,414–5,198 in type Is, 4,414–7,079 in IIa or 4,414–7,083 in IIb; Table S1). The size of this region varied from 785 bp in type I haplotypes to 2,666 and 2,670 bp in type IIa and IIb haplotypes, respectively (Table S1). This region corresponds to the position where the apparent DNA indel that differentiates type I from type II isolates is located (Carter et al. 1990; Gavino and Fry 2002; Fig. 1). The region in both the type I and type II haplotypes was 94% identical at the 5′ region in the first 108 bp and 99.5% identical at the 3′ region in the last 343 bp (Fig. 1b, Table S3). The remaining 334 bp from type I genomes showed identity with the type II genome in small sections from 2 to 42 bp in length (Fig. 1b, Table S3). Similarity among small regions ranged from 72 to 100% except a 12 bp region that showed only 62% identity. Sequence analysis revealed the presence of 7 ORFs of 40–199 codons including the stop codon in type II haplotypes and the absence of these ORFs and stop codons in type I haplotypes (Fig. 1c). The ORFs 39 and 45 were nested in ORF170 and ORF171 in type IIa and IIb haplotypes, respectively. ORF198 present in haplotype IIa was 20 amino acids longer than ORF178 present in haplotype IIb (Fig. 1c).

Two indels were detected downstream of ORF79 (Fig. 1). A 34 bp indel was present in haplotype IIa (9,133–9,166; the 9,132 position in haplotype IIa corresponded to the positions 9,138 and 7,253 in haplotype IIb and type I haplotypes, respectively) but was absent in the other three haplotypes. Likewise, a 36 bp indel was absent in haplotype Ia but present in the haplotypes Ib, IIa and IIb (7,329–7,364 in haplotype Ib, 9,242–9,277 in haplotype IIa and 9,212–9,247 in haplotype IIb; the position 7,328 in haplotype Ia corresponds to the positions 7,328, 9,241 and 9,211 in haplotypes Ib, IIa and IIb, respectively).

Throughout the whole genome, 170 polymorphic sites were detected among all four haplotypes including 149 single nucleotide substitutions and 21 single base pair indels (Table S2). In non-coding regions, 101 polymorphic sites were located and 69 in coding areas. Differences between type I and II haplotypes were found at 109 polymorphic sites. Fourteen polymorphic sites were detected among type I isolates while 50 polymorphic sites were found among type II haplotypes (Table 3). The type IIb haplotype had the highest number of polymorphic sites compared to the other three haplotypes (Table 3).

Twenty-one single nucleotide substitutions that produced amino acid exchanges were observed between the four haplotypes (Table S4). Eleven substitutions differentiated type I from type II isolates. Three sites differentiated Ia from Ib haplotypes, while seven sites differentiated IIa from IIb haplotypes. Furthermore, from the 21 single base pair changes detected among haplotypes, only one polymorphism present in the region coding for the ribosomal protein rps3 in haplotype Ia shifted the ORF.

Several polymorphic sites were linked to restriction enzyme recognition sites, and 38 of them were associated with endonucleases that are available commercially. Twenty polymorphic sites located in restriction enzyme sequences could be useful to separate haplotypes I from II, 14 polymorphic sites to separate haplotypes IIa from IIb and 4 polymorphic sites to separate haplotype Ia from Ib (Tables S5, S6, S7).

Maximum parsimony analysis and neighbor joining trees were constructed using the whole genome sequence data and both showed similar topologies. Two distinct lineages were observed among the whole mitochondrial genome of *P. infestans*. One of the lineages corresponds to the type I haplotypes and the second to the type II haplotypes. Bootstrap support was 99–100% for the tree (Fig. 2).

Coalescent analysis was used to infer the mutational history, time scale of the origin and evolution of polymorphic variation among the haplotypes. The genome sequences were collapsed into unique haplotypes removing indels (insertion/deletion) and excluding infinite-sites violations using SNAP Map (Carbone et al. 2004; Price and Carbone 2005). Also, the whole region flanked by the genes coding for trnY and the small subunit ribosomal RNA (rns) were removed to obtain a higher stringency in the separation of haplotypes. Thus, the analysis included 81 informative mutations (Fig. 3, Table S2). The four haplotypes of *P. infestans* were distinguished by 44 mutations into two lineages corresponding to the type I and type II haplotypes. Through the process of evolution, a transversion from guanine to thymine subdivided the type II haplotypes into the a and b lineages (mutation 1, Fig. 3). More recently during evolution, a transversion of adenine to thymine separated haplotype Ia from Ib (mutation number 70, Fig. 3). Many more mutations occurred giving rise to the IIb than the IIa haplotypes. The type II haplotypes diverged earlier from the common ancestral lineage than the type I haplotypes. The type IIb lineage diverged earlier and has undergone more mutations than the other three lineages.

Table 3 Number of polymorphic sites observed among four mitochondrial DNA haplotypes of *Phytophthora infestans*

Haplotype	Ia	Ib	IIa	IIb
Ia	0	14	131	152
Ib	–	0	127	151
IIa	–	–	0	50
IIb	–	–	–	0

Polymorphic sites include 149 single base pair substitutions and 21 single base pair indels. In non-coding regions, 101 polymorphic sites were found while 69 were found in coding regions of the genome

Discussion

Comparison of the entire mitochondrial genome of the four haplotypes of *P. infestans* corroborated the difference in size determined previously by restriction enzyme digestion (Carter et al. 1990). Variation in genome size has been considered the main difference between the type I and type II mitochondrial haplotypes. Our findings agree with previous reports in the literature that the mitochondrial genome from type II isolates is larger than the mitochondrial genome from type I isolates. Type I and type II haplotypes differed in size by approximately 1.8 kbp located in the region flanked by the genes coding for trnY and the small subunit ribosomal RNA (rns). This region corresponds to the position that some suggest was an insertion point of DNA (Carter et al. 1990; Gavino and Fry 2002).

Comparison of this indel region of DNA among the four haplotypes suggests that this DNA fragment was not acquired as a single insertion, but rather represents a highly variable region of the mitochondrial genome in *P. infestans*. The content and annotation of extra putative genes and ORFs with unknown function in the type II isolates were described. In the type I haplotypes, this region comprises a long intergenic spacer and several small regions that are homologous to sections of some of the ORFs observed in type II isolates. It is thus likely

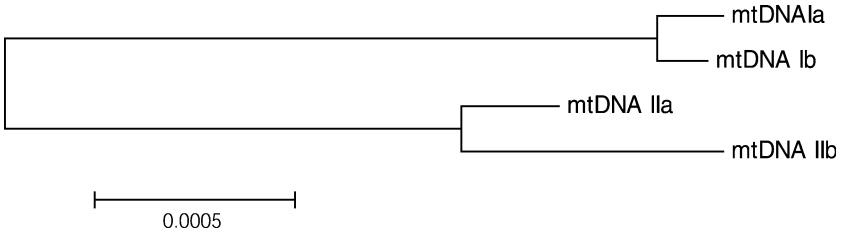

Fig. 2 Phylogenetic relationships among the Ia, Ib, IIa and IIb haplotypes of *Phytophthora infestans* (Carter et al. 1990) inferred by neighbor joining tree reconstruction using the Kimura 2-K parameter correction method. Maximum parsimony analysis with a heuristic tree search showed the same topology. Bootstrapping (1,000 replicates) was conducted for node support

that multiple deletion mutations arose leading to the loss of DNA in this region of the mitochondrial genome in type I haplotypes.

Previous reports postulated that the ancestral mitochondrial genome of *P. infestans* was more similar to the type I haplotypes (Carter et al. 1990; Gavino and Fry 2002). Furthermore, it was suggested that all four haplotypes could have been derived from a single type-Ib-like ancestor (Gavino and Fry 2002). The results presented here indicate that although the type I and type II haplotypes shared a common ancestor, they clearly formed two separate lineages that have evolved independently. The type II lineages did not evolve from the type I lineages as others have suggested (Gavino and Fry 2002). The type II haplotypes diverged earlier in coalescent time than the type I haplotypes. Although the Ib mitochondrial lineage is more similar to the common ancestor than the other mitochondrial lineages since it has undergone fewer mutations, its divergence occurred more recently than the type II haplotypes. Thus the repeated reference in the literature to the Ib haplotype as the "old lineage" or ancestral strain is incorrect (Gavino and Fry 2002; Goodwin et al. 1994a; Grunwald and Flier 2005).

The Ib haplotype was proposed as the ancestral lineage and was also postulated to have been responsible for the nineteenth century potato famine epidemics (Goodwin et al. 1994a). Interestingly, the Ib haplotype was not found in herbarium specimens collected before 1944 from different continents (Ristaino 1998; May and Ristaino 2004), and this haplotype has not been found widely in the Toluca Valley, Mexico, the presumed center of origin of *P. infestans* (Gavino and Fry 2002; Grunwald and Flier 2005; Flier et al. 2003). All the ex-

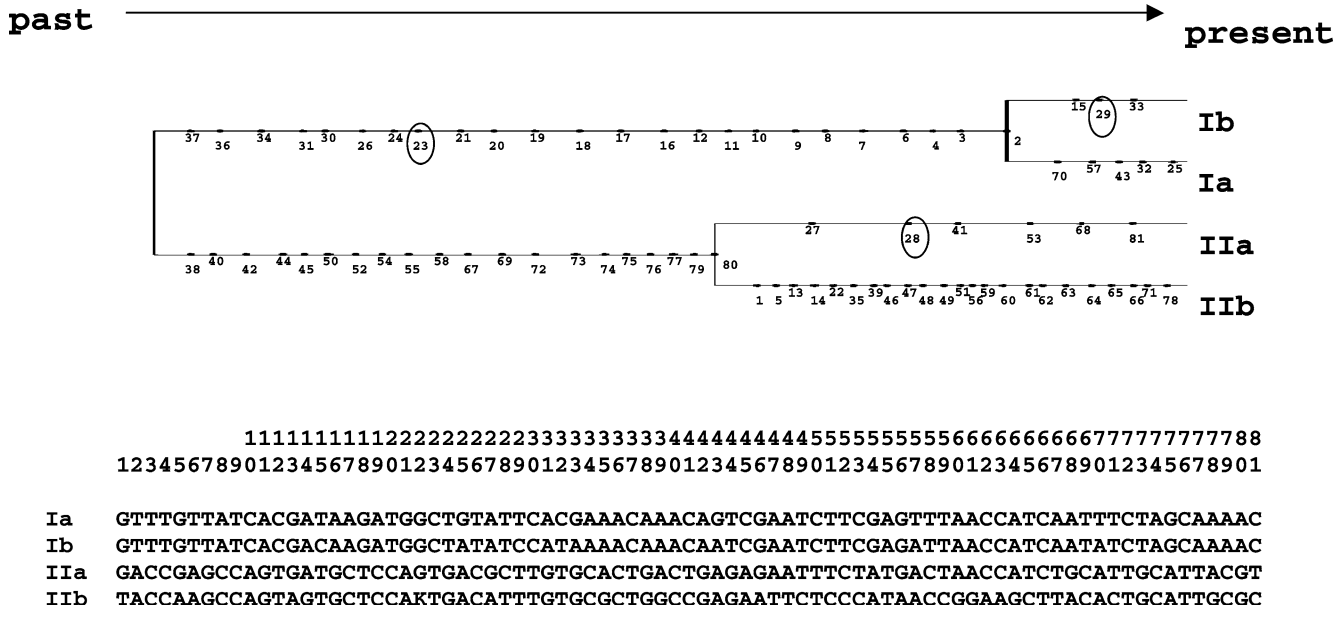

Fig. 3 Coalescence-based genealogy with the highest root probability showing the distribution of mutations among the four mitochondrial lineages of *Phytophthora infestans*. Coalescent analysis to infer the mutational history and the time scale of origin and evolution of polymorphic variation was performed by collapsing the sequences into unique haplotypes, removing indels (insertion/deletion) and excluding infinite sites of violation. The genealogy is based on five million simulations of the coalescent for each run. The direction of divergence is from *left to right* and coalescence is from *right to left*. The nucleotide corresponding to the mutation indicated in the inferred genealogy is presented at the *bottom* of the figure. Mutation *23* indicates the location of the P4 *Eco*R1 restriction site that separates the type I and II haplotypes. Mutations *29* and *28* indicate the location of the P2 *Msp*I restriction sites that separate the a from b haplotypes within the type I and II lineages, respectively. The location of all the mutations in the mitochondrial genome sequence can be found in Table S2

tant mitochondrial lineages of *P. infestans* have been found in the Andean region of South America leading to the hypothesis of an Andean origin for *P. infestans* (Gómez-Alpizar et al. 2004; Ristaino 1998; Ristaino et al. 2001).

The presence of two separate mitochondrial lineages in *P. infestans* was also observed by others recently in independent phylogenetic analysis of species in the genus *Phytophthora* using the cytochrome c oxidase subunits I and II and the *NADH* dehydrogenase subunit I (Kroon et al. 2004; Martin and Tooley 2003). One report showed that one clade included type I isolates and the other clade included type II isolates. Furthermore, combined analysis of nuclear markers, including the β tubulin and the translation elongation factor 1α, led to the same topology (Kroon et al. 2004). Most recently in our lab, analysis of two mitochondrial gene regions from 90 isolates from various locations including Brazil, Bolivia, Ecuador, Peru, Costa Rica, Mexico, the USA and Ireland also demonstrated two distinct mitochondrial lineages (types I and II) that evolved from a common ancestor in the Andean region (Gómez-Alpizar 2004).

We inferred the mutational history and time scale of the origin and evolution of the mitochondrial lineages of *P. infestans* by coalescence analysis. The type II haplotypes diverged from the ancestral strain of *P. infestans* earlier in coalescent time than the type I haplotypes. This divergence occurred before the separation of the type Ia and Ib haplotypes. The number and location of the mutations in the mitochondrial genes and the time of occurrence have been identified. The Ib haplotype is more similar to the ancestral genome of *P. infestans* than the type Ia haplotype. Likewise, the IIa haplotype is more similar to the ancestral strain than the IIb haplotype. Mutation 23, located in the 6 bp sequence that is recognized by the endonucleases *Eco*RI, occurred before the separation of the genome into four haplotypes. This restriction enzyme site has been used to separate type I from type II haplotypes (digestion of the 964 bp PCR product known as P4 region; Carter et al. 1990; Griffith and Shaw 1998). Mutations 29 and 28, located within the type I and II lineages, respectively, have a 4 bp sequence recognized by the endonuclease *Msp*I. Digestion of the 1,070 bp PCR product in the P2 region separates the a haplotypes from b within the type I and II lineages, respectively. These mutations occurred after the divergence that gave rise to the two lineages.

Gene content and arrangement was 100% similar in all four haplotypes suggesting that the mitochondrial genome of *P. infestans* is not highly divergent. Twenty-one single nucleotide substitutions produced amino acid exchanges, but the effect of these amino acid mutations on protein function was not analyzed in this study. The additional ORFs detected in type II haplotypes also distinguished the type I from II lineages.

The proportion and frequency of the four mitochondrial haplotypes are highly variable in nature. The US-1 genotype (Goodwin et al. 1994a, b) with a presumed Ib-type mitochondrial genome (Carter et al. 1990; Griffith and Shaw 1998) was widely dispersed in all potato areas (Goodwin et al. 1994b) except the Toluca Valley in Central Mexico before 1980. All the extant mitochondrial lineages of *P. infestans* do not occur in Toluca Mexico, the presumed center of origin of the pathogen (Gavino and Fry 2002; Gómez-Alpizar 2004). The Ia haplotype predominates in Mexico (Flier et al. 2003; Gavino and Fry 2002). Population studies conducted in different regions of the world have shown that in addition to the Ib haplotypes, the Ia and IIa haplotypes also occur (Day et al. 2004; Ghimire et al. 2003; Knapova and Gisi 2002). In historical samples from the nineteenth century, the Ia haplotypes were dominant in worldwide collections. Although, the Ia haplotype seems to be dominant in most of the modern populations studied today, in countries like Brazil and Taiwan, some haplotypes are more frequently found on tomato or potato plants (Reis et al. 2003; Deahl et al. 2002). The IIb haplotype has been found less frequently in potato fields. This haplotype is the most distant from the common ancestor and contains the highest number of mitochondrial mutations. The rare occurrence of the IIb haplotype in nature may be a result of its mutational history and ancestral divergence.

Evidence from studies in Ecuador indicates that the Andean region is a "hot spot" of mitochondrial diversity and that evolution of new species of *Phytophthora* has occurred there (Kroon et al. 2004; Oliva et al. 2002; Ordoñez et al. 2000). *Phytophthora* species closely related to *P. infestans* have also been reported in Mexico; however, the hosts they infect including *Mirabilis jalapa*, *Ipomoeae longipedunculta* and *Phaseolus vulgaris* were brought to Meso-America from the Andean region. Host specialization may lead to the evolution of novel mitochondrial lineages and/or species in this pathosystem and needs to be explored further. More studies are needed in the Andean region of the world on wild *Solanum* species to study the effect of host selection on mitochondrial genome evolution in nature.

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