Letter to the Editor

Origin of the A2 Mating Type of *Phytophthora infestans* Outside Mexico

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The first report of the A2 mating (compatibility) type of the potato late blight pathogen, *Phytophthora infestans* (Mont.) de Bary, outside Mexico was in Europe during 1984 (32) and, since then, the A2 has been found in many parts of the world (19,23). The four most likely hypotheses to explain the occurrence of the A2 mating type outside Mexico are that it (i) was always present, but undetected (58); (ii) was introduced by migration (62); (iii) arose by mutation or mitotic recombination; or (iv) arose by mating type change, either from exposure to fungicides or by induced selfing (39). Among these, the migration hypothesis is the only one with strong scientific support. However, this subject remains somewhat controversial, and alternative explanations for the origin of the A2 mating type of *P. infestans* outside Mexico still appear occasionally in the literature.

Analyses of allozyme data provided the first unambiguous evidence that the A2 mating type in Europe and Japan was introduced by migration from Mexico (62). Numerous additional population genetic studies have fully supported the migration hypothesis (13, 15,18,23,25,40,44,55,63). In each location studied, the first detection of A2 isolates coincided with the appearance of new alleles at allozyme, DNA fingerprint, and mitochondrial DNA loci. Similar changes occurred with the recent appearance of the A2 mating type in the United States (29). Although the detection of new alleles sometimes preceded the A2 (1,25), the A2 never appeared without new alleles (19,23).

The migration hypothesis was challenged recently by Ko (39), who proposed instead that mating type change was the origin of the A2 mating type of *P. infestans* outside Mexico. Ko's (39) conclusion came from his result that self-fertilization could initiate mating type change. Unfortunately, the mating type change hypothesis in *P. infestans* was not tested using genetic markers, and no genetic mechanism was proposed by which mating type change could occur. Furthermore, the population genetic data that contradict the mating type change hypothesis were ignored. Part of the proof for the mating type change hypothesis was based on a number of early literature reports that supposedly stated that homothallic isolates of *P. infestans* were present outside Mexico prior to the 1950s. Unfortunately, these early references were cited without critical evaluation. It is well-known that heterothallic species of *Phytophthora* produce occasional oospores in single culture (4,21,53,64, 65). It is also quite well documented that oospores of *P. infestans* were found occasionally during the early part of this century in Europe and the United States (8,48). However, because these structures were produced only rarely and under specific conditions, the scientific consensus was that the sexual stage of *P. infestans* remained to be discovered. This did not change until the A2 mating type was found in Mexico during the 1950s (21,47,61).

Because of the strong evidence for migration, the mating type change hypothesis has never been tested directly. Fortunately, this hypothesis provides testable predictions about the genetic background that should be present in A2 isolates outside Mexico. If the A2 originated by mating type change from A1 mating type populations, the first A2 isolates in each location should be identical, or nearly identical except for mating type, to the previously existing A1 isolates. Sexual reproduction after mating type change could generate new genotypes, but they still should contain only the alleles present in the original A1 populations. Because most populations throughout the world, until recently, were composed primarily, or exclusively, of a single clonal lineage (15,23,25), the mating type change hypothesis is easily testable using molecular markers. Identical multilocus genotypes (or changes limited to a rearrangement of alleles) before and after the occurrence of the A2 mating type would confirm the mating type change hypothesis. In contrast, if the A2 mating type originated by migration, A2 isolates could be similar, or very distinct, from the original A1 isolates, depending on the source population for the migrating genotypes. If the first A2 isolates were very different from isolates in the old A1 populations, the mating type change hypothesis would be rejected.

Our purpose was to reanalyze previously published genotypic data to explicitly test the mating type change hypothesis for the origin of the A2 of *P. infestans* outside Mexico. A secondary goal was to evaluate the early literature to test Ko's (39) conclusion that homothallic isolates of *P. infestans* were known outside Mexico prior to the 1950s. Finally, mating type segregations in self-fertilized progenies of *P. infestans* were analyzed to determine whether mating type change has been observed by other investigators.

CLUSTER ANALYSES

Cluster analyses should provide a rigorous test of Ko's (39) hypothesis. If the A2 originated by mating type change, then the first A2s in each location should cluster tightly with the A1 genotypes from which they were derived. The mating type change hypothesis can be rejected if the first A2s in each location are in different clusters from the previously existing A1s.

Data to test the hypothesis were available for six locations where the A2 mating type has appeared recently: northwestern Mexico (28), South America and Costa Rica (25), Eastern Europe (25,63), East Asia (40), Western Europe (25), and the United States and Canada (24,29). To be conservative, isolates scored as homothallic were considered A2 for this analysis. In each location, only data collected within a few years of the initial detection of the A2 mat-

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that also may be suitable.

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ing type were included. Thus, data for East Asia came from isolates collected from 1982 to 1991 (A2 first detected in Japan during 1987 [43]), those in Eastern Europe from 1979 to 1990 (A2 [homothallic] first detected in Estonia during 1983 [25] and in Poland during 1988 [63]), those in Western Europe from 1977 to 1985 (A2 first detected during 1981 [16]), those in the United States and Canada from 1979 to 1993 (A2 first detected during 1987 [10] and during 1991 [24] for the isolates in this data set), those in South America from 1982 to 1990 (A2 first detected during 1984 [5]), and those in northwestern Mexico during 1989 when the A2 was first detected (28). Isolates in each location were scored previously for mating type, for genotype at the two allozyme loci glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*), and for genotype at 26 DNA fingerprint loci revealed by the moderately repetitive probe RG57 (26). In total, the data set for this study contained information on more than 560 isolates.

A multilocus genotype was constructed for each isolate by combining the data for dilocus allozyme genotype and DNA fingerprint. Although the genotype at each DNA fingerprint locus can be determined by analyzing fragment intensities (26), a more conservative approach was used for this study; fragments were scored only as present or absent. Thus, the measures of genetic distance reported here are under-estimates and are more properly referred to as "phenotypic" distances, because the DNA fingerprint data were only scored phenotypically. However, they will be referred to as genotypes for simplicity. DNA fingerprint fragment 4 does not always give repeatable results, so was excluded from all analyses. Two rare fingerprint fragments, 11a (24) and 24a (63), were included in the analysis for a total of 26 DNA fingerprint loci.

Genetic distances of A1 and A2 multilocus genotypes were compared in each of the six regions by cluster analysis. First, the data were analyzed to determine the total number of genotypes in each location (Table 1). Data for each allozyme genotype were converted to binary format by creating a column for each possible allozyme allele (seven alleles for *Gpi* and six for *Pep*). Presence of an allele was marked with 1 and absence with 0. The DNA fingerprint data were in binary format already. Mating type data were not used for this analysis.

Similarity was estimated as the proportion of shared allozyme and DNA fingerprint alleles (i.e., Dice coefficients [51]) between each pair of genotypes in each location (41). This method is the same as Nei and Li's (46) index of genetic similarity (S_{xy}) for restriction fragment length polymorphism (RFLP) comparisons (41). These values were then subtracted from unity to yield genetic distances. The matrix of genetic distances between each pair of genotypes was displayed as a phenogram using the unweighted pair group method for arithmetic averages. All cluster analyses were performed with a SAS (version 6; SAS Institute, Cary, NC) program by M. Levy (Purdue University, West Lafayette, IN).

These analyses indicated that A2 mating type isolates in all locations were very different from those in the old A1 populations (Fig. 1). This was particularly clear in northwestern Mexico, South America and Costa Rica, East Asia, Western Europe, and northern North America (Fig. 1). In Eastern Europe, the analysis was complicated by the large number of genotypes. There were two pairs of identical A1 and A2 genotypes in Eastern Europe: PO-4 and PO-18, and PO-17 and RU-1 (Fig. 1). However, PO-4 and PO-18 differed significantly from the old US-1 (also known as PO-1) genotype, and clearly could not have originated by mating type change. The A2 PO-17 and homothallic EE-2 were in a sister cluster to isolates in the old US-1 clonal lineage (Fig. 1). However, each of these genotypes still would have required changes at two additional unlinked loci besides mating type to have originated by mating type change from US-1. In northwestern Mexico, the A2 US-8 genotype was closest to the A1 LM-2 genotype (distance 0.103) (Fig. 1). However, US-8 and LM-2 each had one allozyme allele not possessed by the other, which excludes an origin by mating type change.

DIFFERENCES FROM US-1

Another way to analyze these data is to determine the number of genetic differences between A1 and A2 isolates. If the A2s originated by mating type change, the number of genetic differences between the A2 genotypes and the previously existing A1s should be extremely low. A large number of genetic differences between A1 and A2 isolates would reject the mating type change hypothesis. Prior to the appearance of the A2 mating type, populations of *P. infestans* worldwide were dominated by a single clonal lineage (15,19,23,25). The most common genotype within this lineage, previously called US-1 (24) or PO-1 (63), was used as the standard for pre-A2 populations. Populations were classified as old (pre-A2) or new (post-A2) depending on when (i) the samples were taken (old samples were earlier); (ii) the A2 mating type was detected (pre-A2 samples were old); and (iii) new molecular marker alleles were detected (if new alleles were detected before the A2). In East Asia, both mating types coexisted when the samples were taken, and the criteria of Spielman et al. (62) were used to define old and new populations. Data for four Mexican populations (28) also were analyzed for comparison. The mean number of differences from the US-1 genotype was calculated for all genotypes in each population using a computer program written by S. B. Goodwin in the C programming language. Genotypes were coded as indicated in Table 1. Significance tests within each location and between the means for all old, new, and Mexican populations were performed with MINITAB (Minitab Inc., State College, PA).

This analysis revealed that isolates from old populations in all locations except the United States and Canada averaged fewer than 0.25 genetic differences from US-1 (Table 2). Isolates from the United States and Canada had a strongly bimodal distribution with a large peak at 0 differences for US-1 isolates and a second peak at 5 differences for US-6 isolates (data not shown). The mean number of differences over all old populations, including the United States and Canada, was 0.59 (Table 2).

In contrast, isolates from new populations were significantly different from US-1. The number of differences from the US-1 genotype among isolates from new populations ranged from 2 to 12 with a mean of 7.54 (Table 2). The ranges of differences from US-1 between old and new populations in the same location were so different that they generally did not even overlap. For example, in South America, isolates from old populations had either 0 or 1 difference, whereas those from new populations had 7 or 8 (Table 2). The ranges overlapped slightly in Eastern Europe and the United States and Canada, but the means were significantly different between old and new populations in each location (*t* tests). The mean number of genetic differences from US-1 in all old populations combined was significantly less than in new populations (*t* test, $P = 0.0000$).

Mexican populations of *P. infestans* had a mean number of differences from US-1 that ranged from 5.38 to 8.52 (Table 2), significantly greater than in old populations (*t* test, $P = 0.0004$). The mean value in Mexican populations was not significantly different from that in new populations worldwide ($t = 0.62$, $P = 0.55$). The new genetic variation in A2 isolates worldwide was a subset of that in central Mexico (13,25). Thus, the new genotypes most likely arose by sexual recombination in Mexico prior to migration.

LITERATURE EVALUATIONS

To test Ko's (39) conclusion that homothallic isolates of *P. infestans* were known outside Mexico prior to the 1950s, all of the early references that Ko (39) cited as proof of homothallism, plus a few others, were analyzed. A difficulty in evaluating early studies is to establish a baseline against which they can be judged. An objective standard for judging the accuracy of the conclusion that early isolates were homothallic is to compare the descriptions of early isolates with the characteristics of homothallic species known

at that time. Therefore, a profile of homothallic isolates was constructed by evaluating the literature for *P. infestans* and the homothallic species *P. erythroseptica* and *P. phaseoli*. Typical and, at that time, known characteristics of homothallic isolates of *Phytophthora* species are (i) antheridia are abundant and easily identified in vitro (8,20,58); (ii) the isolates form large numbers of oospores very quickly on any medium (8,20,48,53,58); (iii) the oospores are well filled with little or no abortion (8,20,48,53); (iv) oosporic cultures have a dense, adpressed colony morphology and produce few, if any, aerial hyphae (20,43); and (v) oospores are produced readily in host tissue or artificial culture with no special physical or nutritional requirements (8,20,48,53). Isolates described in the early literature must match the above features if we are to conclude that they were homothallic.

The analysis showed that none of the isolates described prior to the 1950s matched the characteristics of true homothallics (Table 3). Most of the early reports described oogonia of *P. infestans*, and at least three—those of Clinton (8,9), Hori and Yoshida (34), and

TABLE 1. Multilocus genotypes of *Phytophthora infestans* detected when the first A2 mating type isolates were found at six locations around the world

Location, genotype	Mating type	Allozyme genotype		RG57 fingerprint[c]
		Gpi[a]	*Pep*[b]	
Northwestern Mexico (*n* = 60)				
US-6	A1	*44*	*35*	101111100100110001011001100
US-8	A2	*456*	*55*	100110000100110100011011100
LM-1	A1	*44*	*35*	101110100100110010111001100
LM-2	A1	*26*	*55*	100110000100110100011011100
South America and Costa Rica (*n* = 32)				
US-1	A1	*24*	*35*	101110101100110100011001100
US-1.1	A1	*24*	*55*	101110101100110100011001100
US-1.2	A1	*24*	*35*	101110101000110100011001100
US-1.3	A1	*24*	*35*	101110100100110100011001100
US-1.6	A1	*24*	*35*	101110101100110100011101100
US-1.7	A1	*44*	*35*	101110101100110100011001100
CR-1	A1	*44*	*56*[d]	100100000100110100011001100
BR-1	A2	*44*	*55*	101110100000110000111101100
Eastern Europe (*n* = 72)				
US-1	A1	*24*	*35*	101110101100110100011001100
US-1.1	A1	*24*	*55*	101110101100110100011001100
US-1.2	A1	*24*	*35*	101110101000110100011001100
US-1.8[e]	A1	*24*	*35*	101110001100110100011001100
PO-4	A1	*34*	*55*	100110001100110100011001100
PO-5	A1	*44*	*55*	001110100100110110011000101
PO-6	A1	*34*	*25*	111111110100110100011101100
PO-8	A1	*34*	*25*	101110101000110000011001100
PO-9	A1	*44*	*55*	111111100100110110111101100
PO-10	A1	*44*	*55*	110110001100110100011100101
PO-11	A1	*34*	*45*	100110001100110100011001100
PO-12	A1	*34*	*45*	101110101000110000011001100
PO-13	A1	*34*	*55*	101111110100110100011001100
PO-14	A2	*34*	*55*	100110000100110100011001100
PO-15	A2	*44*	*55*	111111100100110100111100101
PO-16	A1	*34*	*25*	101110100100110000011001100
PO-17	A2	*44*	*55*	101110101100110100011001100
PO-18	A2	*34*	*55*	100110001100110100011001100
PO-19	A1	*44*	*55*	110110011100110010011101100
PO-20	A2	*44*	*55*	101110100100110100111100101
PO-21	SF[f]	*34*	*55*	100110000100110100011001100
PO-22	A2	*44*	*55*	101110101000110010111001100
PO-23	A1	*34*	*55*	110110000100110010010101101
PO-24	A2	*34*	*55*	100110001000110100011001100
PO-25	SF	*44*	*55*	100110001100110100011001100
PO-26	A1	*33*	*55*	100110000100110000111101100
EE-1	A1	*34*	*55*	100110000100110010011001100
EE-2	SF	*34*	*55*	101010101100110100011001100
RU-1	A1	*44*	*55*	101110101100110100011001100
RU-2	A2	*44*	*55*	100110000100110100011001100
East Asia (*n* = 124)				
US-1	A1	*24*	*35*	101110101100110100011001100
US-1 (mt)[g]	A1	*24*	*35*	101110101100110100011001100
US-1.1	A1	*24*[h]	*55*[h]	101110101100110100011001100
JP-1	A2	*44*	*44*	100111000000110110001001101
Western Europe (*n* = 23)				
US-1	A1	*24*	*35*	101110101100110100011001100
US-1.1	A1	*24*	*55*	101110101100110100011001100
US-1.2	A1	*24*	*35*	101110101000110100011001100
NL-1	A1	*44*	*55*	010110001100110100010101100
NL-2	A2	*34*	*25*	111110101100110100110101100
NL-3	A1	*34*	*25*	111111101100110000111101100
NL-4	A1	*34*	*25*	101110100100110110111001100
NL-5	A1	*34*	*25*	111111101000110000111101100
NL-6	A1	*44*	*55*	010010000100110100001001100
NL-7	A1	*44*	*55*	010110000100110100011001100
NL-8	A2	*34*	*55*	110111010100110000010101100
NL-9	A2	*34*	*25*	110111010100110000010101100
NL-10	A1	*44*	*55*	110110000100110110111101100
United States and Canada (*n* = 250)				
US-1	A1	*24*	*35*	101110101100110100011001100
US-1.1	A1	*24*	*55*	101110101100110100011001100
US-1.2	A1	*24*	*35*	101110101000110100011001100
US-1.3	A1	*24*	*35*	101110100100110100011001100
US-1.4	A1	*24*	*55*	101110101000110100011001100
US-1.5	A1	*24*	*35*	101110101100110101011001100
US-2	A1	*24*	*35*	101110100100110101111001100
US-3	A1	*24*	*35*	101110000000110100011001100
US-4	A1	*44*	*33*	101110100100110110011001100
US-5	A1	*44*	*35*	101110100100110101111001100
US-6	A1	*44*	*35*	101111100100110001011001100
US-6.1	A1	*44*	*33*	101111100100110001011001100
US-6.2	A1	*44*	*35*	101110100100110001011001100
US-6.3	A1	*44*	*35*	101111100101110001011001100
US-6.4	A1	*44*	*55*	101101100100110001011001100
US-6.5	A1	*44*	*35*	101111100100110001001001100
US-7	A2	*45*	*55*	100110000100110101011001100
US-8	A2	*456*[i]	*55*	100110000100110100011011100
CA-1[j]	A1	*24*	*35*	111110101100110100111001110
CA-2	A1	*44*	*55*	101100100000110000111001100
CA-3	A2	*24*	*55*	111110100100100110011001100
BC-1[k]	A2	*45*	*55*	100000000100110100011001100
BC-2	A2	*44*	*55*	100011000000110100011001100
BC-3	A2	*44*	*55*	101000100100110001011001100
BC-4	A2	*44*	*55*	100000000000110001011001100

[a] Glucose-6-phosphate isomerase. Alleles at the *Gpi* locus are coded as *86* = 2, *90* = 3, *100* = 4, *111* = 5, and *122* = 6.
[b] Peptidase. Alleles at the *Pep* locus are coded as *83* = 2, *92* = 3, *96* = 4, and *100* = 5.
[c] DNA fingerprint bands revealed by the moderately repetitive probe RG57 (26). Presence of a band is indicated by 1 and absence by 0. Bands 1 to 25 are listed from left to right. The last two numbers indicate presence or absence of bands 11a and 24a, respectively. Band 4 is inconsistent. It was listed here to facilitate comparisons with previous publications, but was excluded from all analyses.
[d] Isolates from Costa Rica were heterozygous for *Pep 100* and a potentially new allele, *Pep 94*.
[e] This genotype was named PO-7 by Sujkowski et al. (63), but was recognized as part of the US-1 clonal lineage and renamed here.
[f] Self-fertile or homothallic.
[g] This variant was identical to US-1, except for mitochondrial DNA haplotype (40). It was listed as a separate genotype here, even though the mitochondrial DNA data were not included in the analysis.
[h] The *Gpi* and *Pep* genotypes for US-1.1 were listed incorrectly by Koh et al. (40). Those listed here are correct.
[i] This genotype had three alleles at the *Gpi* locus.
[j] CA genotypes were identified first in Canada.
[k] These genotypes were from British Columbia, Canada. The BC designation was retained from the paper in which they were described originally (29).

Pethybridge and Murphy (48)—found oospores, at least occasionally. The pictures published by these investigators clearly were *P. infestans* oospores. However, in all cases, antheridia were rare or absent; oospores were few, usually aborted, and required at least 20 days (usually many more) to be produced; oospore production was sporadic and unpredictable; and usually many subcultures were required before oospores appeared (Table 3). Furthermore, the cultures always had a "fluffy" morphology with a large quantity of

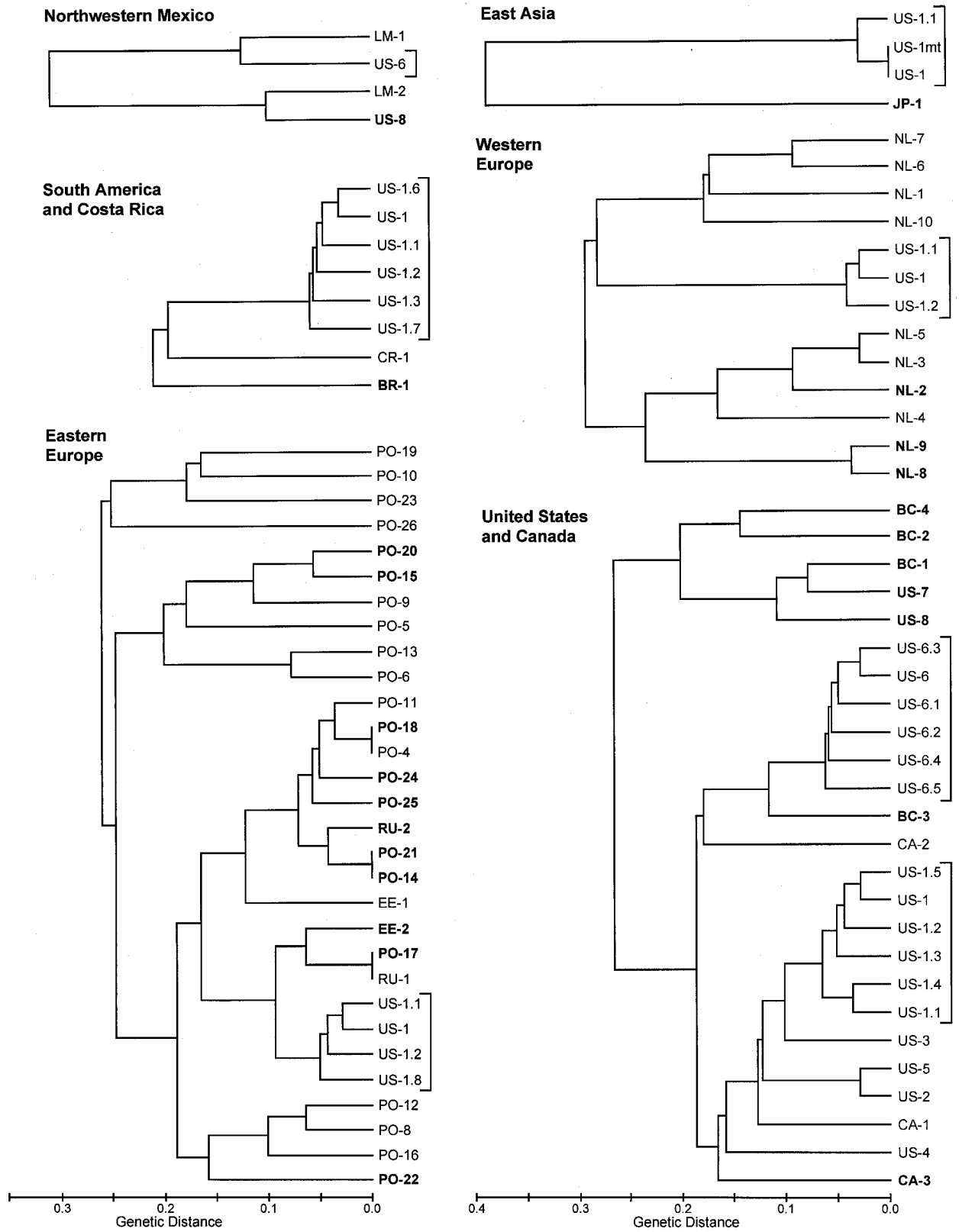

Fig. 1. Cluster analyses of genotypes of *Phytophthora infestans* detected when the A2 mating type first appeared in six locations worldwide. A2 mating type and self-fertile genotypes are indicated in bold. Clusters in which A2 mating type genotypes should appear if they arose by mating type change are indicated by brackets. All phenograms are drawn to the same scale. Genetic (phenotypic) distance (measured by Dice coefficients) is indicated at the bottom. Abbreviations are as follows: BC = British Columbia, Canada; BR = Brazil; CA = Canada; CR = Costa Rica; EE = Estonia; JP = Japan; LM = Los Mochis, Mexico; NL = the Netherlands; PO = Poland; RU = Russia; and US = United States.

aerial mycelia. Oospores were produced only under very specialized conditions, usually on oatmeal agar. In fact, most investigators found no oospores at all until they began using oatmeal agar (2,8,34,48). Thus, although early investigators found oospores, none of their isolates appeared to be homothallic.

MATING TYPE SEGREGATIONS IN SELF-FERTILIZED PROGENIES

To determine whether other investigators found evidence of mating type change due to selfing, previous studies that mentioned self-fertilized progenies of *P. infestans* were revisited. Only those in which self-fertilization was confirmed with molecular markers were included; mating types of parents and their self-fertilized progeny were tabulated. If progeny produced through selfing of one parent produced isolates of the opposite mating type, this would be strong evidence in support of the mating type change hypothesis. If mating types consistently did not change after selfing, this would reject the mating type change hypothesis.

Other investigators occasionally have found apparent segregation for mating type in isolates that arose by self-fertilization. In all cases, self-fertilization was confirmed by analyses using neutral genetic markers. Although apomixis could not be eliminated for some isolates (27,59), hybridization with other isolates, in most cases, was excluded. Mating type segregated in self-fertilized progeny of two A2 isolates: 550 (54) and 618 (27) (Table 4). Mating type did not segregate in all other selfings of A2 isolates and never in any selfings of A1 isolates (Table 4).

DISCUSSION

Based on all available evidence, the mating type change hypothesis for the origin of the A2 of *P. infestans* outside Mexico was rejected. In fact, we have not identified any evidence in support of this hypothesis. If the A2 arose by mating type change, then the first A2 isolates in each location should have been identical, or nearly identical, to A1 isolates in the population where they originated. Minor changes could arise by segregation at heterozygous loci if self-fertilization stimulated the change, but there should be no new alleles in A2 isolates compared with A1s of the old population. This clearly was not the case. A2 isolates were significantly different from previously existing A1 isolates in all locations studied and, therefore, could not have originated by mating type change.

Instead, the migration hypothesis was strongly supported by all available data. The only potentially ambiguous result was in Eastern Europe. Although none of the A2 isolates in Eastern Europe could have arisen by mating type change from the A1 genotypes that occurred there previously, there were two pairs of identical A1 and A2 isolates in new populations. In one pair, the A2 genotype PO-18 was identical to the A1 PO-4. PO-4 was the most commonly detected genotype throughout Eastern Europe (Poland, Estonia, and Belarus) and had the most common alleles at each locus (63). There was strong evidence for sexual recombination in Poland (63), and isolates related by sexual recombination could have the same multilocus genotype as PO-4 for the markers studied by chance. PO-18 also had the A form (22) of mitochondrial DNA, whereas most PO-4 isolates had the B form (L. S. Sujkowski and

TABLE 2. The number of genetic differences from the US-1 multilocus genotype for isolates from old, new, and Mexican populations of *Phytophthora infestans* (based on data at the mating type, and two allozyme and 26 DNA fingerprint loci)

	Old populations			New populations		
Location	Range	Mean	Sample size	Range	Mean	Sample size
Africa (Rwanda)	0	0.00	7	2–6	5.00	4
East Asia	0–1	0.06	53	12	12.00	71
Eastern Europe	0–2	0.23	35	2–12	5.63	158
Western Europe	0–1	0.22	9	5–12	8.33	12
Israel	…	…	…	6	6.00	8
South America	0–1	0.17	29	7–8	7.67	3
United States and Canada	0–7	2.92	173	6–10	8.15	92
Overall range or mean	0–7	0.59	51	2–12	7.54	50
Mexico						
Chapingo	…	…	…	6–10	7.50	8
Los Mochis	…	…	…	5–8	5.93	59
Saltillo	…	…	…	6–10	8.52	27
Toluca (1983 to 1987)	…	…	…	3–9	5.38	47
Overall range or mean in Mexico	…	…	…	3–10	6.83	35

TABLE 3. Analyses of previous reports of oospores of *Phytophthora infestans* outside Mexico prior to the 1950s: Were those isolates really homothallic?

Reference	Antheridia	Oospores	No. of days for oospore production	Frequency of oospore formation	Colony morphology	Special conditions	Homothallic?
Typical homothallic (8,20,48,58)	Always	Huge number, complete	2 days	Always produced	Adpressed, waxy	None	Yes
Berg, 1926 (2)	Few	Not clear	…[a]	Occasional	Fluffy	Oat agar only	No
Clinton, 1911 (8)	Very few	Few, mostly aborted	At least 30, usually many more	Sporadic, unpredictable	Fluffy	Oat agar only	No
de Bruyn, 1926 (12)	Few	Very few, not *P. infestans* type	…	Occasional	Fluffy	Special media only, cereal straw the best	No, probably not *P. infestans* oospores
Hori and Yoshida, 1959 (34)	None	Few, incomplete	At least 20	Sporadic	…	Transfer from corn extract to oatmeal extract agar only	No
Jones et al., 1909 (35), 1912 (37)	No	Very few, thick and spiny	…	Very rare	…	Oogonia-like bodies on potato gelatin or lima bean agar only	No, not *P. infestans* oospores
Pethybridge and Murphy, 1913 (48)	Very few	Few	At least 30, usually more	Sporadic	Fluffy	Oat agar only, mainly in contact with tube	No
Tucker, 1931 (65)	Few	Few	4 months	Very rare, sporadic	…	Lima bean or oat agars only	No

[a] Information unclear or not provided.

S. B. Goodwin, *unpublished data*). Thus, PO-18 could not have originated by mating type change from PO-4. The only possible exceptions were the few PO-4 isolates with the A form of mitochondrial DNA, which theoretically could give rise to PO-18 by mating type change. However, even if this did occur, it was not the original source of the A2, because PO-18 is very different from US-1 (Fig. 1). The other pair of isolates, PO-17 (A2) and RU-2 (A1), clustered near isolates in the old clonal lineage. However, for PO-17 to have originated by mating type change from US-1 would require changes at two additional unlinked allozyme loci besides mating type, which is extremely unlikely. The only explanation that fits the data is that the A2 mating type was introduced into Eastern Europe through massive immigration of new genotypes (including A1 genotypes) (63), and that occasional pairs of similar A1 and A2 genotypes arose by sexual recombination within the new populations.

Other potential mechanisms for the origin of the A2 mating type of *P. infestans* outside Mexico also were contradicted by the population genetic data. The other possible hypotheses are that the A2 mating type (i) was always present, but undetected; or (ii) arose by mutation or mitotic recombination. Lack of detection can be eliminated as an explanation, because old populations worldwide appear to have been founded by a single clonal lineage (15,23,25). The new populations were very different and contained a large number of genotypes (Table 2). There is virtually no possibility that all of these variants could have remained undetected, even in the relatively limited samples available. The population genetic data also rule out the possibility that the A2 arose by mutation or mitotic recombination, because, in either case, the A2s should have been identical, or nearly identical, to the pre-existing A1s as discussed above for mating type change. Therefore, migration is the only hypothesis that cannot be rejected based on all the data available.

Critical analysis of the literature also does not support Ko's (39) conclusion that homothallic isolates of *P. infestans* were recovered from various countries during the 1910s and 1920s. Although it is not possible to perform a completely objective analysis, it is possible to determine whether isolates described in the early literature fit the characteristics of known homothallic isolates. Isolates described in the early literature differed from homothallic isolates in all of the characteristics analyzed, and it is thus unlikely that any of them were homothallic. Oospores of *P. infestans* found during the early part of this century were produced only under special conditions (Table 3), usually when the isolates were grown on oatmeal agar. Even then, oospore production was sporadic (2,8,12,34, 37,48,65), and the numbers of oospores produced were extremely low compared with true homothallic isolates. Isolates in the early literature were self-sterile when cultured originally (8,48,65) and when grown on all other media besides oatmeal or lima bean agar (8,34,37,48,65). Some investigators may have observed structures produced by contaminants in their cultures, rather than by *P. infestans*. For example, de Bruyn (12) reported that the best oospore production occurred when cultures were grown on cereal straw. As far as we are aware, this has never been duplicated by other investigators, and it seems more likely that a contaminant in her cultures grew on cereal straw, not *P. infestans*. Furthermore, the oospores pictured by de Bruyn (12) had extra thickenings around their exteriors, unlike those of *P. infestans*, and the antheridia may have been paragynous rather than amphigynous. Therefore, the oospores seen by de Bruyn (12) probably were not produced by *P. infestans*. Similar criticisms can be made about the work of Jones and coworkers (35,37). The oospores shown in Figures 29 to 38 of Jones et al. (37) were very spiny and were likely produced by a contaminant (possibly a species of *Pythium*, or even a zygospore produced by a Zygomycete), not *P. infestans*. Additional early reports of oospore production (6,7,11,36,45) also were evaluated and found to suffer from some or all of the flaws listed above.

The most likely explanation for the early reports of oospores is that there was something in oatmeal agar that stimulated their production. It is well-known that isolates of heterothallic *Phytophthora* species occasionally produce a few oospores in single culture (3,21,53,64,65). In addition, oospore production can be stimulated by contact with other *Phytophthora* species (60,61), or even true fungi (3,4). Stimulation of oospore production by oatmeal agar was recognized as a possibility by Clinton as early as 1910 (8). Clinton (8) and Pethybridge and Murphy (48) interpreted their results very cautiously, probably because they worked with homothallic species of *Phytophthora*—Clinton with *P. phaseoli* and Pethybridge with *P. erythroseptica*—and realized that the quantity of oospores produced by *P. infestans* in response to stimulation by oatmeal agar was too small to represent the sexual stage as seen in other species. Clinton (8) also reported small numbers of oospores in dried potato leaves. These oospores could have been produced by stimulation from other organisms on the leaf. Although early investigators did find oospores of *P. infestans*, few if any antheridia were produced and most of the oospores were aborted. This is why the scientific consensus was that the sexual stage of *P. infestans* remained to be discovered. Modern analysis of the old reports leads to the same conclusion. A few oospores in dried leaves is simply insufficient evidence to claim the presence of both mating types; more than 60 oospores per square millimeter of potato leaf may be formed in a true mating reaction (14). Hence, unless very large numbers of oospores are found, they do not indicate the presence of both mating types as claimed recently (50).

There also was no evidence for the presence of both mating types of *P. infestans* in Japan during the 1930s, as suggested recently (39). Of the two references cited by Ko (39), the first was an analysis of nine isolates collected from 1936 to 1938 (33). A few oospore-like bodies were found in old cultures of one isolate on oatmeal agar (33), but no true oospores were found. The second report was published in 1959 (34), although it is not clear when the cultures were isolated. A few complete oospores (with antheridia) were found in some pairings of isolates from potato with those from tomato after a very long time in culture (40 to 50 days), and only after transfer from one medium to another (34). Ko (39) interpreted this as proof of the presence of both mating types in contrast to contemporary Japanese investigators who cited the same study only as finding oospore-like bodies (43). Our analysis rejects Ko's (39) conclusion and supports Mosa et al.'s (44) statement that the A2 mating type of *P. infestans* was not known in Japan until 1987.

The number of studies documenting the occurrence of mating types of *P. infestans* between 1958 and 1984 is limited (39). How-

TABLE 4. Segregations of mating type of *Phytophthora infestans* in self-fertilized progeny from previous studies

Reference	Isolate	Mating type	Mating type of selfed progeny[a]		
			A1	A2	SF[b]
Shattock et al. (56)	127	A1	1	0	0
	40/34	A1	9	0	0
Shattock et al. (57)	503	A2	1	0	0
	533	A1	4[c]	0	0
Shattock (54)	550	A2	5	3	2
	1100	A1	2	0	0
Harrison (31,59)	E14	A2	0	28[d]	0
	550	A2	0	8[d]	0
	10/5	A1	6[d]	0	0
	10/6	A1	1	0	0
	16/4	A2	0	15	0
	28/13	A2	0	1[d]	0
Goodwin and Fry (27)	618	A2	2	4[c,d]	0
	P3005[e]	A1	1[d]	0	0

[a] Self-fertilization was verified by analysis of molecular markers (allozymes).
[b] Self-fertile or homothallic.
[c] Includes progeny from two different experiments.
[d] These isolates were either selfs or nonrecombinant parental types (apomicts).
[e] This isolate was *P. mirabilis*, a close relative of *P. infestans*.

ever, we can be fairly certain that as soon as the A2 was reported from Mexico in 1956 (21,47,61), Phytophthora workers worldwide paired their isolates to look for oospore production. We also can be certain that they would have published their results if they found oospores. Therefore, lack of publications probably reflects lack of publishable results rather than lack of effort. For example, Graham and Wright (30) analyzed 103 Canadian isolates in their culture collection from 1958 and earlier, and all were A1. The only reason their study was published was that they occasionally found a few oospores (literally only two per plate compared with from 47 to 579 per square millimeter for a true mating reaction [49]). We will never know how many studies were conducted, but never published, because of negative results.

Segregation of mating type in self-fertilized progenies of two A2 isolates, but not in those of A1 isolates (Table 4), supports the previous hypothesis that the A2 mating type is heterozygous and the A1 homozygous (42,52). However, this runs counter to recent genetic analyses of mating type, which showed that the A1 was heterozygous, not the A2 (38). One possible explanation is that the isolates with "changed" mating type from "selfings" of A2 isolates (27,56) actually were hybrids that appeared to be selfs due to meiotic nondisjunction for the chromosome containing the *Gpi* locus. Previous evidence for a probable high frequency of meiotic nondisjunction in *Phytophthora* (17,27) supports this explanation. Under both genetic models, mating type might "change" in one direction by segregation, but it seems highly unlikely that mating type change would be reversible (38).

A possible explanation for Ko's (39) results is that the isolates with changed mating type actually arose by hybridization rather than self-fertilization. Small perforations in the polycarbonate membranes used in those studies might allow occasional hyphae to penetrate and effect cross-fertilization. Such hybrid progeny would give the appearance of mating type change. This hypothesis could be tested easily with neutral genetic markers. The parent isolates 533 and 550 used by Ko (39) are homozygous for the allozyme alleles *Gpi 122* and *Gpi 86*, respectively (57). Hybrids have the heterozygous genotype *86/122*, and thus can be easily distinguished from selfs (57). S. B. Goodwin requested Ko's isolates on 28 November 1994, within 2 months after his paper (39) appeared in print, to test this hypothesis with molecular markers. Unfortunately, the isolates no longer existed (W. H. Ko, *personal communication*), so it was not possible to directly confirm or refute the origin of isolates with changed mating type. Until it can be confirmed with genetic markers, there is no evidence to support this rather speculative hypothesis. However, it is certain that if mating type change does occur, it is rare, and certainly has not been responsible for the recent occurrence of the A2 mating type of *P. infestans* outside Mexico.

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