Migrations and Displacements of *Phytophthora infestans* Populations in East Asian Countries

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ABSTRACT

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Two clonal lineages of *Phytophthora infestans* were detected in a sample of 124 isolates collected in China, Japan, Korea, the Philippines, and Taiwan. All isolates were analyzed for mating type, genetic variation at two allozyme loci, glucose-6-phosphate isomerase (*Gpi*), and peptidase (*Pep*), nuclear DNA fingerprint as detected with probe RG57, and mitochondrial DNA haplotype. One clonal lineage (A1 mating type, *86/100* for *Gpi*, and *92/100* for *Pep*) was detected in all five countries and was identical to a genotype found throughout the world, previously designated as US-1. The other genotype, JP-1, was A2 mating type, *100/100* for *Gpi* and *96/96* for *Pep*. This genotype has been detected only in recent samples from Korea and Japan, where it is now the predominant genotype. Two variants of the US-1 genotype occurred in the Philippines. Both the US-1 and JP-1 clonal lineages were polymorphic for metalaxyl resistance, indicating that metalaxyl-resistant isolates are probably selected within each clonal lineage in situ. Isolates resistant to metalaxyl were common in the northern part of South Korea, but all isolates tested from the southern part were sensitive. Both A1 and A2 mating type isolates occurred in Korea and Japan, but the A2 genotype appears to be displacing the A1 genotype in both locations. There was no evidence of sexual reproduction in either location.

Phytophthora infestans (Mont.) de Bary is the cause of late blight of tomato and potato and is more destructive on potatoes than any other disease on a worldwide basis (17). Sexual reproduction occurs in this heterothallic oomycete when individuals of opposite mating type (A1 and A2) come in contact, producing oospores that can survive in soil in the absence of a host (7,25). Asexual reproduction occurs via sporangia produced from leaf, stem, fruit, or tuber lesions and is the chief mechanism of reproduction in late blight epidemics. Prior to the 1980s, only the A1 mating type occurred in worldwide populations of *P. infestans* outside of Mexico (4,10,16). The first report of A2 mating type isolates outside of Mexico was in 1984 (16). Since then, this mating type has been found in most parts of the world, including eastern Asia (20). Discoveries of A2 mating types gave the first indications that major changes were occurring in many *P. infestans* populations around the world.

Migrations of *P. infestans* have played a prominent role in the history of late blight. The first worldwide migration occurred during the nineteenth century (10), when *P. infestans* first migrated out of its center of diversity in central Mexico. Recent analyses indicated that this migration was very limited, resulting in the panglobal distribution of a single clonal lineage (14). One result of this migration was the Irish potato famine in the late 1840s (19). Another migration apparently occurred from Mexico to Europe during the late 1970s (26). This new migrating population displaced the previous population in only a few years (26,27). These migrations may have contributed to increased late blight disease problems in many locations worldwide. Based on recent reports of A2 mating type isolates in Japan and Korea, (20,23), it seems likely that populations of *P. infestans* in east Asian countries might also be changing, perhaps because of migration.

A variety of genetic markers have been used to analyze *P. infestans* populations (1,10,11,13,24). In addition to mating type, allozyme alleles at the glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) loci have been used to compare genetic diversity in populations from different locations (8,24,26). DNA fingerprinting (13) has recently contributed significantly to a better understanding of population subdivision than is possible from analysis of mating type and allozymes alone (6,15,27). Finally, mitochondrial DNA (mtDNA) restriction fragment length polymorphisms have provided additional information (2,6,11). The strongly selected phenotype for metalaxyl resistance in combination with other genetic markers also has contributed to a better understanding of population structure (5,8,28).

The goal of this study was to determine the genetic relationships among populations of *P. infestans* from eastern Asian countries and to assess the level of genetic variation within *P. infestans* populations in Korea. A secondary goal was to determine the occurrence and geographic distribution of resistance to the fungicide metalaxyl in Korea and other east Asian countries. Although A2 mating-type isolates had been reported from Korea as well as Japan, relatively little was known about the geographic distribution of A2 mating types in Korea, and nothing was known about the relationships of these populations to those in other east Asian countries. By comparing genotypes found in Asia with those identified in previous collections throughout the world, it should be possible to determine if the changes in east Asian countries were related to those in Europe or were the result of a separate migration, presumably from Mexico.

MATERIALS AND METHODS

Sources of isolates. Isolates came from a variety of sources (Table 1) at different times. Thirty isolates from Japan represent

a selection (equal numbers of A1 and A2 mating types) of individuals collected in Japan during 1988; collection details have been published previously (23,28). The three isolates from Taiwan were provided by G. L. Hartman, then of the Asian Vegetable Research and Development Center, Taiwan. Eighteen isolates collected during 1991 in the Philippines were sent by P. van der Zaag of the International Potato Center (Manila, Philippines) (Table 1). Ten isolates from 1989 were provided by L. Turkensteen (Wageningen, the Netherlands) (Table 1). Most of the Philippine isolates were purified from potatoes, but some were from tomatoes. Six isolates from China were acquired from the American Type Culture Collection (44547–44552) (Beltsville, MD). These isolates had been collected sometime before 1982.

Fifty-seven isolates were obtained from Korea during 1991 and included individuals from most of the major potato growing areas in Korea. Some of the isolates from Chonbuk and Kangwon provinces were obtained from Chonbuk National University, Junju, and The Korean Research Institute of Chemical Technology, Taejeon. Other isolates were obtained from blighted potato leaves. Isolation into pure culture was accomplished by placing a portion of a discrete lesion on 20% nonclarified V8-juice agar (22) containing ampicillin, vancomycin, pimaricin, rifampicin, and benomyl at 500, 200, 100, 50, and 10 ppm, respectively. In our experience, this procedure has never yielded more than a single homokaryotic individual. After isolation into pure culture, isolates were maintained on Rye A agar (3) at 18 C in the dark.

TABLE 1. Sources of isolates of *Phytophthora infestans* from five east Asian countries[a]

Country Location[b]	No. of sites	Year of collection	Host	Sample size
China				
NK[c]	...	before 1982	Potato	6
Japan				
Aomori		1988	Potato	4
Chiba		1988	Potato	1
Fukuoka		1988	Potato	1
Gunma		1988	Potato	2
Hiroshima		1988	Potato	1
Hokkaido		1988	Potato	9
Hyogo		1988	Potato	1
Iwate		1988	Potato	1
Kagawa		1988	Potato	1
Kyoto		1988	Potato	1
Nagasaki		1988	Potato	3
Shimane		1988	Potato	2
Tochigi		1988	Potato	3
Korea				
Chonbak	2	1991	Potato	9
Chonnam	5	1991	Potato	10
Jeju	2	1991	Potato	2
Kangwan	9	1991	Potato	24
Kyungbuk	4	1991	Potato	10
Kyungnam	2	1991	Potato	2
Philippines				
Atok, Luzon	1	1991	Potato	3
La Trinidad, Luzon	1	1989	Potato	5
Macdaim	1	1989	Potato	1
Mountain Province, Luzon	1	1991	Potato	3
Sadsadan, Luzon	1	1989	Potato	1
Swemp	1	1989	Potato	1
Tainidad	2	1991	Potato	9
Tainidad	1	1991	Tomato	3
Upper Sayangan, Luzon	1	1989	Potato	1
...	1	1989	Potato	1
Taiwan				
NK[c]		1991	Tomato	3

[a] Isolates from China were acquired from the American Type Culture Collection (44547–44552) Beltsville, MD; those from the Philippines were provided by L. Turkensteen, IPO, Wageningen, the Netherlands (1989 isolates), and P. van der Zaag of the International Potato Center, Manila, Philippines; those from Taiwan were provided by G. Hartman, then of the Asian Vegetable Research and Development Center, Taiwan.

[b] For Japan, the locations are prefectures; for Korea, they are provinces; for the Philippines, they are towns.

[c] Not known.

Mating type. To determine the mating type of an isolate, an agar disk (8 mm diameter) containing mycelium was cut from the advancing edge of a 7- to 10-day-old colony growing on Rye A agar and placed on 10% clarified (by centrifugation) V8 agar (100 ml of V8 juice, 15 g of agar, 900 ml of H_2O, 1 g of $CaCo_3$, and 0.05 g of β-sitosterol) 4 cm distant from a known A1 isolate (1100) and, on a different plate, 4 cm distant from a known A2 isolate (575). After 5–10 days, the presence or absence of oospores at the hyphal interface between the isolates was examined microscopically. All matings were repeated. Those isolates forming oospores with the A1 tester but not with the A2 tester were designated A2, and those forming oospores with the A2 tester but not the A1 tester were designated A1.

Allozyme analysis. To obtain mycelia for allozyme analysis, isolates were cultured at 18 C for 12–14 days in the dark on Rye A broth in 9-cm petri dishes. Cultures were started with agar disks (8 mm) containing mycelium cut from the edges of actively growing colonies. The excess culture filtrate from three or four petri dishes was removed by vacuum filtration, and the resulting mycelial mat was ground with glass beads in one to four drops of water. A small square of tissue paper was placed on top of the macerated mycelium, and paper wicks were placed directly on the tissue paper. Starch gel electrophoresis was carried out using the standard buffer systems described by Sujkowski et al (27). Allozyme phenotypes were scored according to the relative mobilities of individual active enzymes, with the most common allele designated as 100 as described by Tooley et al (29) and using standard isolates maintained at Cornell as controls. Because GPI and PEP are both dimeric enzymes and *P. infestans* is a diploid, heterozygotes had three bands (one band for each homodimer and one band for the heterodimer).

DNA fingerprinting. DNA was extracted from mycelia cultured and harvested as described above. However, instead of grinding with the glass beads, the mycelia were frozen at −80 C and lyophilized overnight. The freeze-dried tissue was then placed in a mortar, frozen with liquid nitrogen, ground with a pestle, and stored at −80 C. DNA was extracted from 1–2 ml of powdered lyophylized mycelium. The powdered mycelium was suspended in 2.6 ml of extraction buffer (1 ml of water-saturated phenol, 670 µl of triisopropylnaphthalene at 20 mg/ml, 670 µl of 4-aminosalicylic acid at 20 mg/ml, and 330 µl of 5× RNB [Tris-HCl, 0.76 M; NaCl, 1.2 M; and EDTA, 0.25 M]) in a 16-ml Falcon tube at 55 C and vortexed briefly. After a 2-min incubation in a 55 C water bath, 670 µl of chloroform/isoamyl alcohol (24:1) was added, and the mixture was again incubated at 55 C for 2 min. The mixture was then centrifuged at 8,000 rpm for 10 min, and the water phase was extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1) and once with chloroform/isoamyl alcohol (24:1). The final extract was transferred into a 2.5-ml microfuge tube. The DNA was then precipitated with 0.6 volumes of 2-propanol centrifuged at 13,000 rpm in a microcentrifuge. The supernatant was discarded, and the pellet was resuspended in 200 µl of Tris-EDTA buffer containing RNAse at 20 µg/ml.

The samples were digested with the restriction enzyme *Eco*RI according to the manufacturer's directions except that spermidine was added to each reaction to a final concentration of 4 mM. Gel electrophoresis in 0.9% agarose gels, alkaline blotting to nylon membranes, hybridization with ^{32}P random-primed probe RG57 (13), and autoradiography were all performed according to standard techniques (21). All filters were hybridized at 65 C and washed three times for 10 min each, first in 2× SSC (1× SSC is 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0) and 0.1% SDS (sodium dodecyl sulfide), then in 1× SSC and 0.05% SDS, and finally in 0.5× SSC and 0.025% SDS. After autoradiography, the DNA was removed from the filter by washing three times at 42 C, for 10 min each time: first in 0.1 N NaOH, then in 100 mM Tris, pH 7.7, 0.1× SSC, and 0.1% SDS, and finally in 0.1× SSC and 0.1% SDS.

Probe RG57, a 1.2-kb moderately repetitive fragment of *P. infestans* genomic DNA and purified mtDNA from central

TABLE 2. Genotypes of *Phytophthora infestans* from five east Asian countries

Country	Mating type	Allozyme genotype *Gpi*	Allozyme genotype *Pep*	RG57 fingerprint[a]	mtDNA[b]	Genotype code[c]	Met[d]	Host	Sample size
China	A1	*86/100*	*92/100*	10101010110011010001100110	A	US-1	S	Potato	6
Japan	A1[e]	*86/100*[e]	*92/100*[e]	10101010110011010001100110	A	US-1	S[e]	Potato	15[e]
	A2[e]	*100/100*[e]	*96/96*[e]	10001100000011011000100111	B	JP-1	S[e]	Potato	10[e]
	A2[e]	*100/100*[e]	*96/96*[e]	10001100000011011000100111	B	JP-1	I[e]	Potato	3[e]
	A2[e]	*100/100*[e]	*96/96*[e]	10001100000011011000100111	B	JP-1	R[e]	Potato	2[e]
Korea	A1	*86/100*	*92/100*	10101010110011010001100110	A	US-1[f]	S	Potato	1
	A2	*100/100*	*96/96*	10001100000011011000100111	B	JP-1	S	Potato	10
	A2	*100/100*	*96/96*	10001100000011011000100111	B	JP-1	I	Potato	23
	A2	*100/100*	*96/96*	10001100000011011000100111	B	JP-1	R	Potato	23
Philippines	A1	*86/100*	*92/100*	10101010110011010001100110	A	US-1	S/R	Potato	22
	A1	*86/100*	*92/100*	10101010110011010001100110	E	US-1	S	Potato	3
	A1	*100/100*	*92/100*	10101010110011010001100110	F	US-1.1	S	Tomato	3
Taiwan	A1	*86/100*	*92/100*	10101010110011010001100110	A	US-1	S	Tomato	3

[a]The nuclear DNA fingerprint was determined with probe RG57 as described in text. 1 = presence of band (heterozygous or homozygous); 0 = absence of band; data for the bands are from left to right for bands 1–25. The last digit is band 24a (Fig. 2). RG57 fingerprint-band 4 is inconsistent, depending on the plasmid preparation rather than on the isolate. Band 4 was not detected in the batch of probe used in this study and was thus scored "absent" (0). In a previous study (12), band 4 was scored as "present" (1). However, data for band 4 were not used in the analyses.

[b]Mitochondrial DNA (mtDNA) types are described in Goodwin (11) and in Figure 2.

[c]The US-1 and US-1.1 genotypes were first named in Goodwin et al (12). This is the first description of JP-1.

[d]Met = reaction to metalaxyl (S = sensitive; I = intermediate; R = resistant) as defined in the text.

[e]Also reported in Mosa et al (24) and Therrien et al (28).

[f]Found near Wando in Chonnan Province.

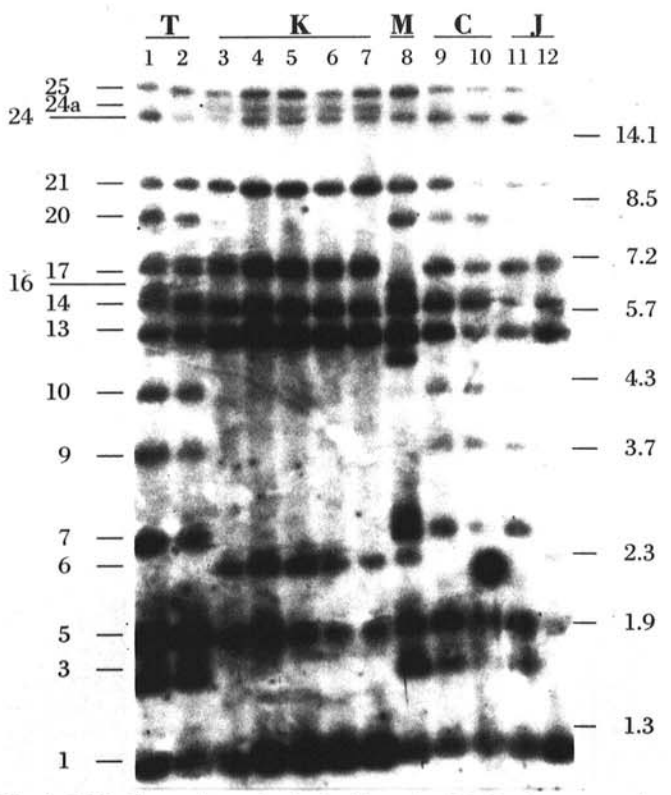

Fig. 1. DNA fingerprints obtained with probe RG57 of representative isolates from Taiwan (T), Korea (K), China (C), and Japan (J). Mexican isolate 568 (M) was included as a reference. Numbers along the left margin indicate band numbers, and numbers along the right margin indicate approximate molecular weights in kilobases. Isolates 1, 2, and 9–11 are genotype US-1, and isolates 3–7, and 12 are genotype JP-1 (Table 2).

Mexican isolate 580 were used in hybridizations to produce DNA fingerprints and to identify mitochondrial haplotypes for each isolate. Each DNA fingerprint locus was scored for either the presence or absence of a band. When comparing fingerprint patterns among isolates, band 4 was omitted because its appearance seemed to depend on the batch of plasmid used as probe rather than on the isolate. Probe RG57 produces a large number of patterns (13). The mitochondrial probe produces a limited number of banding patterns, (1,6,11,27)

Metalaxyl sensitivity. Response to metalaxyl was determined by an in vitro growth test. Radial growth of an isolate on 10% V8 agar with metalaxyl at final concentrations of 5, 100, and, in some tests, 500 μg/ml was compared to growth in the absence of metalaxyl. One liter of medium was treated with 1 ml of dimethylsulfoxide (DMSO) containing the appropriate concentration of technical grade metalaxyl to achieve the desired concentration. The control (no metalaxyl) received 1 ml of pure DMSO. Agar disks (8 mm) containing mycelium from the edge of an actively growing colony were transferred to the center of petri dishes (9 cm) containing the test medium. Cultures were incubated at 18 C in the dark for 10–20 days, until the colony in the absence of metalaxyl was at least 3 cm in diameter. The mean radial growth of two replicates of each isolate was measured. Some tests also were done on Rye B agar (3) similarly amended with metalaxyl.

Two types of statistics relating to metalaxyl sensitivity were calculated. The effective concentration of metalaxyl for inhibiting 50% of mycelial growth of an isolate (EC_{50}) was calculated on the basis of linear regression of the response (mean radial growth) plotted against dose (log concentration of fungicide). Classes of responses similar to those described by Dagget et al (5) and Therrien et al (28) also were calculated: sensitive (S = relative growth at 5 and 100 μg/ml, less than 40% that of the control); intermediate (I = relative growth at 5 μg/ml, more than 40% that of the control, but at 100 μg/ml relative growth was less than 40% of the control); and resistant (R = relative growth at 5 and 100 μg/ml, more than 40% that of the control). All assays were performed twice.

RESULTS

Mating type. Only A1 mating-type isolates were found in the samples from China, the Philippines, and Taiwan, whereas both A1 and A2 mating types were found in the population from Korea (Table 2). Previous reports of both A1 and A2 mating types in Japan (23) were confirmed (Table 2). Among the 57 isolates from Korea, only one was A1 (Table 2). No self-fertile isolates were detected.

Population structures based on mating type, allozyme genotype, nuclear fingerprint, and mitochondrial haplotype. Only four multilocus genotypes were detected in the total sample of 121 isolates. One A1 genotype (US-1 [Table 2; Fig. 1]) is the same one that has been detected all over the world and that has

dominated most worldwide populations until recently (9,14,27). This genotype was the only one found in the small samples from Taiwan and China (the Chinese isolates were deposited with the ATCC some time before 1982); it dominated the collection from the Philippines, was present in Japan, and was present in very low frequency in Korea (one of 57 isolates) (Table 2). The Korean isolates were sampled randomly, but the sample from Japan was selected from a larger collection (23) to provide equal numbers of A1 and A2 isolates. Therefore, the actual frequency of the various genotypes in Japan cannot be determined from this collection.

The second common genotype (JP-1), detected in Korea and Japan, was an A2 mating type with a dilocus allozyme genotype of *100/100* and *96/96* for *Gpi* and *Pep*, respectively, as reported previously for Japan by Mosa et al (24). This genotype also was monomorphic for nuclear DNA fingerprint and for mtDNA haplotype (Table 2; Figs. 1 and 2). Thus, it appears that the same clone of *P. infestans* detected previously in Japan also was detected in Korea.

The only other genotypes were variants of the US-1 lineage detected in the Philippines. Three isolates with the same mating type, *Pep* genotype, and nuclear DNA fingerprint as US-1 had a single change at the *Gpi* locus and were described previously as US-1.1 (12). These were the only isolates obtained from tomatoes in the Philippines, and they also had a novel mtDNA haplotype, F (Table 2; Fig 2), which appeared to be the result of an insertion into the third largest mtDNA band. Three other isolates in the Philippines otherwise identical to the US-1 genotype had another novel mtDNA haplotype, E (Table 2; Fig. 2). This haplotype was identical to haplotype A, except the third largest band was missing.

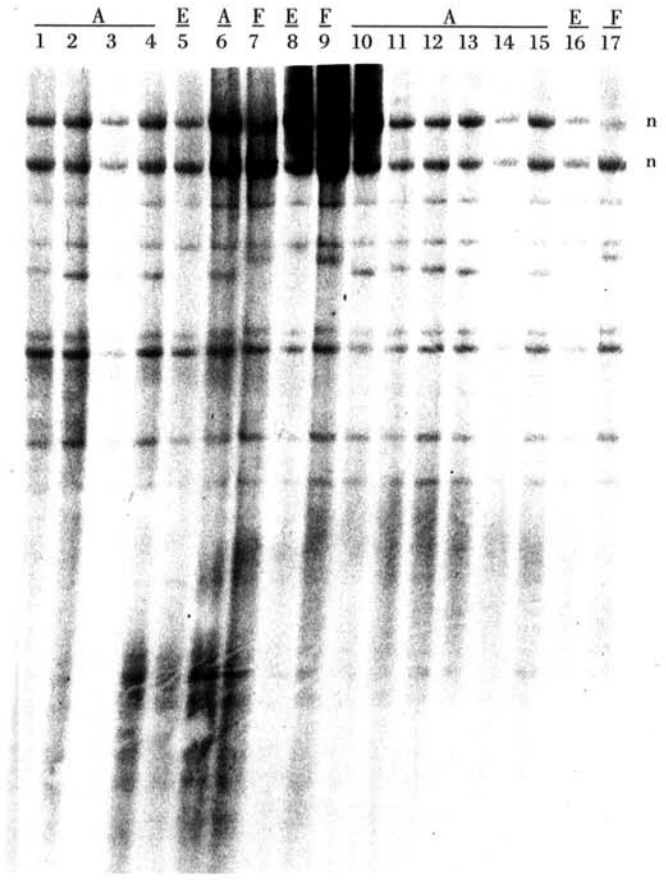

Fig. 2. Mitochondrial haplotypes detected in isolates from the Philippines. Lanes 1–4, 6, and 10–15 are type A (11); lanes 5, 8, and 16 are type E; and lanes 7, 9, and 17 are type F (Table 2). The two largest molecular weight bands (n) are repetitive nuclear DNAs visible due to slight contamination with nuclear DNA of the purified mitochondrial DNA preparation used as a probe.

Metalaxyl sensitivity. There was diversity for metalaxyl sensitivity in both the JP-1 and US-1 genotypes. In Korea, metalaxyl-resistant isolates of JP-1 were obtained from the northern provinces of Kangwon, Kyungbuk, and Chonbuk but not from the southern provinces of Chonnam, Kyungnam, and Jeju (Table 3; Fig. 3). However, metalaxyl-sensitive isolates of JP-1 were

TABLE 3. Degree of resistance to metalaxyl among 57 isolates of *Phytophthora infestans* collected from potato farms at different locations in Korea during 1991

Province[a]	Nearest town	EC_{50} (μg/ml)[b] range (no. of isolates)				
		0–5	5–49	50–99	100–499	500+
Jeju	Hallim	1	1			
Kyungnam	Milyang	1	1			
Chonnam	Wando	1[c]				
	Haenam	2	1			
	Kangjin	2	3			
	Muahn		1			
Chonbuk	Kimje		1		1	
	Muju	4	2			1
Kyungbuk	Cheongsong	1	1			
	Youngyang	3	2	1		2
Kangwon	Jinbu	3	1	1		
	Kangneung			1	1	
	Hoenggye		1	1	6	9
Total		18	15	4	8	12

[a] Provinces are listed from south (Jeju) to north (Kangwon).
[b] EC_{50} is the concentration of fungicide that inhibits 50% of mycelial growth and is calculated from a linear regression of the response (mean radial growth) plotted against dose (log concentration of metalaxyl). The broad classes of resistant (R), intermediate (I), and sensitive (S), (5) correspond approximately to EC_{50} values of 100–500^{+}, 5–99, and 0–5 μg/ml, respectively.
[c] This was the only isolate with an A1 mating type.

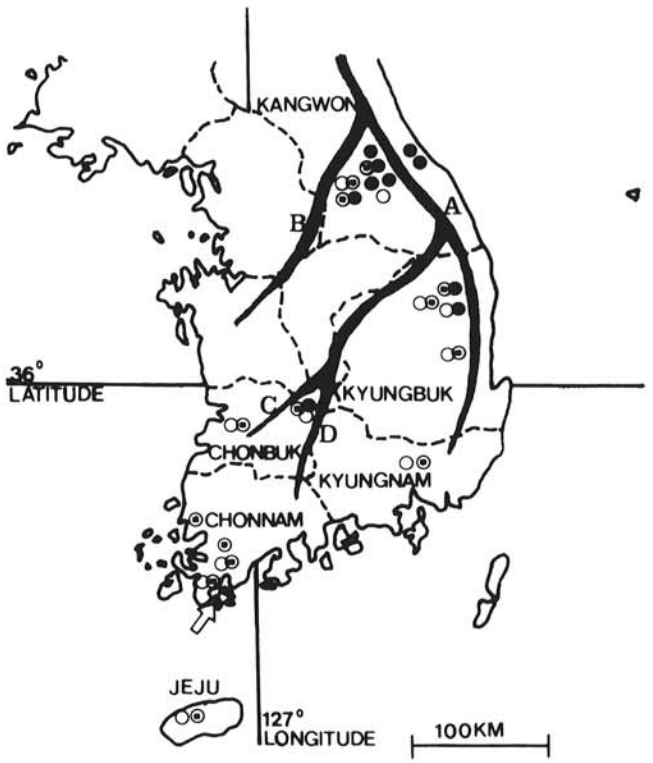

Fig. 3. Locations in Korea of sites from which isolates of *Phytophthora infestans* were obtained during 1991. Open circles = metalaxyl-sensitive isolates; concentric circles = intermediately sensitive isolates; and closed circles = metalaxyl-resistant isolates. The arrow indicates the site at which the single isolate with an A1 mating type was found. The heavy lines describe the locations of mountain ranges; locations where moderate temperatures and rainy periods favoring late blight are common.

detected in all provinces. For Korea as a whole, metalaxyl-sensitive isolates constituted approximately 30% of the population (Table 3). Similarly, there was diversity for sensitivity to metalaxyl among isolates with the JP-1 genotype from Japan. However, because this was a selected collection, the frequencies cannot provide reliable inferences. All isolates with the US-1 genotype from China, Japan, Korea, and Taiwan and most from the Philippines were sensitive to metalaxyl, but a few US-1 genotypes from the Philippines were resistant to metalaxyl (Table 2).

The genotypes in this study were compared to genotypes in a database of worldwide isolates maintained at Cornell University, Ithaca, NY. The US-1 genotype has been detected on all continents where *P. infestans* has been identified, except Australia. The JP-1 genotype has not been reported before in Europe, the United States, Canada, or northern Mexico.

DISCUSSION

The limited number of genotypes detected in samples of *P. infestans* from east Asian countries indicates that these populations are genotypically quite simple. The US-1 clonal lineage was found in all five countries and was the only lineage detected in the limited samples from China, Taiwan, and the Philippines. This lineage was present in China before 1982. Some isolates from Japan collected as early as 1958 had the same mating type and dilocus allozyme genotype as US-1 (24). These findings provide support for the hypothesis (14) that this clonal lineage was distributed panglobally in a very early migration of *P. infestans*. The JP-1 genotype in Japan and Korea is probably the result of a recent migration. Isolates with the same mating type and dilocus allozyme genotype as JP-1 were detected first in Japan during 1987 (24). JP-1 is now the predominating genotype in South Korea, where it is probably displacing the US-1 genotype. As of 1991, US-1 was prevalent in the Philippines, and it certainly was present in Taiwan. However, with the small sizes of our samples, it is not possible to know with certainty that JP-1 has not been introduced also to those locations.

At this time, it is impossible to identify the route of immigration into eastern Asia. The JP-1 genotype was compared with genotypes collected from all over the world (S. B. Goodwin, B. A. Cohen, and W. E. Fry, *unpublished data*), and there were no matches. Thus, the JP-1 genotype may represent a unique migration unrelated to recent migration(s) into Europe. Presumably, the original source of JP-1 was Mexico, but whether the path of migration was directly to Asia from Mexico is unclear. Because JP-1 is so well established in Japan and Korea, it seems likely that it may have been introduced also into China. Recent isolates from China are needed to test this hypothesis. The common exchange of potatoes between Japan and Korea probably explains why this lineage is prevalent in both countries.

Based on previous analyses of isolates from Japan, other investigators have concluded that sexual reproduction probably did not occur (24,28). This was confirmed for Japan and Korea by DNA fingerprint analysis. All isolates tested from both countries had one of only two different multilocus genotypes. The occurrence of a single clone in most locations precludes sexual reproduction; none of the isolates analyzed in this study appeared to have arisen by recombination between the two predominating genotypes.

Despite the current lack of evidence for sexual recombination among isolates of *P. infestans* in east Asian countries, the existence of both mating types in the same region makes sexual reproduction a possibility. In fact, the situation in eastern Asia may be similar to that in Europe, where evidence for sexual reproduction was not reported until more than 10 yr after the probable introduction of A2 genotypes (7,27). If sexual reproduction occurs, it might change the epidemiology of late blight in east Asian countries. Oospores survive over winter (7), and could serve as a source of inoculum as well as a source of new genetic variation. Mosa et al obtained F1 hybrids between the predominating A1 (US-1) and A2 (JP-1) genotypes in Japan (24). Because the genetic structure of populations of *P. infestans* in Japan and Korea is so simple, it should be possible to easily identify the products of sexual reproduction should they occur in the future.

One possible factor affecting the survival of a genotype is its metalaxyl sensitivity. In this study, we found that all individuals of the US-1 clonal lineage outside of the Philippines were sensitive to metalaxyl, but the JP-1 genotype was polymorphic for metalaxyl resistance. There appeared to be a strong association in Korea between geographic origin and occurrence of metalaxyl resistance. Highly resistant isolates with EC_{50} values >50 μg/ml were detected only in the northern provinces of Kangwon, Kyungbuk, and Chonbuk, not in the southern provinces of Chonnam, Kyungnam, and Jeju. Most of the sampling sites in the northern provinces have weather favorable to late blight and have had a history of severe epidemics (Fig. 3). Consequently, metalaxyl use in these areas has been intensive. The province of Kangwon had isolates with the greatest resistance to metalaxyl (Table 3). Metalaxyl resistance has been detected there since the late 1980s (Y. I. Ham, *personal communication*). Because this area includes some seed-tuber production areas, it might be the center of origin of metalaxyl-resistant isolates in Korea.

The immigrating genotype is probably more fit than the previous population. Kim et al (18) reported that a composite fitness index of metalaxyl-resistant isolates (presumably only JP-1) was higher than that for metalaxyl-sensitive isolates (containing both US-1 and JP-1 isolates). Although not conclusive, this result is consistent with the concept that the immigrating genotypes are more fit than the previous isolates with the US-1 clonal genotype. It is unlikely that specific virulence (ability to overcome R-gene resistance) plays a role in fitness. Most potato cultivars have no or very few R-genes, and indigenous populations already contain specific virulences enabling compatibility.

Two variants of the US-1 clonal lineage were detected in isolates collected from the Philippines. One variant (US-1.1) was *100/100* for *Gpi* and had mtDNA haplotype F, whereas the other variant was identical to US-1, except it had mtDNA haplotype E (Table 2; Fig 3). The *100/100* genotype for *Gpi* most likely arose by mitotic recombination; the original US-1 genotype is heterozygous *86/100* at this locus. The mtDNA haplotypes E and F probably arose by mutations. Haplotype E was the result of the loss of a fragment, whereas haplotype F appeared to be an insertion into the third largest mtDNA fragment. As far as we are aware, these two mtDNA haplotypes have not been reported previously (1,6,11). All of the isolates ($n = 3$) collected from tomatoes in the Philippines were US-1.1 and were distinct from the potato isolates. This may represent a separate lineage that is evolving independently of the potato isolates due to specialization for tomato. Further experiments are required to test this hypothesis.

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