Genetic Structure of *Phytophthora infestans* Populations in China Indicates Multiple Migration Events

Liyun Guo, Xiao-Qiong Zhu, Chia-Hui Hu, and Jean Beagle Ristaino

First and second authors: Department of Plant Pathology, China Agricultural University, Beijing 100193; and third and fourth authors: Department of Plant Pathology, Box 7616, North Carolina State University, Raleigh 27695.
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ABSTRACT

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One hundred isolates of *Phytophthora infestans* collected from 10 provinces in China between 1998 and 2004 were analyzed for mating type, metalaxyl resistance, mitochondrial DNA (mtDNA) haplotype, allozyme genotype, and restriction fragment length polymorphism (RFLP) with the RG-57 probe. In addition, herbarium samples collected in China, Russia, Australia, and other Asian countries were also typed for mtDNA haplotype. The Ia haplotype was found during the first outbreaks of the disease in China (1938 and 1940), Japan (1901, 1930, and 1931), India (1913), Peninsular Malaysia (1950), Nepal (1954), The Philippines (1910), Australia (1917), Russia (1917), and Latvia (1935). In contrast, the Ib haplotype was found after 1950 in China on both potato and tomato (1952, 1954, 1956, and 1982) and in India (1968 and 1974). Another migration of a genotype found in Siberia called SIB-1 (*Glucose-6-phosphate isomerase* [*Gpi*] 100/100, *Peptidase* [*Pep*] 100/100, IIa mtDNA haplotype) was identified using RFLP fingerprints among 72% of the isolates and was widely distributed in the north and south of China and has also been reported in Japan. A new genotype named CN-11 (*Gpi* 100/111, *Pep* 100/100, IIb mtDNA haplotype), found only in the south of China, and two additional genotypes (*Gpi* 100/100, *Pep* 100/100, Ia mtDNA haplotype) named CN-9 and CN-10 were identified. There were more diverse genotypes among isolates from Yunnan province than elsewhere. The SIB-1 (IIa) genotype is identical to those from Siberia, suggesting later migration of this genotype from either Russia or Japan into China. The widespread predominance of SIB-1 suggests that this genotype has enhanced fitness compared with other genotypes found. Movement of the pathogen into China via infected seed from several sources most likely accounts for the distribution of pathogen genotypes observed. MtDNA haplotype evidence and RFLP data suggest multiple migrations of the pathogen into China after the initial introduction of the Ia haplotype in the 1930s.

Additional keywords: genetic diversity, late blight, mtDNA haplotypes.

Late blight caused by the oomycete pathogen *Phytophthora infestans* is the most devastating disease of potato in China (54). Late blight occurs in all the major potato-growing regions in China, including the northeast provinces (Heilongjiang, Jilin, Liaoning, and the eastern part of Inner Mongolia), north and northwest China (Inner Mongolia, Gansu, Shaanxi, Ningxia, Shanxi, and Hebei), southwest China (Sichuan, Chongqing, Yunnan, and Guizhou), and south and southeast China (Fujian, Guangdong, and Guangxi). The earliest record of a serious loss caused by late blight in China was in November 1940. More than 90% of the potato crop was lost in the Chongqing area in that year (53). The first nationwide outbreak of this disease was in the early 1950s, which led to the intensive investigations of control measures for late blight (57). By using effective fungicides and growing resistant cultivars, late blight was managed in China for nearly three decades, until its reemergence in the early 1990s (57).

P. infestans (Mont.) de Bary is a heterothallic oomycete with two mating types, designated as A1 and A2. The increase of disease occurrence in many countries in the last two decades has been attributed to changes in the genetic structure of pathogen populations (15). DNA evidence suggests that the initial lineage of *P. infestans* introduced to the United States and Europe was the Ia mitochondrial DNA (mtDNA) haplotype and that this haplotype was subsequently replaced by the Ib mtDNA haplotype (37,41). In the mid 1970s, new populations, comprising both the A1 and A2 mating types, were introduced into Europe and the United States from Mexico, and displaced the initial A1 lineages (15,21). New, aggressive genotypes of the pathogen have been found in the United States and Europe (14).

Populations of *P. infestans* have been replaced by more aggressive new strains that often are characterized as A2 mating type and metalaxyl resistant (10,15). The presence of the A2 mating type in China was first reported in 1996 (56). The fungicide metalaxyl was introduced experimentally into China in the late 1980s but used more widely in the 1990s (35). Strains insensitive to metalaxyl were first reported in China in 1998 (34). Subsequently, many investigations on the detection of mating types and fungicide resistance, especially the response to metalaxyl of isolates of *P. infestans* from different geographic regions, have been done (24,29,32,44,50,51,55,58–60). Only a few studies have examined the genetic structure of populations in China (1,22, 24,27,32,36,60). However, these studies failed to examine the disease on a national scale and did not explore the first introductions of the pathogen or consequences of the migrations due to the lack of historical specimens and reference isolates of known genotypes.

Oospores now play a role in the disease cycle in some areas of Europe and Canada (11). Understanding variation in pathogen populations of *P. infestans*, including the potential for sexual reproduction, is clearly a significant factor in deploying effective control strategies. For example, populations in Scandinavian countries can survive via oospores, and epidemic onset now occurs earlier in the season (5). Populations of *P. infestans* continue to change and late blight management remains a significant challenge to the potato and tomato industry. Little is

Corresponding author: J. B. Ristaino; E-mail address: Jean_Ristaino@ncsu.edu

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known about the role of sexual reproduction in the pathogen's biology in China (32).

The role of the movement of seed potato tubers in the distribution of pathogen genotypes in the China has not been investigated previously in China but has been studied elsewhere (17). Potato was probably introduced into China in the 17th century (48). Potato was not an important crop under the commune system (1958 to 1978), although peasants were allowed to grow potato for personal consumption. Potato production increased significantly after the 1960s and now China is the largest producer in the world, with over 72 million tons produced in 2007 (13). Potato breeding was done in Inner Mongolia for over 30 years and breeding materials from Russia, South America, and elsewhere were introduced into the country and subsequently moved within China (F. Ezeta and F. Wang, *personal communication*) (47). Because seed potato tubers are produced in the north of China and are distributed throughout the country, there is the possibility of movement of the pathogen in infested seed (33). Long-distance dispersal of aerial inoculum or movement of seed potato tubers between other Asian countries, including Japan and Russia, could also contribute to epidemic development because shared genotypes have been found among European, Asian, and Russian populations (3,6,12).

An extensive sampling of populations of *P. infestans* collected from potato and tomato fields in 10 provinces in China was conducted in 1998 to 2004. Archival herbarium samples of infected potato leaves were also examined from the first major outbreaks of the disease in China, Russia, and other Asian countries. We studied the phenotypic and genotypic diversity among modern and historic populations of the pathogen in China in order to ask three questions. First, what mtDNA haplotype was first introduced into China? Second, have there been multiple migrations of the pathogen into China? Third, has movement of potato seed affected the population structure of *P. infestans* in China? Preliminary reports of a portion of this work have been published (27,42).

MATERIALS AND METHODS

Isolates of *P. infestans*. In all, 100 isolates of *P. infestans* from potato (n = 97) or tomato (n = 3) were collected from 10 provinces in China, including Beijing (n = 3), Chongqing (n = 5), Fujian (n = 4), Gansu (n = 12), Hebei (n = 1), Heilongjiang (n = 4), Jilin (n = 3), Inner Mongolia (n = 46), Sichuan (n = 10), and Yunnan (n = 12), and some isolates were kindly donated by D. L. Liang, A. Ogoshi, W. F. Luo, or C. H. Li (Table 1; Fig. 1) (26,41,59,60). Because sampling was done by many collaborators, sample size is not equal across all provinces and years. Single-zoospore cultures were made and maintained on tomato-rye agar which was modified from V8 juice-rye agar (8). Isolates YLM13-5, YLM18-2, and YLM22-4 were single-zoospore isolates of potential self-fertile isolates YLM13, YLM18, and YLM22, respectively. Potential self-fertile isolates formed oospores in low numbers in single culture in the absence of the opposite mating type, as described previously by Fye and Shaw (16). Rye juice was made by soaking 50 g of rye grain in 1,000 ml of distilled water at 24°C for 24 to 36 h followed by autoclaving for 30 min and filtering through four layers of cheesecloth. In each liter of the medium, 50 ml of V8 juice and 0.2 g of $CaCO_3$ were replaced by 100 ml of tomato juice with 0.4 g of $CaCO_3$ (25). The final volume was adjusted to 1,000 ml with distilled water. Cultures were maintained at 18°C in darkness.

Mating type. The mating type of each isolate was determined by pairing an A1 tester isolate (DN111) or an A2 tester isolate (DN107) on a block (≈10 by 15 by 3 mm) of tomato-rye agar with each test isolate (8,39). Tester isolates were kindly provided by A. Ogoshi, Hokkaido University, Japan. An unpaired test isolate was used as a control. Pairings between the opposite mating types of tester isolates was used as a positive control. Five blocks were placed in a petri dish at equal distances from each other along the edge of the petri dish. After incubation at 17 to 19°C in darkness for 14 days in a moist chamber, oospore formation on each block was examined microscopically. Isolates that produced oospores with the known A1 tester isolates were designated as the A2 mating type and isolates that produced oospores with the known A2 tester isolate were designated the A1 mating type.

Fungicide sensitivity. The sensitivities of isolates to metalaxyl were tested as described previously (9). The level of sensitivity of each isolate to metalaxyl was determined as described by Shattock (45). Sensitive, intermediate, and resistant phenotypes were defined as isolates exhibiting <10, 10 to 60, and >60% growth, respectively, on media amended with metalaxyl at 10 μg liter^{-1} relative to growth on metalaxyl-free media (Table 2).

DNA extraction. Mycelium of each isolate was obtained by culturing the pathogen for 9 days in darkness at 17°C on tomato-rye agar overlaid with a cellophane membrane (preboiled in distilled water for 10 min before autoclaving). Six plates were used for each isolate. The resulting mycelia were then collected from the membranes and stored at –20°C. The frozen mycelium (≈350 mg) was ground in liquid nitrogen and the genomic DNA was prepared using methods described previously (40). Dried DNA was dissolved in Tris-EDTA containing RNAse overnight at 37°C, then stored at –20°C before amplification.

mtDNA haplotype analysis. All isolates were analyzed for mtDNA haplotype. Four mitochondrial haplotypes have been described in *P. infestans*: Ia, Ib, IIa, and IIb (4,7,23). Polymerase chain reactions (PCRs), digestion with restriction enzymes, and determination of haplotypes were according to the methods of Griffith and Shaw (23). The mitochondrial haplotypes of isolates were determined by comparing their patterns to reference isolates of Ia, Ib, IIa, and IIb and by DNA sequencing. The mtDNA haplotypes of samples from archival herbarium collections (Table 3) were determined by PCR amplification and sequencing of smaller targets within the P2, P3, and P4 regions of the mitochondrial genome by the methods described previously (37). Work with archival materials was done in the Phytotron containment lab at North Carolina State University.

Allozyme analysis. Allozyme genotypes were determined for a subset of isolates at the *Glucose-6-phosphate isomerase* (*Gpi*) (n = 48) and *Peptidase* (*Pep*) (n = 21) loci (20). Mycelium obtained from pea broth culture (120 g of frozen peas in 500 ml of distilled water, autoclaved for 5 min, filtrate brought to 1 liter, and reautoclaved 25 min) was cut into small pieces (0.5 to 1 cm^2) and placed in sterile 1.5-ml microcentrifuge tubes. The mycelium was centrifuged at 13,000 rpm for 1 min to remove excess water. Extraction buffer (50 μl; 20% sucrose, 2% Triton X-100, 0.01% bromophenol blue, and 9.8 ml of H_2O) was added to each tube and the mycelia were ground for 30 to 60 s with a hand drill equipped with sterile Konte pestles. Samples were centrifuged for 2 min at 10,000 rpm, and the supernatant was collected for allozyme analysis.

Allozyme genotypes were determined at the *Gpi* and *Pep* loci by cellulose-acetate electrophoresis (CAE) (20). Isolate 188.1.1 of the clonal genotypes US-1 (*Gpi* 86/100), isolate CR120 of the clonal lineage CR-2 (*Gpi* 100/111/122), and isolate 94-53 of the clonal genotype US-7 (*Gpi* 100/111) were used as standards on each acetate plate for the *Gpi* assay, and 188.1.1 (*Pep* 92/100) and isolate 93-5 (*Pep* 100/100) were used as standards for the *Pep* assay. Isolate 94-53 was provided by W. Fry, Cornell University. Migration distances of proteins from the unknown isolates were compared with migration distances of proteins from the tester genotypes. Alleles in individual isolates were scored based on the migration of their proteins relative to the protein produced at the 100 allele, which is the most common allele (20).

Restriction fragment length polymorphism genotype. Restriction fragment length polymorphism (RFLP) analysis using

the RG57 probe was carried out using the methodology described by Goodwin et al. (19) on a subset of isolates (n = 21) from China (Table 1). Isolates were grown in pea broth for 14 days at 18°C. The mycelium was vacuum filtered through Whatman no. 1 filters, lyophilized at –5°C overnight, and ground in liquid nitrogen. Total DNA was extracted following the miniprep procedure described by Lee et al. (31) as modified by Jacobson and Gordon (28). Transfer to Hybond –N^+ nylon membrane (Amersham Biosciences), hybridization with nonradioactive RG-57 probe, and autoradiography were all according to the manufacturer's instructions (Renaissance nonradioactive kit; NEN Life Science Products, Inc., Boston) and methods described previously (52). The genotype of the isolates was determined by comparing their patterns with those of reference isolates US-1 (188.1.1), US-7 (2.1.3), US-8 (94-8-4), and US-15 (80787-94L) (19,21,50). US1.1 (188.1) and US-7 (2.1.3) were provided by Zamir Punja, University of Vancouver, British Columbia and US-15 (80787-94L) was provided by Seong Kim, Pennsylvania Department of Agriculture.

RESULTS

Of the 100 isolates of *P. infestans* from China, 97 were A1 mating type, with the exception of 3 isolates from Yunnan that were A2 mating type but self-fertile (Table 1). Self-fertile isolates produced a few oospores in single culture but formed more oospores with A1 isolates in crosses.

Metalaxyl resistance was found among the isolates collected (Table 2). Among the 84 isolates tested for resistance to metalaxyl, 42% were resistant, 33% were intermediate resistant, and 25%

TABLE 1. Isolates of *Phytophthora infestans* collected in China and characterized for phenotypic and genotypic traits in this study

Field location							
Province	County	Isolate name[a]	Host[b]	Sensitivity[c]	mtDNA[d]	Year[e]	Supplier[f]
Beijing	Changping	BC1*	T	ND	Ib	2004	A
Beijing	Daxing	BT02*, BDX4*	T	1I, 1ND	Ib	2002	A
Chongqing	Wanxian	CWX3104, CWX4102	P	ND	Ia	2000	B
Chongqing	Youyang	CYY1, CYY4	P	S	Ia	2001	A
Chongqing	Yunyang	CYY1175*	P	R	IIb	2000	B
Fujian	Dehua	FDH10-2, FDH11*, FDH4	P	R	IIb	2002	A
Fujian	Zhouning	FZN1-1*	P	R	IIb	2002	A
Gansu	Huichuan	GHC4	P	S	Ia	2001	A
Gansu	Lanzhou	GLZ4, GLZ9	P	R	IIa	2001	A
Gansu	Minxian	GMX10, GMX1-2, GMX17, GMX2-1*, GMX26	P	2I, 2S, 1ND	IIa	2001	A
Gansu	Minxian	GMX15, GMX19	P	S	Ia	2001	A
Gansu	Weiyuan	GAN1, GAN1-2	P	1R, 1ND	IIa	2000	A
Hebei	Weichang	HWCH3*	P	ND	IIa	2004	A
Heilongjiang	Harbin	HHB1, HHB11, HHB2*, HHB5	P	2R, 2S	IIa	2001	A
Inner Mongolia	Baotou	N2396	P	I	IIa	1998	C
Inner Mongolia	Baotou	N2397	P	S	IIa	2000	C
Inner Mongolia	Hohhot	N2400, N2406, N2431	P	1I, 2ND	IIa	1998	C
Inner Mongolia	Hohhot	NHL	P	I	IIa	1998	A
Inner Mongolia	Hohhot	N2398	P	I	IIa	1999	C
Inner Mongolia	Hohhot	N2338	P	I	IIa	2000	C
Inner Mongolia	Hohhot	NN15P, NN25, NN26, NN27	P	1R, 1I, 1S, 1ND	IIa	2000	A
Inner Mongolia	Hohhot	N2447, N2451, N2454, N2499, N2511-1*, N2451-3, N2499-2	P	5I, 2S	IIa	2001	C
Inner Mongolia	Hohhot	N87110*	P	ND	IIa	2004	C
Inner Mongolia	Jiagedaqi	NJQ10, NJQ11, NJQ12, NJQ14, NJQ15, NJQ3, NJQ5, NJQ7, NJQ8	P	R	IIa	2000	A
Inner Mongolia	Jiagedaqi	N2468	P	R	IIa	2001	C
Inner Mongolia	Jining	NJN15, NJN17, NJN18, NJN24, NJN32, NJN33, NJN4, NJN6, NJN7	P	R	IIa	2001	A
Inner Mongolia	Siziwangqi	NSW1, NSW3, NSW5, NSW6	P	I	IIa	2000	A
Inner Mongolia	WuMeng	N2394	P	I	IIa	1998	C
Inner Mongolia	Zhalantun	N2389-2	P	R	IIa	1999	C
Inner Mongolia	Zhalantun	NNJ3-1*	P	S	IIa	2000	A
Jilin	Yanbian	JYB1, JYB3*	P	R	IIa	2001	A
Jilin	Yanbian	JYB5	P	R	Ia	2001	A
Sichuan	Liangshan	SLS4*	P	S	Ib	2000	A
Sichuan	Liangshan	SLS5, SLS9	P	1I, 1S	Ia	2000	A
Sichuan	Sangou	SSG1, SSG3, SSG4	P	I	IIa	2000	A
Sichuan	Shifang	SSF2, SSF3-1*, SSF8	P	2S, 1ND	IIa	2000	A
Sichuan	Shifang	SSF4	P	ND	Ia	2000	A
Yunnan	Ganhaizi	YGHZ1	P	ND	IIa	1999	A
Yunnan	Huize	YPH13-1*, YPH5	P	1R, 1ND	IIa	1999	A
Yunnan	Kunming	YXSR11	P	S	Ia	1999	A
Yunnan	Luliang	YLM13-5▲*, YLM18-2▲, YLM22-4▲*	P	I	Ia	2001	D
Yunnan	Tonghai	YLT04	P	R	IIa	1999	A
Yunnan	Tonghai	YLT06	P	ND	Ia	1999	A
Yunnan	Xundian	M-5	P	S	Ia	2004	E
Yunnan	Xundian	XA-4*	P	R	IIb	2004	E
Yunnan	Zhaotong	Zt-2-12*	P	S	Ia	2004	E

[a] Isolates marked with ▲ are A2 self-fertile isolates and the rest are A1 isolates; * indicates isolates used for the restriction fragment length polymorphism (RFLP) genotype. Isolates from outside China were used as controls in RFLP or allozyme genotype assays.
[b] P = potato, T= tomato,.
[c] Sensitivity to metalaxyl: ND, not determined; I, intermediate; S, sensitive; R, resistant.
[d] Mitochondrial DNA.
[e] Year collected.
[f] Suppliers are indicated as follows: A, stored in Mycology Lab, China Agricultural University; B, Z. K. Wang, Chong Qing University; C, D. L. Liang, Inner Mongolia Academy of Agricultural Science; D, W. F. Luo, Yunnan Agricultural University; E, C. H. Li, Yunnan Normal University.

were sensitive. The metalaxyl-resistant isolates were found in populations of *P. infestans* from many provinces, including Chongqing, Fujian, Gansu, Heilongjiang, Inner Mongolia, Jilin, and Yunnan. Sichuan and Beijing provinces were the only provinces where metalaxyl-resistant isolates were not found, although only a few isolates were tested from Beijing. No isolates were tested from Hebei.

The Ia mtDNA haplotype of *P. infestans* was found earlier in China than other mtDNA haplotypes. Samples collected by different researchers in China from tomato in 1938 in Kunming and from potato in 1940 in Chengjiang and Chongqing were the Ia mtDNA haplotype (Table 3). Similarly, the oldest samples from other Asian countries, including India, Japan, Peninsular Malaysia, Nepal, the Philippines, Russia, and Australia, were the Ia haplotype. In contrast, the earliest record of the Ib haplotype of *P. infestans* in China was later: in 1952, on potato in the Sichuan region; in 1954, on potato in Hebei; and in 1956, on tomato in Beijing. The Ib haplotype was also found later in India in 1968 and 1974 (Table 3). Interestingly, the Ib mtDNA haplotype was also found in 1982 on *Solanum lyratum* in the Sichuan province, documenting a host shift to a wild species (Table 3) (46).

In contrast, four mtDNA haplotypes were detected among modern isolates from potato, including the Ia, Ib, IIa, and IIb haplotypes. The IIa mtDNA haplotype was the most frequent haplotype detected (72%), followed by the Ia (18%), IIb (6%), and Ib (4%) haplotypes (Table 1). The geographic distribution of the mtDNA haplotypes varied. Both type Ia and IIa mtDNA haplotypes were widely distributed across China, in Gansu, Jilin, Sichuan, and Yunnan provinces (Fig. 1). Only the IIa mtDNA haplotype was detected in Inner Mongolia, Hebei, and Heilongjiang (Fig. 1). In contrast, the distribution of the IIb mtDNA haplotype was limited to the southern part of China, in Chongqing, Fujian, and Yunnan (Fig. 1). The Ib mtDNA haplotype was found both in Sichuan on potato and in Beijing on tomato. DNA sequences from P3 and P4 regions of the mitochondrial genome (data not shown) confirmed the identity of the mtDNA haplotypes.

Four allozyme genotypes, including *Gpi* 86/100, *Pep* 92/100; *Gpi* 86/100, *Pep* 100/100; *Gpi* 100/100, *Pep* 100/100; and *Gpi* 100/111, *Pep* 100/100, were found (Fig. 2). Three isolates from tomato and one isolate from potato were either *Gpi* 86/100, *Pep* 92/100 or *Gpi* 86/100, *Pep* 100/100 and the Ib mtDNA haplotype and identified as the US-1 genotype (21) by RG57 fingerprinting (Table 4). All isolates that were the Ia and IIa mtDNA haplotype were *Gpi* 100/100, *Pep* 100/100. Isolates with the IIb mtDNA haplotypes were *Gpi* 100/111, *Pep* 100/100 (Table 4).

Variation was observed for the RG57 DNA fingerprint and isolates with unique RFLP fingerprints were observed (Fig. 3; Table 4). Among *Gpi* 100/100, *Pep* 100/100 isolates with the Ia mtDNA haplotype, one isolate was a previously described genotype, MO-6 (Moscow-6) (12), and the rest were unique genotypes and named CN-9, CN-9.1, and CN-10. All isolates with *Gpi* 100/100, *Pep* 100/100 allelles and the IIa mtDNA haplotype were

TABLE 2. Relative sensitivity to metalaxyl among isolates of *Phytophthora infestans* collected from China, 1999–2004

Province	Metalaxyl sensitivity[a]			Total no.
	Sensitive	Intermediate	Resistant	
Beijing	–	1	–	1
Chongqing	2	0	1	3
Fujian	0	0	4	4
Gansu	5	2	3	10
Hebei	ND	–	–	0
Heilongjiang	2	–	2	4
Inner Mongolia	5	17	20	42
Jilin	–	–	3	3
Sichuan	4	4	–	8
Yunnan	3	3	3	9
	21	28	35	84

[a] Level of sensitivity of each isolate to metalaxyl was determined as described by Shattock (45). Sensitive, intermediate, and resistant phenotypes were defined as isolates exhibiting <10, 10 to 60, and >60% growth, respectively, on media amended with metalaxyl at 10 μg liter^{-1} relative to growth on metalaxyl-free media; – = none found and ND = not determined.

Fig. 1. Sampling locations of *Phytophthora infestans* and mitochondrial DNA haplotype distribution in major potato production areas in China.

identified as SIB-1 or variants of SIB-1 (SIB-1.1 or SIB-1.2 that differed by 1 or 2 bands, respectively) (12). Two other isolates were *Gpi* 100/111 and *Pep* 100/100, with the IIb mtDNA haplotype and unique RFLP fingerprints, and were named CN-11 and CN-11.1. Two isolates with *Gpi* 100/111 and *Pep* 100/100 genotypes and the IIb mtDNA haplotype were identical to the US-16 genotype (or US-16.1) (Table 4).

Among the genotypes found in this study, the levels of resistance to metalaxyl of US-1 isolates varied from sensitive to intermediate resistance. The isolates of CN-10 and MO-6 were sensitive to metalaxyl. Isolates of CN-9 were intermediate. Isolates of CN-11 and US-16 were resistant. The level of metalaxyl resistance among isolates of the SIB-1 genotype varied from sensitive to resistant.

The US-1 genotype was only identified in three isolates from tomato in Beijing and one isolate from potato in Sichuan (Table 4). The predominant genotype was SIB-1 (IIa mtDNA haplotype) and was found in seven provinces in China (Table 4). These isolates were widely distributed in China in Gansu, Hebei, Heilongjiang, Inner Mongolia, and Jilin provinces in the north and Sichuan and Yunnan province in the south (Fig. 1). The CN-11 genotype was found only in the south of China, in Chongqing and Fujian. The CN-9, CN-10, and MO-6 genotypes were found in Yunnan and the US-16 genotype was also found in Yunnan and Fujian (Table 4). More diverse genotypes were found in Yunnan than other provinces.

A cluster analysis of the genotypes was done with the RFLP data using an unweighted pairgroup method with arithmetic means analysis and NTSYS-PC (43). The dendrogram illustrates the matrices of similarity between the newly described genotypes and other previously reported RFLP genotypes in China (Fig. 4). The SIB-1 genotype is most similar to genotypes reported previously in Japan, including JP-2 and JP-3 and SIB-2 from Japan and Russia, respectively. The previously reported Chinese genotypes CN-1 to CN-5, CN-7, and CN-8 formed a cluster that was nearest to the new Chinese lineages CN-9 and

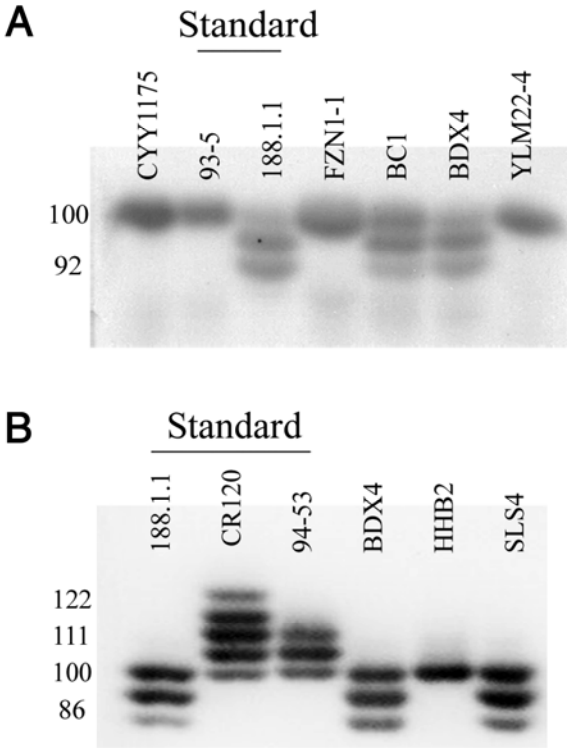

Fig. 2. Allozyme genotypes of *Phytophthora infestans* on cellulose-acetate gels stained for **A,** Peptidase (*Pep*) and **B,** *Glucose-6-phosphate isomerase* (*Gpi*). *Pep* genotypes of isolates are in lanes 1 to 7 from left to right: Chinese isolate CYY1175 (*Pep* 100/100), standard US-7 93-5 (*Pep*100/100), standard US-1 188.1.1 (*Pep* 92/100), and Chinese isolates FZN1-1 (*Pep*100/100), BC1 (*Pep* 92/100), BDX4 (*Pep* 92/100), and YLM22-4 (*Pep* 100/100). *Gpi* genotypes of *P. infestans* isolates are in lanes 1 to 6 from left to right: standard US-1 188.1.1 (*Gpi* 86/100), standard Costa Rican isolate CR120 (*Gpi* 100/111/122), standard US-7 94-53 (100/111), and Chinese isolates BDX4 (86/100), HHB2 (100/100), and SLS4 (86/100).

TABLE 3. Identity of the mitochondrial DNA haplotypes of *Phytophthora infestans* in archival herbarium samples from China, southeast Asia, Russia, and Australia, 1901 to 1987

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

[a] *Lycopersicon esculentum* is now called *Solanum lycopersicum*.

[b] Specimens were sampled from the collections housed at the United States Department of Agriculture National Fungus Collection, Beltsville, MD (BPI); The Institute of Microbiology, Chinese Academy of Sciences, People's Republic of China, Beijing (HMAS); the Farlow Herbarium, Harvard University, Cambridge, MA (FH); the Royal Botanic Gardens Mycological Herbarium, Kew, England (K); and the International Mycological Institute, Egham, England (IMI).

[c] Mitochondrial DNA (mtDNA) haplotype determined by the methods of May and Ristaino (37).

CN-10 and are Ia mtDNA haplotypes. US-16 and CN-11 genotypes were closely related and are IIb mtDNA haplotypes. The US-1 genotypes formed a separate lineage that clustered with MO-6 and CN-3.

DISCUSSION

Only a few studies have examined the population structure of *P. infestans* in China previously. The US-1 genotype was the only genotype reported in China in a small sample (n = 6) of isolates studied in 1994 (36). A comparative study of Japanese (n = 12) and Chinese (n = 9) populations of *P. infestans* revealed one allozyme genotype (*Gpi* 100/100 and *Pep* 100/100) and three RFLP genotypes present in north China in the provinces of Gansu and Hebei in 1996 (1). In that study, a genotype commonly found in Japan called Japanese A1-A (now JP-2) was recognized as similar to a genotype found in Hebei and Gansu called He-Gan A1-A and was a IIa mtDNA haplotype (1). They noted that the same genotype was found in Russia previously and was called SIB-1 (1,2,12). Another study of larger populations (n = 82) of *P. infestans* from the four regions of China in Wei Chang, Lanzhou, Kunming, and Chengdu in 1996 and 1997 were the same allozyme genotype (*Gpi* 100/100 and *Pep* 100/100) and 8 unique RFLP genotypes were named CN-1 to CN-8 (22). These isolates were all the Ia mtDNA haplotype (22).

Greater genetic variation in populations of *P. infestans* in China was observed in our study than in previous work (1,2,22, 32,36,39). Four mtDNA haplotypes of *P. infestans* were detected in populations from potato in China (Fig. 1). The Ib mtDNA haplotype was found mostly in Beijing on tomato but was also found in one field on potato in Sichuan. Host adaptation to potato and tomato within the US-1 clonal lineage has been reported previously in Africa and South America. Although we did not examine host adaptation in our work, it is likely that these introductions of Ib mtDNA haplotype in China may have been from different sources because variants within the US-1 lineage were found (49). In contrast, the other mtDNA haplotypes were geographically separated. Isolates of the IIb mtDNA haplotype were restricted to the south of China in three provinces and the IIb mtDNA haplotypes has also been reported in Taiwan (30), which is close to Fujian province. In contrast, the IIa mtDNA

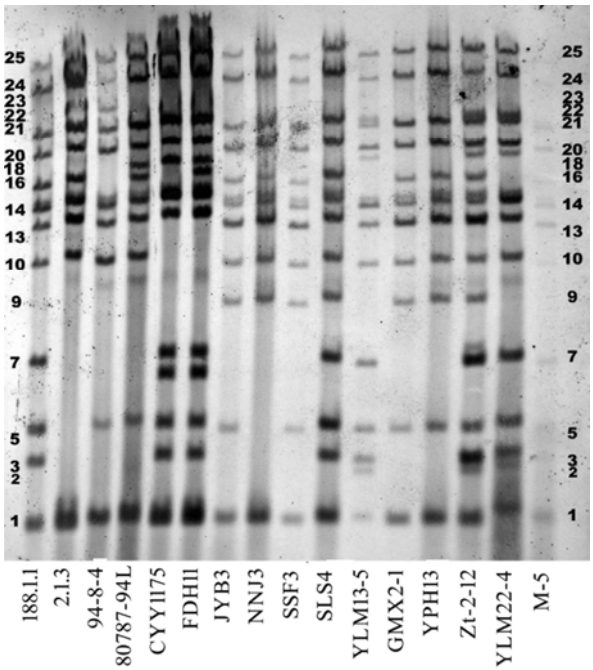

Fig. 3. DNA fingerprint patterns with RG57 probe of *Phytophthora infestans*. Lane 1, US-1.1 genotype (188.1.1); lane 2, US-7 genotype (2.1.3); lane 3, US-8 genotype (94-8-4); lane 4, US-15 genotype (80787-94L); lanes 5 to 16, Chinese isolate number (genotype) CYY1175 (CN-11), FDH11 (CN-11.1), JYB3 (SIB-1), NNJ3-1 (SIB-1.1), SSF3-1 (SIB-1), SLS4 (US-1.1), YLM13-5 (CN-9.1), GMX2-1 (SIB-1), YPH13-1 (SIB-1), Zt-2-12 (CN-10) , YLM22-4 (CN-9), and M-5 (MO-6). RG57 fingerprint band numbers are indicated on the right and left.

TABLE 4. Allozyme genotypes, mitochondrial DNA (mtDNA) haplotype, and restriction fragment length polymorphism (RFLP) fingerprint of Chinese isolates of *Phytophthora infestans*[a]

Province	Isolate no.	Host	*Gpi*	*Pep*	mtDNA[b]	Sens.[c]	RFLP fingerprint[d]																							
Beijing	BC1	Tomato	86/100	92/100	Ib	ND	1	0	1	0	1	0	1	0	0	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	US-1.3
Beijing	BDX4	Tomato	86/100	92/100	Ib	ND	1	0	1	0	1	0	1	0	0	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	US-1.3
Beijing	BT02	Tomato	86/100	100/100	Ib	I	1	0	1	0	1	0	1	0	0	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	US-1.1
Sichuan	SLS4	Potato	86/100	100/100	Ib	S	1	0	1	0	1	0	1	0	1	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	US-1.1
Yunnan	YLM13-5	Potato	100/100	100/100	Ia	I	1	1	1	0	1	0	1	0	0	1	0	0	1	1	0	0	0	1	0	1	1	1	0	1	1	CN-9.1
Yunnan	YLM22-4	Potato	100/100	100/100	Ia	I	1	1	1	0	1	0	1	0	0	1	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	CN- 9
Yunnan	M-5	Potato	100/100	100/100	Ia	S	1	0	1	0	1	0	1	0	0	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	MO-6
Yunnan	Zt-2-12	Potato	100/100	100/100	Ia	S	1	1	1	0	1	0	1	1	1	1	0	0	1	1	0	1	0	1	0	1	1	0	0	1	1	CN-10
Heilongjiang	HHB2	Potato	100/100	100/100	IIa	S	1	0	0	0	1	0	0	0	1	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	SIB-1
Gansu	GMX2-1	Potato	100/100	100/100	IIa	ND	1	0	0	0	1	0	0	0	1	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	SIB-1
Hebei	HWCH3	Potato	100/100	100/100	IIa	ND	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	1	1	0	1	1	1	SIB 1.2
Jilin	JYB3	Potato	100/100	100/100	IIa	R	1	0	0	0	1	0	0	0	1	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	SIB-1
Inner Mongolia	N2511-1	Potato	100/100	100/100	IIa	I	1	0	0	0	1	0	0	0	1	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	SIB-1
Inner Mongolia	N87110	Potato	100/100	100/100	IIa	ND	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	1	1	0	1	1	1	SIB-1.2
Inner Mongolia	NNJ3-1	Potato	100/100	100/100	IIa	S	1	0	0	0	0	0	0	0	1	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	SIB-1.1
Sichuan	SSF3-1	Potato	100/100	100/100	IIa	S	1	0	0	0	1	0	0	0	1	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	SIB-1
Yunnan	YPH13-1	Potato	100/100	100/100	IIa	R	1	0	0	0	1	0	0	0	1	1	0	0	1	1	0	1	0	0	0	1	1	0	0	1	1	SIB-1
Chongqing	CYY1175	Potato	100/111	100/100	IIb	R	1	0	1	0	1	1	1	0	0	0	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	CN-11
Fujian	FDH11	Potato	100/111	100/100	IIb	R	1	0	1	0	1	1	1	0	0	0	0	0	1	1	0	1	0	1	0	1	1	0	0	1	1	CN-11.1
Fujian	FZN1-1	Potato	100/111	100/100	IIb	R	1	0	0	0	1	1	1	0	0	1	0	0	1	1	0	1	0	1	0	1	1	0	0	1	1	US-16.1
Yunnan	XA-4	Potato	100/111	100/100	IIb	R	1	0	0	0	1	1	0	0	0	1	0	0	1	1	0	1	0	1	0	1	1	0	0	1	1	US-16

[a] Genotype identified by mating type, allozyme analysis at glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) loci, and mtDNA haplotype and RFLP fingerprint (19).

[b] MtDNA haplotype determined by the methods of Griffith and Shaw (23).

[c] Sensitivity to metalaxyl: ND, not determined; I, intermediate; S, sensitive; R, resistant.

[d] Presence (1) or absence (0) of RG57 fingerprint bands. Bands 1 to 25 are indicated from left to right. Band 4 did not appear in isolates tested and is not diagnostic. Genotypes US-1.1, US-1.3, US-16 (21), MO-6, and SIB-1 (12) and CN-1 to CN-8 (22) were described previously. The genotypes CN-9, CN-9.1, CN-10, CN-11, CN-11.1 US-16.1, and SIB-1.2 are named here.

haplotype was more widely distributed than reported previously (1), and found in seven provinces in China. Other investigations have also reported the IIa mtDNA haplotype (SIB-1) in several countries, including Japan, Korea, and Russia (1,2,12), but this haplotype has not been reported in countries south of China. Likewise, the Ia mtDNA haplotype was widely distributed in five of eight provinces. In India and Nepal, the Ia haplotype is dominant and has been present in these countries since at least 1913 and 1954, respectively (Table 3) (17,22). Because late blight was only reported in the 20th century in China and China is far from the center of origin of late blight pathogen (18), the presence of all four mtDNA haplotypes of the pathogen and their spatial distribution in China in different regions suggests that the pathogen was probably introduced into China from multiple sources at different times. Clearly, the earliest introductions of *P. infestans* into China included Ia mtDNA haplotypes. More and different mtDNA haplotypes were found in Yunnan province, where multiple sources of seed potato have been introduced from both the north and south of China in recent years (Fig. 5).

Data from the archival herbarium samples supports the hypothesis of multiple migrations of *P. infestans* into China and also suggests the time periods when these migrations occurred. The Ia mtDNA haplotype of *P. infestans* was found earlier in China than other mtDNA haplotypes. Samples collected by different researchers in China from the first outbreaks from tomato in 1938 in Kunming and from potato in 1940 in Chengjiang and Chongqing were the Ia mtDNA haplotype. In contrast, the earliest record of the Ib haplotype (US-1 genotype) of *P. infestans* in China was in 1952 on potato in the Sichuan region, in 1954 on potato in Hebei, and in 1956 on tomato in Beijing. In samples from other Asian countries (India, Japan, Peninsular Malaysia, Nepal, and The Philippines), the Ia mtDNA haplotype was also found earlier than the Ib haplotype. Our current data indicate that the Ia haplotype is present in five of the eight provinces sampled in China (Table 1; Fig. 1). The Ia haplotype was most likely the founder haplotype introduced into China and the Ib mtDNA haplotypes migrated later in the mid-20th century, refuting other reports of the Ia displacing the "old" Ib populations in this region (14,17). There were multiple introductions of the Ia mtDNA haplotypes into other Asian countries (Table 3). We cannot rule out multiple introductions of the Ia haplotype into China. The expanded number of RFLP genotypes that are Ia haplotypes suggest that this may be the case (22). We previously documented that the Ia and Ib mitochondrial lineages arose independently and that the Ib is not derived from the Ia (4). Our data suggest a post-WWII spread of the Ib mtDNA haplotype into Asia, most likely from South American seed potato, and could explain previous reports of its widespread occurrence in the 1970s to 1990s worldwide (14,15,21).

Interestingly, the Ib mtDNA haplotype was also found in 1982 on *S. lyratum*, an herbaceous species that grows wild throughout China and Asia adjacent to cultivated fields (Table 3). This represents an example of a host shift for the pathogen to a related wild species (46). Because the US-1 genotype (Ib mtDNA haplotype) is highly sensitive to the fungicide metalaxyl in some areas of the world (10,21,45), perhaps *S. lyratum* acted as a reservoir for the metalaxyl-sensitive genotype to survive in a wild, unsprayed host and then spread into cultivated potato or tomato. Although populations of US-1 in North America are sensitive to metalaxyl, South African populations have developed resistance to phenylamides (45). It would be interesting to survey *S. lyratum* in China for the presence of *P. infestans* and test isolates

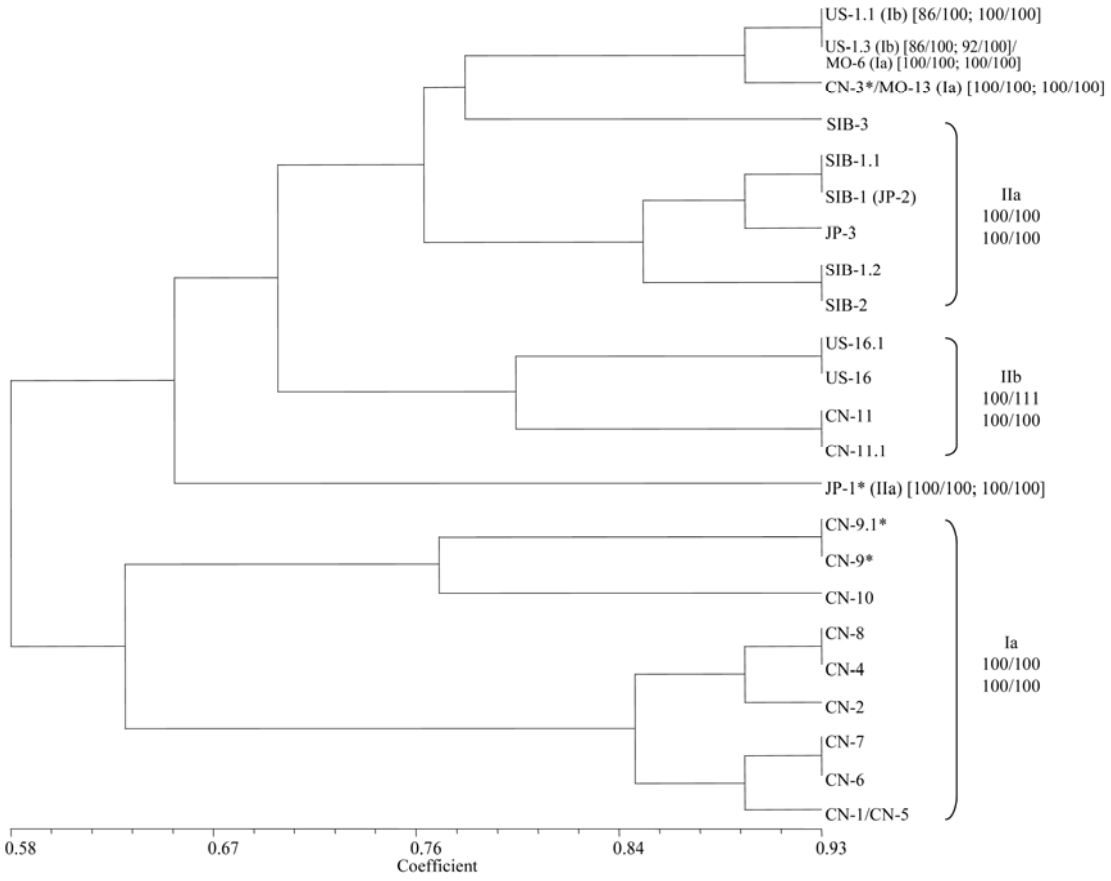

Fig. 4. Dendrogram of newly described Chinese restriction fragment length polymorphism genotypes and previously reported genotypes of *Phytophthora infestans* based on matrices of similarity using unweighted pairgroup method with arithmetic means analysis in NTSYS-PC (43). Asterisks indicate A2 mating types. Similarity coefficient shown at the bottom of the figure.

recovered for genotype and sensitivity to metalaxyl to confirm or refute this hypothesis.

Previous workers have described eight RFLP genotypes of *P. infestans* in China, including CN-1 to CN-8 (22). Our findings suggest that the genetic diversity of *P. infestans* populations in China is greater than previously detected. Seven RFLP genotypes, including US-1 (21), US-16 (21), MO-6 (12), SIB-1 (12), and the three newly named genotypes CN-9, CN-10, and CN-11, were identified. The genotype SIB-1 was the most predominant genotype detected among the isolates we tested and was widely distributed in both the north and south in China in seven provinces. SIB-1 may have fitness traits that have led to its widespread adaptation in China, similar to US-8 in the United States and Blue 13 in Europe, and this possibility needs further examination (14). SIB-1 was also reported as the dominant genotype in Siberia and the Russian Far East Sakhalin Islands in another study (12). The spatial distribution of SIB-1 in Russia suggests that the genotype was most likely spread via the trans-Siberian railway because this is a common method of transporting potato tubers in Russia (12). Potato tubers were introduced from Russia into China through collaboration and nongovernmental trade (Fig. 5) in the north of China in Helongjiang and also in the south to Yunnan in 1950s (33,47,48).

The most predominant RFLP genotype identified in our study was SIB-1. This genotype is also called Japanese A1-A and JP-2 in Japan (22). SIB-1 has also been found in Russia and some other European countries (22), which suggests several possible migrations pathways of this clonal lineage into China from other neighboring countries, including Siberia or Japan. The cluster analysis also indicated a close relationship between SIB-1, JP-2, and JP-3. SIB-1 is widely distributed within China from the north to the south, likely as a result of migration of the late blight pathogen within seed tubers (60) (Fig. 5). Historical movement of potato tubers as breeding materials from outside China and from north China to Yunnan has been documented (60). From the late 1950s to 1990, several hundred potato breeding lines, including both local cultivars and cultivars from outside China and from the International Potato Center, were collected and planted in the experimental plots of Wumeng Agricultural Institute, Inner Mongolia for breeding purposes (60). Some of this breeding material was later introduced into Yunnan, Guizhou, and Sichuan. The experimental plot of Wumeng Agricultural Institute, Inner Mongolia was one of the places that the A2 mating type was first detected in China (56).

Before mid-1980s, farmers in China used to use potato tubers from the previous year's crop as seed tubers, until yields declined due to the accumulation of tuberborne viruses. In the late 1980s, techniques for growing virus-free tubers were adopted in China and a majority of the base for "virus-free seed potato tubers" was set up in the northern part of China, including Inner Mongolia, Heilongjiang, Hebei, and Gansu, where the cool weather and isolation is desirable for virus-free seed potato cultivation. Thereafter, the general path of movement of seed potato tubers yearly within China is from north to south although, more recently, several provinces in the south such as Yunnan and Sichuan have established their own seed potato production locally at high elevations (56). Yunnan province now has ≈90% locally produced seed potato and ≈10% imported potato from northern China. A

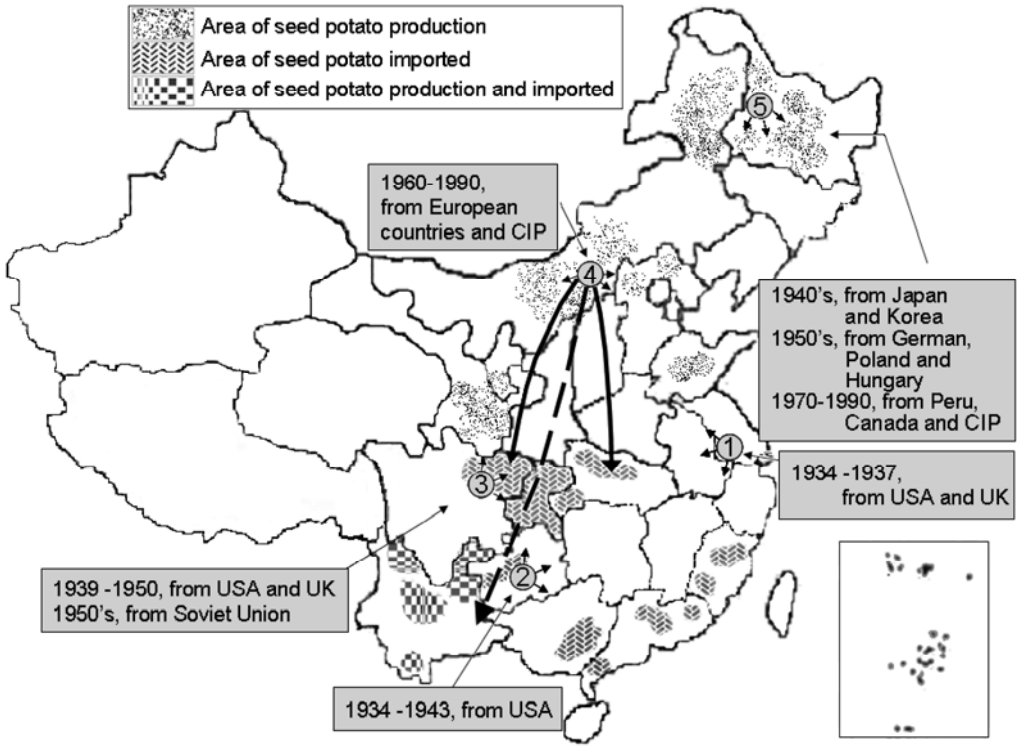

Fig. 5. Map of China indicating the locations of potato seed production, seed importation, or both and general dates and countries from which seed was imported (small arrows) and paths of flow of seed within the country (large arrows). Numbers refer to Agricultural Experiment Stations, as follows. 1, Nanjing National Agricultural Experiment Station (49). From 1934 to 1936, potato tubers from the United States and United Kingdom were imported as breeding materials. Several cultivars were grown in Jiangsu, Shannxi, and Hebei provinces. 2, Guizhou Agricultural Experiment Station (49). From 1937 to 1944, at least 52 potato cultivars were introduced from the United States as breeding material. 3, Chengdu Agricultural Improvement Station, Sichuan (49). From 1939 to the 1950s, potato tubers from the United States, United Kingdom, and the previous Soviet Union were introduced and grown continuously in this area, and some materials with better performance were distributed to Sichuan, Guizhou, and Gansu provinces. 4, Wumeng Agricultural Experiment Station, Inner Mongolia (48,49). From 1960 to 1990, potato tubers from European countries and CIP were imported and grown here. Some of the cultivars and some breeding materials were later introduced into Hubei, Guizhou, and Sichuan provinces and then introduced to Yunnan. 5, Keshan Potato Research Institute and the Northwest Agricultural College (33,48). Potato tubers from Japan and Korea were first introduced into Heilongjiang by the Japanese. In the 1950s, potato tubers from Germany, Poland, and Hungary were imported. From 1970 to 1990, potato tubers from Peru, Canada, and CIP were imported.

large quantity of potato seed produced there are also exported to Vietnam and Burma. With increased growth of potato as a winter crop in south China provinces, SIB-1 has now dominated and is the main clonal genotype in that region. The migrations of potato tubers from north to south throughout China, some of which were infected with SIB-1, may have led to the wide distribution of SIB-1 in China and is likely one of the major causes of resurgence of late blight in China.

Populations of *P. infestans* have undergone genetic changes because samples collected after 1999 contained fewer A2 isolates than was reported previously (1,22). Moreover, reports from other labs recently have shown that the A2 isolates were not detected in areas where A2 mating types were reported earlier (5,32,55,58). During our extensive survey of the mating type distribution of *P. infestans* in nine provinces in China, only a few isolates of A2 mating type were detected and only in Yunnan province. Asexual reproduction and dispersal of clonal lineages in seed is the predominant method of pathogen reproduction in most areas of China. However, because isolates of *P. infestans* from Yunnan province had the greatest number of different genotypes, further sampling in this region is in order. A few self-fertile isolates were found among A1 isolates in Yunnan (16). The potential for the pathogen to undergo sexual reproduction in Yunnan in the future should also be investigated.

Our data suggest multiple migrations of *P. infestans* into China. The Ia mtDNA haplotype was first introduced in the 1930s and still occurs but other mitochondrial lineages have also been introduced into the country over time. The Ib mtDNA haplotype was found in historic samples in China and other Asian countries after 1950, suggesting a second migration. Movement of seed potato tubers through the country has played a major role in the current dominance of the SIB-1 genotype throughout China, most likely via infected potato tubers from Russia and Japan. Populations of *P. infestans* are dynamic and displacement of genotypes and continued monitoring of the genetic structure, mating types, pathotypes, and fungicide sensitivity of isolates will be necessary in the future in China over wider areas of the country to both aid the late blight potato breeding programs and improve disease management.

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