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Diversity in and evidence for selection on the mitochondrial genome of *Phytophthora infestans*

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Abstract: Two extant nomenclature systems were reconciled to relate six mitochondrial DNA (mtDNA) haplotypes of *Phytophthora infestans*, the oomycete pathogen causing late blight disease on potato and tomato. Carter's haplotypes I-a and I-b were included in Goodwin's haplotype A, while Carter's haplotypes II-a and II-b were included in Goodwin's haplotype B. In addition, haplotypes E and F were included in Carter's haplotype I-b. The mutational differences separating the various haplotypes were determined, and we propose that either haplotype I-b(A) or haplotype I-a(A) is the putative ancestral mtDNA of *P. infestans*, because either can center all the other haplotypes in a logical stepwise network of mutational changes. The occurrence of the six haplotypes in 548 isolates worldwide was determined. Haplotypes I-a and II-a were associated with diverse genotypes worldwide. As previously suggested, haplotype I-b was found only in the US-1 clonal lineage and its variants (n = 99 isolates from 16 countries on 5 continents), and haplotype II-b was limited to the US-6 clonal lineage and its derivatives (n = 36). In a confirmation of a previous suggestion, the randomly mating population in the Toluca Valley of central Mexico (n = 78) was monomorphic for mtDNA haplotype I-a(A). We hypothesize that selection there may be driving the dominance of that single mtDNA haplotype.

Key Words: mtDNA haplotypes, oomycete, population genetics

INTRODUCTION

Most hypotheses concerning the population genetics of *Phytophthora infestans* emerged from analyses of nuclear DNA (nDNA), but recently available data on mitochondrial DNA (mtDNA) may generate new findings. During the last decade, much has been learned about mtDNA of oomycetes in general and *P. infestans* in particular. For example, the circular 37 914 bp mtDNA of *P. infestans* has been completely sequenced (Lang and Forget 1993). The *P. infestans* mtDNA is A + T-rich (76%) and is predominantly composed of coding sequence (Paquin et al 1997). Chesnick and colleagues (1996) analyzed the sequence of the mitochondrial *nad 4L* gene and affirmed that the oomycete *Phytophthora* is more closely related to the chromophytic algae *Chrysodisymus synuroides* and *Ochromonas danica* (both groups are placed in the Kingdom Stramenopila) than to filamentous fungi. In addition, stramenopile mtDNAs have low variability in genome size and gene complement (Chesnick et al 2000).

An initial report of limited mitochondrial diversity in *P. infestans* (Klimczack and Prell 1984) has been confirmed several times via two systems of nomenclature. Carter et al (1990) used RFLP analysis to describe four mtDNA haplotypes (I-a, I-b, II-a, and II-b) among 24 isolates from 11 countries. In a subsequent study, Griffith and Shaw (1998) used mtDNA sequence information (Paquin et al 1997) to devise a polymerase chain reaction (PCR)-RFLP method to detect Carter's four haplotypes among 90 isolates (with the majority from Russia (n = 57) and the United Kingdom (n = 16)). At nearly the same time as Carter's work, Goodwin (1991) described by Southern analysis four haplotypes (A, B, C, and D) among 173 isolates, mainly from Mexico (n = 86), Peru (n = 15), and Europe (n = 36). In addition, Koh et al (1994) found two other haplotypes (E and F) using Goodwin's method to assay 124 isolates from east Asian countries. Although many isolates have been tested for mtDNA diversity, two unrelated methods with two autonomous nomenclatures have been used on different isolates from various locations—creating some confusion about generalities from these studies.

In general, the two nomenclature systems exhibit common themes. Carter's haplotype groups I and II seem to correspond to Goodwin's haplotypes A and B, respectively (Carter et al 1991, Goodwin 1991). This means that Goodwin's haplotype A includes both of Carter's haplotypes I-a and I-b; and Goodwin's haplotype B includes both of Carter's haplotypes II-a and II-b. However, it is not yet explicitly clear how Goodwin's haplotypes C and D, and Koh's haplotypes E and F, relate to Carter's haplotypes.

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The identification of mutational changes in mtDNAs may provide insights into their relationships. In *P. infestans*, Carter et al (1990) concluded that the group II haplotype arose by an insertion of a 2 Kb fragment into group I. This suggests that the ancestral mtDNA does not have the 2 Kb insert. On the other hand, Goodwin (1991) observed that haplotype C differed from haplotype A by an insertion, and haplotype D might have undergone several mutations. Koh et al (1994) inferred that haplotype E had a deletion whereas haplotype F had an insertion when compared to haplotype A. These comparisons point to the possibility that haplotype A (group I) may be the ancestral mtDNA from which other haplotypes were generated through mutations. Additional markers may reveal other associations among the diverse mtDNA haplotypes.

An investigation of isolates from the probable center of origin of *P. infestans* in central Mexico may unravel clues on the evolution of its mitochondrial genome. One would expect to find diverse haplotypes, and perhaps the ancestral mtDNA, in the Toluca Valley of central Mexico where the *P. infestans* population has the greatest diversity in nDNA (Goodwin et al 1992b, Goodwin 1997). Goodwin and coworkers (Goodwin 1991, Goodwin et al 1992b) had sampled the populations of *P. infestans* in different parts of Mexico. Interestingly, they found only two haplotypes, A and D, among 33 diverse (nDNA) isolates from central Mexico (Goodwin 1991, Goodwin et al 1992b). Haplotype B was absent from central Mexico, yet it was more common than haplotype A among 64 isolates from northern Mexico (Goodwin 1991).

Knowledge of mitochondrial haplotypes has led to some interesting observations concerning populations of *P. infestans*. First, some haplotypes seem to have limited geographical distribution. For example, haplotypes I-a and II-a have been associated with the populations introduced into Europe during the 1970s (Carter et al 1991, Day and Shattock 1997, Griffith and Shaw 1998). Haplotype II-b has been limited to North America (Carter et al 1991, Griffith and Shaw 1998). Additionally, certain mtDNA haplotypes have been associated with particular nDNA genotypes. Haplotype I-b has been associated with the US-1 clonal lineage (Griffith and Shaw 1998). It is hypothesized that the US-1 lineage survived an extreme genetic bottleneck during its initial migration from Mexico into the United States and the rest of the world (Fry et al 1993, Goodwin et al 1994). Haplotypes E and F were found only among variants of the US-1 genotype in the Philippines (Koh et al 1994). These haplotypes may be closely related to haplotype I-a or I-b. Finally, the II-b or B haplotype was initially found only in the US-6 clonal lineage (Gavino 1999), and has recently been identified in sexual progeny of the US-6 lineage (Gavino et al 2000).

The present study was undertaken to expand our knowledge of the population genetics of *P. infestans* through analysis of mitochondrial diversity. First, the two mitochondrial nomenclature systems were experimentally reconciled and the relationships among haplotypes were determined. Then we tested the hypotheses that: (i) haplotype I-b is ancestral to the other haplotypes; (ii) haplotype I-b is extant in the population from central Mexico; and (iii) all haplotypes occur in many different nuclear backgrounds. Our initial approach was to apply both the system devised by Carter et al (1990) as modified by Griffith and Shaw (1998) and the Southern analysis of Goodwin (1991) to a common set of isolates known to be diverse for mtDNA haplotype. We also examined other regions of the mitochondrial genome to find additional polymorphisms that might help determine relationships among the various haplotypes.

MATERIALS AND METHODS

Reconciliation of two nomenclature systems.—Twenty-six isolates representing individuals of the haplotypes of Carter (I-a, I-b, II-a, and II-b), Goodwin (A and B), and Koh (E and F) were obtained from the *Phytophthora* Culture Collection at Cornell University (TABLE I). Representatives of Goodwin's mtDNA haplotypes C and D were originally identified in single isolates that have since died, and were therefore not available. Each isolate was grown on pea broth and lyophilized before extracting total DNA as previously described (Goodwin et al 1992a).

Southern analyses to identify haplotypes A, B, E, and F were done according to Goodwin (1991, Koh et al 1994). Briefly, total DNA was extracted from isolates to be assayed, digested with *Eco* RI, subjected to agarose gel electrophoresis and blotted to nylon membrane. The mtDNA, to be used as a probe, was isolated by centrifugation with cesium chloride-bisbenzimide (Garber and Yoder 1983) and labeled with ^{32}P. Haplotypes were identified based on RFLP fingerprints as described by Goodwin (1991) and Koh et al (1994).

Carter's haplotypes were determined using the PCR-RFLP method devised by Griffith and Shaw (1998). Primer pairs 1(F5′ GCAATGGGTAAATCGGCTCAA 3′ and R5′ AAACCATAAGGACCACACAT 3′), 2 (F5′ TCCCTTTGTCCTCTACCGAT 3′ and R5′ GCTTATGCTTCAGTTGCTCAT 3′), and 4 (F5′ TGGTCATCCAGAGGTTT ATGTT 3′ and R5′ CCGATACCGATACCAGCACCAA 3′) designed by Griffith and Shaw (1998) were used to amplify mtDNA by PCR. Primer pair 3 by Griffith and Shaw (1998) was not used in this study because it did not provide any additional information. Each PCR reaction (100 μL) consisted of 1 × Thermo buffer (50 mM KCl, 10 mM Tris-HCl), 2.75 mM $MgCl_2$, 200 μM dNTPs, 0.325 μM each primer, 5 units *Taq* DNA

TABLE I. Reconciliation of the two nomenclature systems for mitochondrial haplotypes of *P. infestans*

Carter's mtDNA haplotype[a]	Goodwin's mtDNA haplotype[b]	Location	Isolate accession number[c]	PCR product (P) sequenced
I-a	A	Mexico-central (Calimaya)	ME885015	P1, P2
I-a	A	Mexico-central (Calimaya)	ME885070	
I-a	A	Mexico-central (Calimaya)	ME885113	P2
I-a	A	Mexico-central (Banco Metepec)	ME885280	P6
I-a	A	Mexico-central (Banco Metepec)	ME885284	P1, P2
I-a	A	Mexico-central (Banco Metepec)	ME885317	P2
I-a	A	Mexico-central (Atizapan)	ME885420	P2
I-a	A	Mexico-northeast (Saltillo, Coahuila)	ME880155	P2
I-a	A	United States	US920210	
I-a	A	Peru	PE960040	P2
I-a	A	Poland	PO880058	
I-b	A	Philippines	PH910011	
I-b	A	Philippines	PH910015	
I-b	A	Philippines	PH910018	
I-b	A	Philippines	PH910023	
I-b	A	Philippines	PH910024	
I-b	E	Philippines	PH910014	
I-b	E	Philippines	PH910021	
I-b	F	Philippines	PH910016	
I-b	F	Philippines	PH910019	
I-b	F	Philippines	PH910020	
II-a	B	Mexico-northeast (Saltillo, Coahuila)	ME880161	P1, P2, P4
II-a	B	Bolivia	BO96009	P6
II-a	B	Israel	IS840009	
II-b	B	Mexico-northwest (Los Mochis, Sinaloa)	ME890182	P6
II-b	B	United States	US920159	P2

[a] Data obtained in this study.
[b] Haplotypes E and F were described by Koh et al. (1994).
[c] Source: *Phytophthora* Culture Collection at Cornell University.

polymerase, and 100 ng DNA. Amplification was conducted with one cycle of 94 C for 90 s followed by 40 cycles of 94 C for 40 s, 55 C for 60 s, and 72 C for 90 s (Griffith and Shaw 1998). The PCR products were separated on 2% agarose gels and visualized with ethidium bromide using standard techniques. The products (P1, P2, and P4) were numbered according to the primer pairs used for amplification and digested with specific restriction enzymes. The following sizes were expected for products: P1 = 1.1 Kb, P2 = 1.2 Kb, and P4 = 0.96 Kb. Products P1, P2, and P4 were digested with *Cfo* I, *Msp* I, and *Eco* RI, respectively. Haplotypes I-a, I-b, II-a, and II-b were identified by their restriction patterns as described by Griffith and Shaw (1998) following the mtDNA nomenclature system of Carter et al (1990).

Relationships among haplotypes.—Specific mutations distinguishing the different haplotypes were identified. First, fragment sizes cleaved by *Eco* RI during Southern analysis were compared with those expected based on analysis of the full-length mtDNA sequence (haplotype I-b) provided by Dr. Franz Lang (http://megasun.bch.umontreal.ca/People/lang/FMGP). Second, the length mutations in haplotypes E and F were verified by Southern analysis using the PCR product P4 as probe. This probe was chosen because it had the potential to detect insertions or deletions in three *Eco* RI fragments (5.7 Kb, 0.4 Kb, and 6.5 Kb) as shown in FIG. 1.

Nucleotide substitutions resulting in gain or loss of restriction sites were ascertained by sequence analysis. DNA sequences of PCR products (P1, P2, and P4) from selected isolates in TABLE I were compared with the full-length sequence of a mtDNA (haplotype I-b) from *P. infestans*. For all sequence analysis, the PCR products were purified using QIAquick PCR Purification Kit (QIAGEN Inc., California) prior to automated sequencing at the BioResource Center, Cornell University. The sequence data were edited (EDITSEQ) and aligned (MEGALIGN) by computer programs in the Lasergene Biocomputing Software package (DNASTAR 1994).

Analysis of additional regions of the mtDNA.—Additional diversity was sought by analyzing other portions of the mitochondrial genome in a subset of the isolates from central Mexico. Three additional primer pairs were designed: primer pair 5 (F5′ AAGGGAAGGTAGCATAGTCTGG 3′ and R5′ AAAAGTTTGGGCCGTGTCTC 3′), primer pair 6 (F5′ GAGTATTAGCCTTCTAAGC 3′ and R5′ CTGTTTTACCGA-

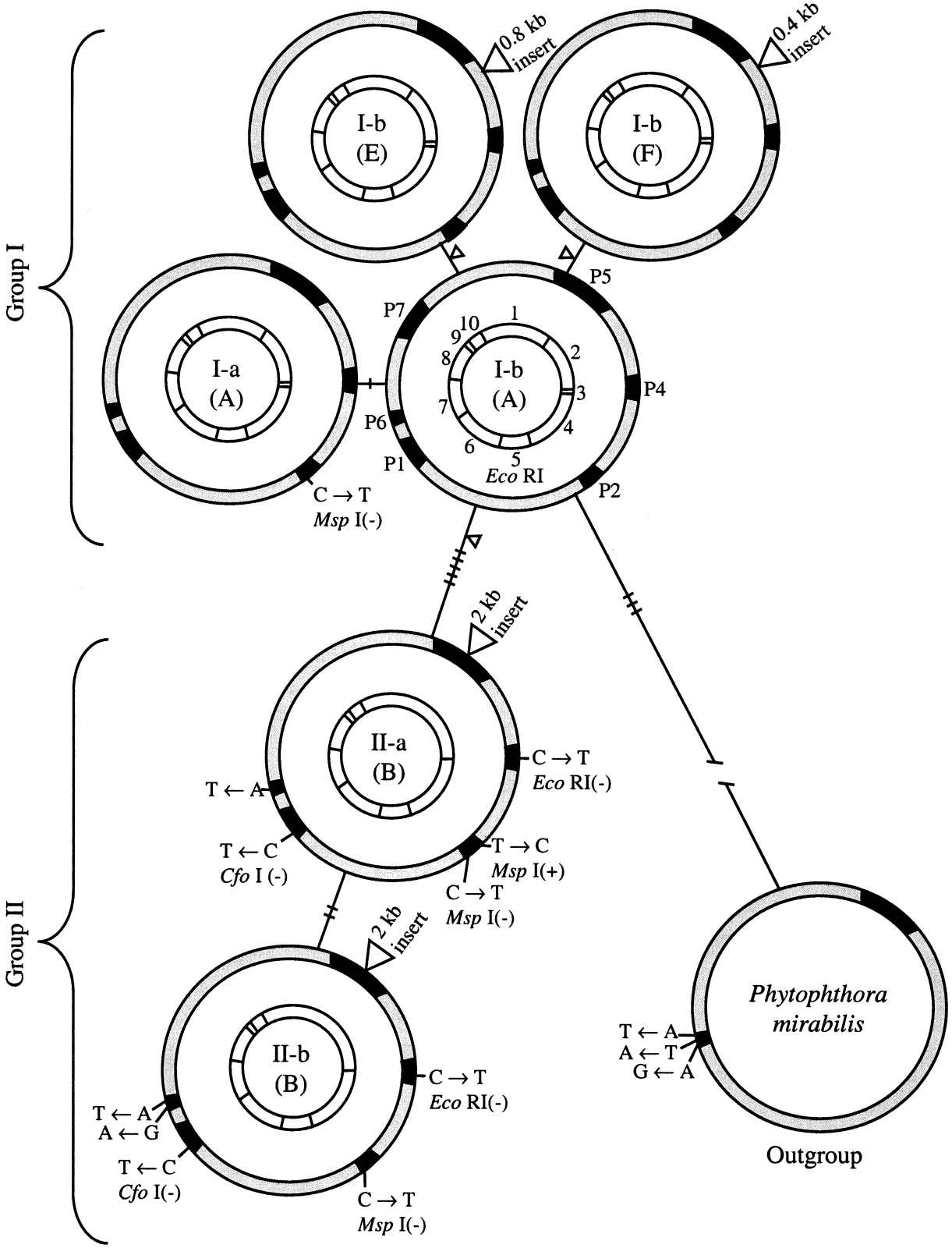

FIG. 1. Proposed relationships among the mtDNAs of *P. infestans*. Carter's haplotypes I-a, I-a, II-a, and II-b and Goodwin's haplotypes A, B, E, and F were reconciled and grouped (I and II). The mtDNA diagram was adapted from Griffith and Shaw (1998). Haplotype I-b(A) shows an *Eco* RI restriction map (inner circle) based on the full sequence of *P. infestans* mtDNA (Paquin et al 1997). This is a minimum length tree with no character reversals inferred. Fragments 1–10 corresponding to the following sizes: 1 = 7.6 kb, 2 = 5.7 kb, 3 = 0.4 kb, 4 = 6.5 kb, 5 = 3.3 kb, 6 = 4.4 kb, 7 = 4.7 kb, 8 = 2.9 kb, 9 = 0.6 kb,

GATATCG 3′), and primer pair 7 (F5′ TCCTCTAACGGG TAATCCTTGAC 3′ and R5′ AACGACCTTTAAATTGTA-GACGA 3′). The following sizes were expected for products P5 = 2.5 Kb, P6 = 0.64 Kb, and P7 = 1.98 Kb. Primer pair 5 was designed to amplify a large region including the site for the 2 Kb insertion (FIG. 1) and was also used to amplify *P. mirabilis* (isolates OS-16 and OS-18), a close relative that served as an outgroup for comparisons. The conditions to amplify P5 were as follows: 1 cycle of 94 C for 90 s and 40 cycles of 94 C for 40 s, 55 C for 3 min, and 72 C for 90 s. Primer pair 6 was designed to amplify the region containing inverted repeats which is thought to be variable in mtDNAs of oomycetes and chromophytic algae (Coleman et al 1991). P6 includes the *nad* 4L and *nad* 1 genes in *P. infestans* and *P. mirabilis*. The conditions to amplify P6 and P7 were the same as those used for P1, P2, and P4. Some PCR products were digested with restriction enzymes to assay more mtDNA sites in the subset of 30 isolates from central Mexico: P4 + *Dra* I, P5 + *Dra* I, P7 + *Dra* I, P7 + *Eco* RI, P7 + *Rsa* I, and P7 + *Taq* I. The digested fragments were separated by electrophoresis on 2% agarose gel and visualized by staining with ethidium bromide.

Search for additional mitochondrial haplotypes among additional isolates.—A large sample of isolates (n = 548) obtained from the *Phytophthora* Culture Collection at Cornell University (Forbes et al 1998) was analyzed for mtDNA haplotype. The sample included isolates from 24 countries on six continents (TABLE II). The largest number of isolates had been obtained from Mexico (n = 137) with 18 from northeast Mexico, 32 from northwest Mexico, and 87 from central Mexico. Haplotypes Ia, Ib, IIa, and IIb were determined using the PCR-RFLP method (Griffith and Shaw 1998) as described above. The haplotypes according to Goodwin were determined by Southern analysis, using purified mtDNA (Goodwin 1991, Koh et al 1994). The nuclear genetic background (allozymes and RG57 fingerprint) of approximately 75 isolates had not been determined previously, so these data were generated using routine methods (Goodwin et al 1992a, 1995).

RESULTS

Relationships among nomenclature systems.—There was similarity but not complete coincidence between the two nomenclature systems as shown in TABLE I. A combination of the two systems permitted unambiguous designation. FIGURE 1 shows a minimum length tree with no character reversals inferred. Carter's haplotypes I-a and I-b were contained in Goodwin's haplotype A and the combined designations are I-a(A) and I-b(A) (FIG. 1). The only polymorphism detected between haplotypes I-a(A) and I-b(A) was a single nucleotide substitution from C→T that resulted in the loss of a *Msp* I site in P2 region of haplotype I-a(A) (FIG. 1).

Carter's haplotype I-b included haplotypes E and F as well as A (TABLE I) for which new designations are I-b(E) and I-b(F) as well as I-b(A) in FIG. 1. As Koh et al (1994) noted, the *Eco* RI restriction patterns of haplotypes I-b(A), I-b(E), and I-b(F) were identical, except that the 5.7 Kb band was absent in both haplotypes I-b(E) and I-b(F) (FIG. 2). Moreover, haplotype I-b(F) has a unique 6.1 Kb band as shown in FIG. 2A (Koh et al 1994). In this study, probe P4 hybridized more strongly to the 5.7 Kb band than the 6.5 Kb band in haplotype I-b(A) as expected because of sequence similarity (FIG. 2B). In contrast, probe P4 hybridized intensely to the 6.5 Kb band of haplotype I-b(E) suggesting that there could be two fragments of the same 6.5 Kb size. It may be possible that a 0.8 Kb fragment inserted into the 5.7 Kb fragment yielding the second 6.5 Kb band (FIG. 1). On the other hand, probe P4 hybridized equally to bands 6.5 Kb and 6.1 Kb in haplotype I-b(F) (FIG. 2B) indicating that a 0.4 Kb fragment may have inserted into the 5.7 Kb fragment generating the 6.1 Kb band (FIG. 1).

Carter's haplotypes II-a and II-b were included in Goodwin's haplotype B (TABLE I, FIG. 1). The 2 Kb insertion described by Carter et al (1990) for haplotype group II was confirmed by amplification with primer pair 5. The size of P5 for haplotypes II-a and II-b was 4.5 Kb compared to 2.5 Kb for haplotypes I-a, I-b, and *P. mirabilis* (data not shown).

Nucleotide substitutions differentiated other haplotypes. II-a and II-b were additionally distinguished from I-b(A) by several substitutions: (i) in the P1 region a change from C→T resulted in the loss of a *Cfo* I site; (ii) in the P2 region a change from C→T resulted in the loss of an *Msp* I site; (iii) in the P4 region a change from C→T resulted in the loss of an *Eco* RI site; and (iv) in the P6 region there was a change from A→T (FIG. 1). Only two nucleotide substitutions distinguished haplotype II-a from II-b (FIG. 1). In P2 region, a substitution from T→C resulted in the presence of a second *Msp* I site in haplotype II-a but not in II-b.

Our search for additional variation in *P. infestans* mtDNAs had limited success. Sequences for genes

←

and 10 = 1.5 kb. Regions (black) P1, P2, P4, P5, P6, and P7 were amplified and assayed for polymorphisms. Insertions (∇) were found in haplotypes I-b(E), I-b(F), II-a(B), and II-b(B). Single nucleotide substitutions (→) resulting in the gain (+) or loss (−) of restriction sites were detected in P1, P2, P4, and P6 by sequencing. The number of nucleotide substitutions (|) and insertions (∇) that differ between haplotypes are indicated in the branches connecting the haplotypes.

TABLE II. Geographic locations for *P. infestans* samples (n = 548) assayed for mitochondrial diversity

Location	Year	Sample	Source
North America			
Mexico			
Toluca (central)	1983–1989	18	W. E. Fry, J. Matuszak
Atizapan (central)	1988	12	J. Matuszak
Metepec (central)	1988	36	J. Matuszak
Calimaya (central)	1988	12	J. Matuszak
Puebla (east central)	1989	9	J. Matuszak
Saltillo, Coahuila (northeast)	1988	18	P. W. Tooley, W. E. Fry
Los Mochis, Sinaloa (northwest)	1989–1993	32	S. Bergeron
United States	1979–1996	28	H. D. Thurston, J. Dufel, L. J. Spielman, N. T. Young, S. B. Goodwin
Canada	1992–1994	15	G. McCollum, D. Ormrod
South America			
Argentina	1994	20	H. Hohl
Bolivia	1994	17	E. Fernandez Northcote
Brazil	1984–1987	3	S. H. Brommenschenkel
Colombia	1990	4	G. A. Forbes
Ecuador	1990–1992	46	G. A. Forbes
Peru	1984–1997	66	P. W. Tooley, G. A. Forbes, R. Nelson
Europe			
France	1993	2	—
Germany	1990	27	S. Daggett
Netherlands	1970–1989	11	L. Davidse, W. E. Fry
Poland	1987–1990	64	L. S. Sujkowski
United Kingdom	1989	19	P. W. Tooley
Asia			
Japan	1988	17	N. Sato, P. W. Tooley
South Korea	1991	7	Y. J. Koh
Taiwan	1991	3	G. L. Hartman
Philippines	1990–1996	19	P. van der Zaag, Z. Ganga
Isreal	1993–1997	5	S. Klopman
Soviet Union	1983–1990	14	Y. Dyakov
Africa			
Kenya	1995	7	G. A. Forbes
Rwanda	1985	8	H. Hohl
Uganda	1995	8	G. Forbes
Australia	Late 1980s	1	T. Swiech
Total		548	

nad 4L and *nad 1* in P6 were identical for all four haplotypes (I-a, I-b, II-a, and II-b) of *P. infestans* and the two isolates of *P. mirabilis*. However the region of inverted repeats flanking the two genes provided limited nucleotide differences (FIG. 1). Haplotypes I-a and I-b were identical in the inverted repeat region of P6, but differed from haplotypes II by a common nucleotide (A→T) (FIG. 1). Additionally, haplotype II-b differed from II-a by one nucleotide (G→A) in this inverted repeat region of P6 (FIG. 1).

Relationships among haplotypes.—The characterizations of the six mtDNA haplotypes illustrate how they differ from each other. The 2 Kb insert characteristic of haplotype group II was absent in *P. mirabilis*, suggesting that the ancestral mtDNA of *P. infestans* belongs to group I (FIG. 1). Haplotypes I-a(A), I-b(E), and I-b(F) each differed from I-b(A) by one unique change. No other haplotype was related to all of the others by only a single difference. Thus, each haplotype could have been derived from I-b(A). Haplotypes II-a and II-b differed from I-b(A) by several common changes, but they differ from each other by only a few changes, and appear to be closely related.

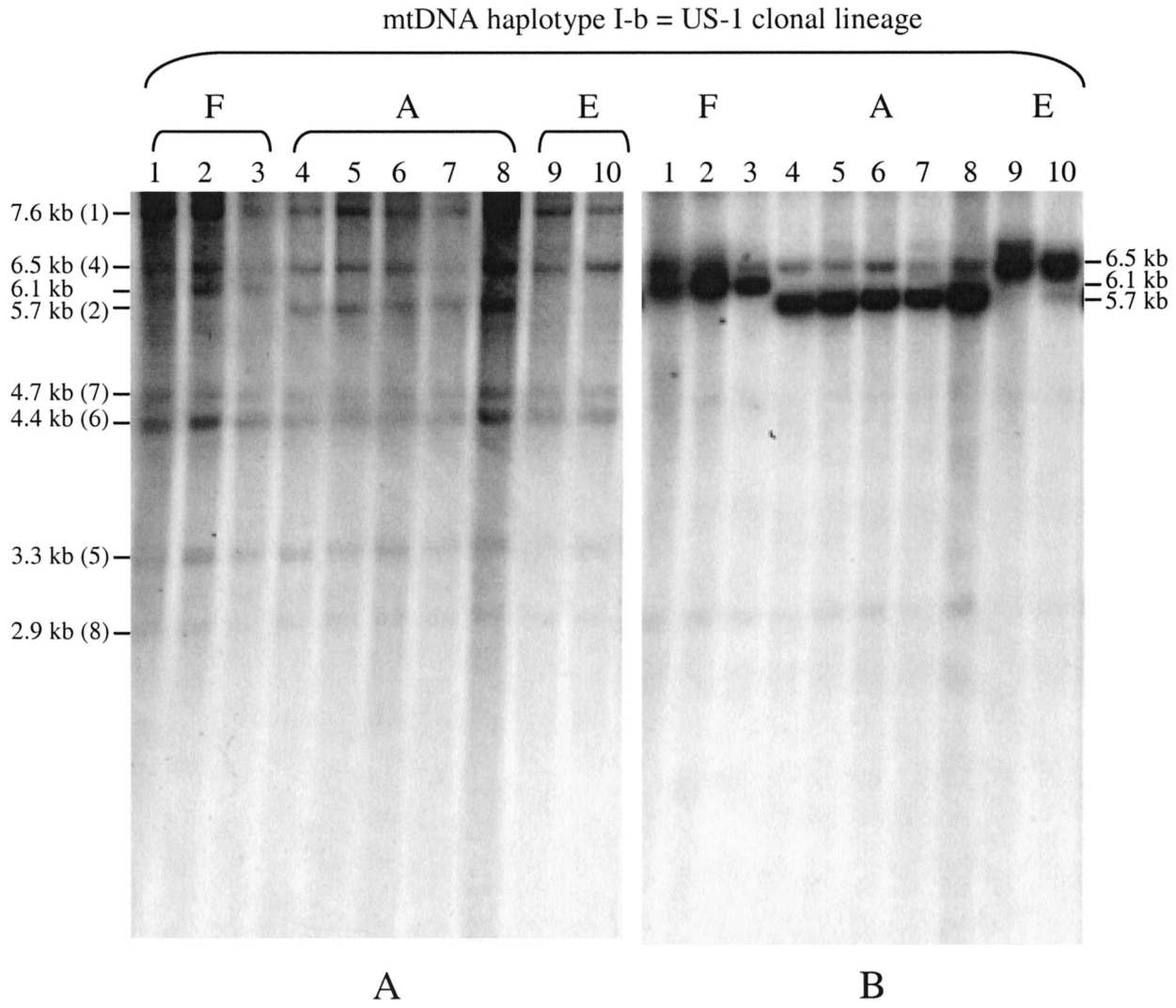

FIG. 2. RFLP patterns of Goodwin's haplotypes A, E, and F included in Carter's haplotype I-b. Total DNA digested with *Eco RI* was hybridized with (A) mtDNA purified from isolate IS840009 and (B) PCR product P4 from isolate ME880128. Isolates are of the US-1 clonal lineage from the Philippines. Lanes 1 through 10 correspond to DNA from the following isolates: 1. PH910020, 2. PH910016, 3. PH910019, 4. PH910015, 5. PH910018, 6. PH910011, 7. PH910023, 8. PH910024, 9. PH910014, and 10. PH910023. Numbers in parenthesis correspond to the fragments in the *Eco* RI restriction map of haplotype I-b(A) in FIG. 1.

mtDNA in the Toluca Valley.—There was only a single mtDNA haplotype (I-a) detected in isolates from the region of the Toluca Valley (TABLE III). This confirms the earlier suggestion by Goodwin (1991) that this population (with the greatest nDNA diversity) has very limited mtDNA diversity. The isolates from Toluca were obtained mainly from commercial potato cultivars such as Alpha and Rosita, while a few isolates (n = 5) were from wild species (data not shown). The monomorphic nature of haplotypes in the isolates from Toluca was maintained even after additional mtDNA restriction sites were assayed in a subset of 30 isolates. Uniform restriction patterns were observed when the following PCR products were digested with the following restriction enzymes: P4 + *Dra* I, P5 + *Dra* I, P7 + *Dra* I, P7 + *Eco* RI, P7 + *Rsa* I, and P7 + *Taq* I. Moreover, five isolates from Calimaya, Metepec, and Atizapan in Toluca had exactly the same 182 bp sequence of the P2 region.

Mitochondrial diversity in other parts of Mexico.—Three of Carter's haplotypes (I-a, II-a, and II-b) were found in various regions of Mexico. Haplotype I-b, however, was not found in any of the Mexican isolates (n = 137) (TABLE IV). Just east of the Toluca Valley in Puebla (east central), haplotypes I-a and II-a were observed among diverse nDNA genotypes (TABLES I, V). In Saltillo (northeast), both haplotypes I-a and II-

TABLE III. Distribution of mitochondrial haplotype I-a(A) associated with diverse nuclear backgrounds

Location	Year	nDNA genotype	Allozyme genotype		Mating type	Sample size
			Gpi	*Pep*		
North America						
Mexico	1983–1989	Diverse	Diverse	Diverse	A1 & A2	18
Toluca (central)	1983–1989	Diverse	Diverse	Diverse	A1 & A2	18
Atizapan (central)	1988	Diverse	Diverse	Diverse	A1 & A2	12
Metepec (central)	1988	Diverse	Diverse	Diverse	A1 & A2	36
Calimaya (central)	1988	Diverse	Diverse	Diverse	A1 & A2	12
Puebla (east central)	1989	Diverse	Diverse	Diverse	A1 & A2	4
Saltillo, Coahuila (northeast)	1988	Diverse	100/100	100/100	A1 & A2	8
Los Mochis, Sinaloa (northwest)	1989	US-8	100/111/122[a]	100/100	A2	8
Los Mochis, Sinaloa (northwest)	1989	LM-1	100/100	92/100	A1	1
United States	1992	US-7	100/111	—	A2	5
United States	1992	US-8	100/111/122[a]	—	A2	2
Canada	—	BC-1	—	—	A2	5
Canada	—	US-7	100/111	—	A2	8
South America						
Peru	1994	clonal	100/100	—	A1	3
Peru	1995–1997	clonal	100/100	100/100	A1	9
Argentina	1994	AR-1	100/100	92/100	A2	2
Argentina	1994	AR-3	100/100	100/100	A2	1
Brazil	—	—	100/100	—	A2	1
Europe						
Netherlands	1984	Diverse	90/100	—	A1	5
Netherlands	1984	Unique	100/100	—	A1	1
Netherlands	1984	NL-4	90/100	—	A1	1
Netherlands	1987	Unique	—	—	A2	1
Netherlands	—	NL-6	100/100	—	A1	1
United Kingdom	1989	Diverse	—	—	—	7
Poland	1988–1990	Diverse	90/100	—	A1 & A2	7
Poland	1988–1990	Diverse	100/100	—	A1 & A2	7
Poland	1989	PO-26	90/90	—	A1	1
Germany	1990	Diverse	—	—	A1	20
France	1993	—	—	—	—	2
Africa						
Rwanda	—	RW-2	90/100	—	A1	3
Asia						
Israel	—	Clonal	—	—	—	5
Soviet Union	1983	—	90/100	—	A1	1
Australia	Late 1980s	—	—	—	—	1

[a] Data on mating type, nDNA genotypes, and allozyme genotypes were obtained from the *Phytophthora* database at Cornell University (Forbes et al 1998).

[a] Trisomic for the chromosome encoding *Gpi*.

a were among diverse nDNA genotypes (TABLES I, V). In contrast, haplotype II-b (n = 23) was found only in Los Mochis (northwest) and was associated with the US-6 genotype.

Genetic and geographic distribution of mtDNA haplotypes.—Two mtDNA haplotypes (I-b(A) and II-b(B)) were tightly associated with particular clonal lineages. Haplotype I-b was entirely coincident with the US-1 clonal lineage and its variants (TABLE IV). It was present in the oldest collections from countries such as the United States (1979), Peru (1984) and the Netherlands (1970). From a total of 99 isolates with haplotype I-b, 84 had exactly the US-1 nuclear genotype and 15 were closely related (one or two differences) and previously regarded as variants of the US-1 clonal lineage (TABLE IV). Haplotypes I-b(E) and I-b(F) were found only in the Philippines. No other isolates

TABLE IV. Distribution of mtDNA haplotype I-b(A, E, F) associated with the US-1 clonal lineage

Location	Year	nDNA genotype	Allozyme genotype		Mating type	Sample size
			Gpi	*Pep*		
North America						
United States	1979	US-1.1	86/100	—	A1	1
United States	1987–1993	US-1	86/100	—	A1	7
Canada	1980	US-1.1	86/100	100/100	A1	1
Canada	1992	US-1	86/100	92/100	A1	1
South America						
Peru	1984–1997	US-1	86/100	—	A1	18
Brazil	1986	US-1.4	86/100	100/100	A1	1
Brazil	1987	US-1.7	100/100	92/100	A1	1
Colombia	1990	US-1	86/100	92/100	A1	1
Ecuador	1995–1996	US-1	86/100	92/100	A1	4
Ecuador	1995		86/100	100/100	A1	2
Europe						
Netherlands	1970	US-1	—	—	—	1
United Kingdom	—	—	86/100	—	A1	1
Poland	1987–1988	US-1	86/100	—	A1	8
Asia						
Japan	1988	—	86/100	92/100	A1	8
Taiwan	1991	US-1	86/100	—	A1	3
Philippines	1990–1996	US-1	86/100	—	A1	19
Soviet Union	1990	US-1	86/100	100/100	A1	2
Africa						
Kenya	1995	—	86/100	92/100	A1	4
Kenya	1995	—	100/100	92/100	A1	3
Rwanda	—	US-1	86/100	—	A1	5
Uganda	1995	—	86/100	92/100	A1	4
Uganda	1995	—	100/100	92/100	A1	4
Total						99

Data on mating type, nDNA genotypes, and allozyme genotypes were obtained from the *Phytophthora* database at Cornell University (Forbes et al 1998).

had the I-b mtDNA. Haplotype II-b was found only in isolates with the US-6 genotype (n = 13), and was found primarily in the western part of North America.

Other mtDNA haplotypes were found in association with many different nuclear genomes. Haplotype I-a(A) was found among diverse nDNA genotypes and clonal lineages worldwide (TABLE IV). In the United States and Canada, haplotype I-a(A) was associated with the nDNA genotypes US-7 and US-8. It was also found among several clonal lineages from Peru, Argentina, Brazil, Rwanda, and Israel. Haplotypes I-a and II-a were present in Europe among diverse nDNA genotypes consisting of A1 and A2 mating types (TABLES III, V). Haplotype II-a was found in Japan and Korea (TABLE V) associated with the JP-1 genotype. In South America, haplotype II-a was found in several clonal lineages however II-a was not found in the USA or Canada (TABLE V).

DISCUSSION

We confirm that Carter's haplotypes I-a and I-b are included in Goodwin's haplotype A, and Carter's haplotypes II-a and II-b are included in Goodwin's haplotype B (TABLE I). Moreover, Koh's haplotypes E and F are included in Carter's haplotype I-b (TABLE I). A combination of nomenclatures is presented in FIG. 1 with group I comprising haplotypes I-a(A), I-b(A), I-b(E), and I-b(F) and group II comprising haplotypes II-a(B) and II-b(B).

Knowledge of insertions and nucleotide substitutions allowed us to propose relationships among the haplotypes (FIG. 1), and to test our first hypothesis that haplotype I-b(A) is ancestral to the other known haplotypes. The relationships are shown in FIG. 1 as a minimum length tree with no character reversals inferred. Haplotypes in group I were distinguished from those in group II by a 2 Kb insertion (Carter et

TABLE V. Distribution of mitochondrial haplotype II-a(B) associated with diverse nuclear backgrounds

Location	Year	nDNA genotype	Allozyme genotype		Mating type	Sample size
			Gpi	*Pep*		
North America						
Mexico						
Puebla (east central)	1989	Diverse	86/100	92/100	A1	2
Puebla (east central)	1989	Unique	86/100	92/92	A1	1
Puebla (east central)	1989	Unique	100/100	92/92	A1	1
Puebla (east central)	1989	Unique	100/122	92/100	A1	1
Saltillo, Coahuila (northeast)	1988	Diverse	100/100	100/100	A1 & A2	10
South America						
Peru	1994–1997	Clonal	100/100	—	A1	17
Peru	1994–1997	Clonal	100/100	—	A1	19
Argentina	1994	AR-2	100/100	92/100	A2	1
Argentina	1994	AR-5	100/100	96/100	A2	1
Argentina	1994	AR-4	100/100	100/100	A2	1
Argentina	1994	Clonal	—	—	A2	11
Argentina	1996	Clonal	100/100	100/100	—	3
Bolivia	1994	Clonal	100/100	96/100	A2	4
Bolivia	1994	Clonal	100/100	100/100	A2	5
Bolivia	1994	Clonal	100/100	—	A2	2
Bolivia	1994	Clonal	—	—	—	6
Colombia	1990	Clonal	90/90	—	A1	1
Colombia	1990	Clonal	—	100/100	A1	1
Colombia	1990	EC-1	90/100	96/100	A1	1
Ecuador	1990–1996	EC-1	90/100	96/100	A1	36
Ecuador	1990–1995	Clonal	100/100	100/100	A1	2
Ecuador	1995	Clonal	100/100	92/100	A2	2
Europe						
Poland	1989–1991	PO-4	90/100	—	A1	12
Poland	1989–1991	Diverse	90/100	—	A2	15
Poland	1989	PO-14	90/100	—	A2	2
Poland	1989–1991	Diverse	100/100	—	A1 & A2	8
Poland	1990	PO-29	90/90	—	A1	3
Poland	1991	Unique	—	—	A1	1
Netherlands	1984	NL-3	90/100	—	A1	3
Netherlands	1987–1989	Diverse	90/100	—	A1 & A2	3
Germany	1990	Diverse	—	—	A1	7
United Kingdom	1989	Diverse	100/100	—	A1	11
Asia						
Japan	1988	JP-1	100/100	100/100	A2	9
South Korea	1991	JP-1	100/100	100/100	A2	7
Soviet Union	1990	PO-4	90/100	100/100	A1	7
Soviet Union	1990	RU-2/PO-57	100/100	100/100	A2	4

Data on mating type, nDNA genotypes, and allozyme genotypes were obtained from the *Phytophthora* database at Cornell University (Forbes et al 1998).

al 1990) localized in the P5 region (FIG. 1). Because we did not find the 2 Kb insert in the P5 region of *P. mirabilis* mtDNA (FIG. 1) we believe that the ancestral mtDNA belongs to group I. Further, it is plausible that haplotypes I-b(E) and I-b(F) were derived from haplotype I-b(A) especially since they all have the same nDNA background (FIG. 2) (Koh et al 1994). Intriguingly, three insertions were located in the same mtDNA region within the 5.7 Kb *Eco* RI fragment (FIG. 1). This region may be less constrained in contrast to the rest of *P. infestans* mtDNA. From all of these data, we cannot reject our first hypothesis that haplotype I-b(A) is the putative ancestral mitochondrial haplotype of *P. infestans*. However,

while we expect that haplotype I-b(A) is the ancestral form, we cannot completely reject the hypothesis that haplotype I-a(A) might be the ancestral haplotype since haplotypes I-a(A) and I-b(A) differ from each other by only a single nucleotide. Sequence comparison based on a larger part of the mitochondrial genome of *P. infestans* and its close relatives will be necessary to determine the actual ancestral relationships.

We were unable to find haplotype I-b(A) in any isolates recently obtained from Mexico, causing us to tentatively reject our second hypothesis (that central Mexico would contain this haplotype). While a sample size of 137 isolates cannot enable us to state absolutely that haplotype I-b(A) is absent from Mexico, we can certainly conclude that if present, it is at low frequency. Our detection of only haplotype I-a (A) in isolates from central Mexico contrasts with our expectation of finding some mitochondrial diversity coincident with the greatest nuclear diversity. Indeed, these data lead to contrasting hypotheses, including this one: that the widespread distribution of a single mtDNA haplotype in the Toluca Valley of central Mexico is perhaps due to selection. There are reports of non-neutral mutations in mtDNA in several organisms (Fos et al 1990, Rand and Kann 1996, Rand et al 1994), so that this hypothesis is certainly not without precedent.

While our data are consistent with selection acting on mtDNA in *P. infestans* in central Mexico we have not confirmed a mechanism. One possible mechanism might be that domestic potatoes provide a different environment and therefore selected different mtDNA than did wild *Solanum* spp. A precedent has been suggested in the fungus *Mycosphaerella graminicola*. The limited mtDNA variation in that fungal pathogen might have been caused by a selective sweep that occurred as populations became specialized to infect bread wheat (McDonald et al 1999). If a similar event occurred in the *P. infestans*–*Solanum* spp. pathosystem it needs to explain the previous worldwide occurrence of haplotype I-b (A) on potatoes, and the current occurrence of haplotype I-a (A) on diverse *Solanum* spp. in central Mexico. First, the worldwide distribution of haplotype I-b(A) on potatoes could be explained by hitchhiking with a particular nuclear genotype. Second, the occurrence of haplotype I-a (A) on diverse native *Solanum* spp. in central Mexico (n = 5 in our sample) could be explained by the relatively recent (latter half of the 20th century), but currently strong, influence of very extensive and intensive production of domestic potatoes in central Mexico.

A test of the non-neutral effect of mtDNA haplotype I-b(A) relative to other haplotypes is possible experimentally. Progeny of a cross between an isolate containing haplotype I-b(A) and an isolate containing I-a (A) would produce a population of genetically diverse individuals containing either mtDNA I-b (A) or I-a (A). Pathogenic fitness tests done on these progeny in a manner similar to that done by Day and Shattock (1997) would reveal fitness differences attributable to mtDNA haplotype. Such tests await the development of the proper progeny.

Our third hypothesis was that all haplotypes would occur in a variety of nuclear backgrounds. While haplotypes I-a and II-a certainly occurred in many different nDNA genotypes, this was not the situation for haplotypes I-b and II-b. Haplotype I-b was found only in isolates and variants of the US-1 clonal lineage (TABLE IV). This lineage contains some nuclear diversity, but all individuals appear to be related and to differ from each other only by a small number of mutations (TABLE IV). Variant haplotypes I-b(E) and I-b(F) appeared to have arisen from haplotype I-b(A) by independent mutations. In addition, haplotype II-b was also found in a very limited number of nuclear backgrounds mainly involving the US-6 clonal lineage (A1 mating type), or its derivatives (Gavino 1999, Gavino et al 2000). This lineage had a distribution of western Canada, western United States and northwestern Mexico (Fry and Goodwin 1997, Goodwin et al 1998). Previously, Goodwin et al (1998) reported that US-6 lineage was introduced into the United States and Canada from northwestern Mexico in the 1970s. Griffith and Shaw (1998) also found haplotype II-b only in the United States and Canada, but they found it among A1 and A2 mating types. Their sample from these two countries was different from ours, and the reports of sexual reproduction in northern North America are certainly consistent with the distribution of this haplotype in diverse nuclear backgrounds in this region. In fact, in a subsequent study, we learned that haplotype II-b occurs in the US-11 lineage, a lineage that has continental distribution and which probably arose very recently via sexual recombination involving the US-6 lineage (Gavino et al 2000).

A pattern of slow evolution in the mtDNA of oomycetes is emerging. The small size and conservation of mtDNAs appear to be universal in oomycetes and their algal relatives (Chesnick et al 1996, 2000). The uniformity in oomycete mtDNAs is in contrast to the non-uniformity in mtDNAs of some true fungi (McNabb and Klassen 1988). Many fungi possess isolate- and species-specific variations in mtDNAs (Garber and Yoder 1983, Taylor 1986). Low mtDNA variation, however, also exists in some fungi such as *Fusarium oxysporum* f. sp. *canariensis* (Plyler et al 2000), *Chondrostereum purpureum* (Ramsfield et al 1999), and *Ceratocyctis fagacearum* (Kurdyla et al 1995). Un-

like fungal mtDNAs, mtDNAs of *P. infestans* and *P. megasperma* do not have introns (Förster et al 1987, Paquin et al 1997). The sequences of four mtDNA genes (l-rRNA, s-rRNA, oxi-2, and cob) are the same for *P. megasperma* f. sp. g*lycine*, *P. megasperma* f. sp. m*edicaginis*, and *P. parasitica* var. *nicotianae* (Förster et al 1987). In another study, microsatellites for *P. cinnamomi* mtDNA were not highly polymorphic (Dobrowolski et al 1998). Möeller et al (1993) even considered *P. mirabilis* a forma specialis of *P. infestans* because of very high similarity in their mtDNA restriction patterns. Sequence conservation in *P. infestans* mtDNA is consistent with the fact that more than 95% of the genome codes for genes (Paquin et al 1997).

Our study has used the mitochondrial genome as a tool to investigate questions concerning the evolution and population genetics of *P. infestans*. We reconciled two existing nomenclature systems for mtDNA haplotypes and suggested a hypothetical common ancestor. The diversity in the I-b haplotype is consistent with the hypothesis that this is the oldest of the extant haplotypes and is also consistent with the hypothesis that the US-1 lineage in which it is found is the oldest of extant lineages. Our study with the mitochondrial genome confirmed previous hypotheses concerning migrations of *P. infestans*, but it also generated novel hypotheses. The most surprising of these was the hypothesis that selection might be driving mtDNA haplotype I-a(A) to approach fixation in the Toluca Valley of central Mexico. Identification of the forces causing that selection awaits further investigation.

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