Increased Genotypic Diversity via Migration and Possible Occurrence of Sexual Reproduction of *Phytophthora infestans* in Poland

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Supported by USDA OICD and by Cornell University Hatch Project 153430.
Accepted for publication 2 November 1993.

ABSTRACT

Sujkowski, L. S., Goodwin, S. B., Dyer, A. T., and Fry, W. E. 1994. Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. Phytopathology 84:201-207.

Genetic changes in populations of *Phytophthora infestans* in Poland were determined by analyzing 247 isolates collected from 1985 through 1991. All isolates collected from 1985 through 1987 were A1 mating type and consisted of a single clonal lineage based on allozyme and DNA fingerprint analyses. The appearance of new genotypes in Poland, presumably due to migration, was first detected in 1988 with the identification of the A2 mating type, three allozyme, and a number of DNA fingerprint alleles that had not been detected previously. This migration resulted in dramatic changes. Gene and genotype diversity and the frequency of uniquely detected genotypes all increased beginning in 1988, and reached a peak in 1990. A new clonal genotype, first detected in 1988, increased in frequency and made up almost 50% of all isolates collected in 1991. In contrast, the old clonal lineage that dominated populations from 1985 to 1987 was not detected after 1988. The frequency of A2 isolates reached 44% by 1989, then decreased to 12% in 1991. There was almost no genetic differentiation among regions within Poland. In addition to the major impact caused by migration, four lines of evidence indicated that sexual reproduction also might have occurred in Poland: genotypes at most loci were in Hardy-Weinberg equilibrium; there was no genetic differentiation between A1 and A2 isolates; there was a high proportion of genetically unique individuals that would not be expected in populations restricted to asexual reproduction; and a new genotype (*90/90*) at the allozyme locus glucose-6-phosphate isomerase was detected that could have been produced from sexual recombination.

Additional keywords: DNA fingerprinting, genetic diversity, population genetics, potato late blight.

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is one of the most devastating potato diseases worldwide (14), and in Poland, epidemics often cause severe losses (1). Because fungicides have had limited use in Poland, there has been great emphasis on developing resistant cultivars. Unfortunately, a high degree of variability in virulence and aggressiveness in Polish populations of *P. infestans* (25) makes resistance breeding extremely difficult. A better understanding of the genetic structure of *P. infestans* populations may provide insight into the mechanisms by which this genetic diversity is generated and maintained.

There have been major changes in *P. infestans* populations in Europe during the past two decades. These changes were probably caused by migration from Mexico, which brought additional genetic variation including the A2 mating type (13,15,19,22,24); the *90* and *83* alleles at the allozyme loci glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*), respectively (5); and a number of DNA fingerprint bands that had not been detected previously in Europe (4,9). There was a concomitant change to increased complexity of virulence phenotypes in Europe beginning in the late 1970s (21). The number of virulence genes detected in Poland also increased over this period; for instance, corresponding virulences to the potato resistance genes R7, R10, and R11 were rare prior to the mid-1970s (20) but were common by the late 1980s (25).

The recent development of molecular markers (6) has greatly enhanced the resolution of genetic variation in *P. infestans* populations. In particular, the DNA fingerprinting probe RG57 provides data on over 25 probable genetic loci (8,10). Each band appears to identify a specific locus, and alleles are either "present" or "absent" (8). Analyses with allozyme and DNA fingerprint markers revealed that in geographic regions where sexual reproduction is probably a normal part of the life cycle, such as central Mexico, almost every individual had a unique genotype (12). In contrast, there was very little genotypic diversity in northwestern Mexico (only four genotypes among 88 isolates sampled) (12), indicating that reproduction in this location was predominately asexual even though both mating types were present. Until very recently, worldwide populations of *P. infestans* outside of Mexico were limited to asexual reproduction due to the presence of only one (A1) mating type; most of these populations were composed of a single clonal genotype (9). Intermediate levels of genetic diversity in recent European populations, along with the A2 mating type, indicate the potential for sexual reproduction. In the Nether-

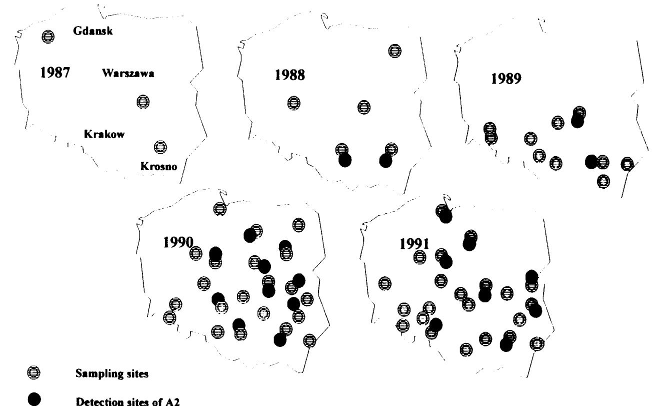

Fig. 1. Sampling site locations of A1 and A2 isolates of *Phytophthora infestans* in Poland from 1985 to 1987 through 1991. Locations of four major cities in Poland are indicated in the 1985–1987 map.

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lands, *P. infestans* collections from some fields were monomorphic, whereas others had levels of genotypic diversity approaching some Mexican populations (4,5). Thus, the genetic structure of *P. infestans* populations as revealed by allozyme and DNA fingerprint markers in conjunction with mating type may provide insight into the degree to which sexual reproduction is a factor in the life history of *P. infestans* in Poland.

The recent migrations into Europe have already affected the genetic structure of *P. infestans* populations in Poland (24); however, previous characterizations of these populations have been hindered by a limited number of markers and/or small sample sizes. There is still a great need for additional sampling to determine the full effects of migration. If the rate of migration was uneven, there might be genetic differences among regions in Poland due to the presence of new migrants in some areas but not in others. The presence of the A2 mating type since 1988 (24) provides the potential for sexual reproduction in Poland. Sexual reproduction could greatly increase the number of genotypes present in this obligately outbreeding oomycete. In contrast, genotypic diversity in a completely asexual population might decrease in subsequent years as rare genotypes are lost through genetic drift. The purposes of this study were to quantify the genetic changes that occurred in populations of *P. infestans* in Poland from 1985 through 1991, to test for differences in genetic diversity among regions (geographic substructuring) in Poland, and to determine whether the observed patterns of genetic diversity are consistent with the hypothesis that sexual reproduction may be occurring in these populations.

MATERIALS AND METHODS

Collection and maintenance of isolates. Isolates were obtained from commercial fields and experimental plots throughout Poland from 1985 through 1991. Summaries of the mating type and allo-

TABLE 1. Genotypes of 175 isolates of *Phytophtora infestans* in Poland by year

Genotype	Mating type	*Gpi*[a] genotype	*Pep*[b] genotype	Fingerprint pattern[c]	District	Region[d]	Number of occurrences
1985–1987							
PO-1	A1	24	35	10111010110011010001100110	...[e]	...[e]	12
PO-2	A1	24	55	10111010110011010001100110	...	...	3
PO-3	A1	24	35	10111010100011010001100110	Rzeszow	SE	1
1988							
PO-1	A1	24	35	10111010110011010001100110	...	...	13
PO-2	A1	24	55	10111010110011010001100110	Warszawa	EC	1
PO-4	A1	34	55	10011000110011010001100110	Suwalki	NE	3
PO-5	A1	44	55	00111010010011011001100011	...	SE	2
PO-6	A1	34	25	11111111010011010001110110	...	...	2
PO-7	A1	24	35	10111000110011010001100110	Rzeszow	SE	1
PO-8	A1	34	25	10111010100011000001100110	Krosno	SE	1
PO-9	A1	44	55	11111110010011011011110110	Krosno	SE	1
PO-10	A1	44	55	11011000110011010001110011	Krosno	SE	1
PO-11	A1	34	45	10011000110011010001100110	Suwalki	NE	1
PO-12	A1	34	45	10111010100011000001100110	Suwalki	NE	1
PO-13	A1	34	55	10111111010011010001100110	Suwalki	NE	1
1989							
PO-4	A1	34	55	10011000110011010001100110	...	...	9
PO-6	A1	34	25	11111111010011010001110110	Krosno	SE	1
PO-14	A2	34	55	10011000010011010001100110	Warszawa	EC	3
PO-15	A2	44	55	11111110010011010011110011	Rzeszow	SE	2
PO-16	A1	34	25	10111010010011000001100110	Krosno	SE	1
PO-17	A2	44	55	10111010110011010001100110	Rzeszow	SE	1
PO-18	A2	34	55	10011000110011010001100110	Warszawa	EC	1
PO-19	A1	44	55	11011001110011001001110110	Krosno	SE	1
PO-20	A2	44	55	10111010010011010011110011	Rzeszow	SE	1
PO-21	H3[f]	34	55	10011000010011010001100110	Warszawa	EC	1
PO-22	A2	44	55	10111010100011001011100110	Rzeszow	SE	1
PO-23	A1	34	55	11011000010011001001010111	Skierniewice	EC	1
PO-24	A2	34	55	10011000100011010001100110	Warszawa	EC	1
PO-25	H3	44	55	10011000110011010001100110	Walbrzych	SW	1
PO-26	A1	33	55	10011000010011000011110110	Jelenia Gora	SW	1
1990							
PO-4	A1	34	55	10011000110011010001100110	...	...	9
PO-11	A1	34	45	10011000110011010001100110	Rzeszow	SE	1
PO-27	A1	34	55	10011000110011000001100110	Krakow	SE	1
PO-28	A1	44	55	10011000110011010001100110	...	...	3
PO-29	A1	33	55	10011000110011010001100110	...	...	2
PO-30	A2	44	45	11111011010011000001100110	...	...	2
PO-31	A1	34	55	10011101110011011011110110	—[g]	—[g]	1
PO-32	A2	44	55	10011100010011001001110110	Walbrzych	SW	1

(*continued on next page*)

[a] Alleles at the *Gpi* (Glucose-6-phosphate isomerase) locus are coded as *83* = 1, *86* = 2, *90* = 3, *100* = 4.

[b] Alleles at the *Pep* (Peptidase) locus are coded as: *78* = 1, *83* = 2, *92* = 3, *96* = 4, *100* = 5.

[c] The fingerprint pattern was determined via Southern analysis with probe RG57 as described in the text. The code is: 1 = presence of band (heterozygous or homozygous); 0 = absence of band; data for the fingerprint bands are ordered from left to right for bands 1–25. The rightmost DNA fingerprint column has data for band 24a (Fig. 2). The "present" alleles were only detected at 22 of the 26 loci. Two loci were fixed for the "present" allele, so only 20 loci were polymorphic.

[d] Regions are: Northeast (NE); East Central (EC); Southeast (SE); Southwest (SW); West Central (WC); Northwest (NW).

[e] ... indicates more than one district or region.

[f] Homothallic.

[g] A dash indicates lack of information.

zyme data from the isolates collected from 1985 through 1989 have been reported previously (24). Samples were taken from susceptible and moderately resistant potato cultivars in commercial production fields and from research plots containing advanced breeding material highly resistant to *P. infestans*. In addition to late blight resistance, the breeding program emphasized starch content, virus resistance, and high yield. The goal was to obtain five to 15 isolates from each location. In some fields, low disease pressure precluded the acquisition of larger samples, and some samples did not survive, so the final sample sizes varied from one to 10 isolates per location per year. Three fields were intentionally sampled in sequential years to determine if the same genotypes persisted from year to year. The minimum distance between sampling sites was about 25 km, and the maximum distance was about 800 km (Krosno in the southeast and Gdansk in the northwest) (Fig. 1).

P. infestans was isolated from single lesions occurring on leaves or stems as described in Goodwin et al (12). Previous tests indicated that discrete single lesions from field-infected leaves contain only one genotype of the fungus (S. B. Goodwin, *unpublished data*). Subsequent culturing was carried out on rye A agar (2). After isolation into pure culture, each isolate was sent to Cornell University, Ithaca, NY, and stored cryogenically at −130C and/or under mineral oil on rye A agar slants at 18 C.

Characterization of isolates. Mating types were determined by pairing each isolate with known A1 and A2 testers on clarified rye A or B agar according to Fry et al (5). Mycelium for allozyme analysis and for DNA extraction was obtained from mycelial mats (usually 10–14 days old) grown on liquid rye medium or in pea broth (10) in 9-cm petri plates. After removal of excess liquid by vacuum filtration, the mycelia were immediately frozen (−80 C) and then lyophylized, ground to a powder, and stored at −80 C until further use. Proteins for allozyme analysis for most samples were extracted from the ground mycelium as

TABLE 1. (*continued from preceding page*)

Genotype	Mating type	*Gpi*[a] genotype	*Pep*[b] genotype	Fingerprint pattern[c]	District	Region[d]	Number of occurrences
PO-33	H3	34	55	11011001110011011001110110	Torun	NW	1
PO-34	A2	44	55	10010000000011010001100110	Czestochowa	SW	1
PO-35	A1	34	55	10011000110011010011110010	Ostroleka	NE	1
PO-36	H3	33	55	10011000010011010001100110	Suwalki	NE	1
PO-37	A1	44	44	10011001010011000001100110	Siedlce	EC	1
PO-38	A1	44	55	11011000010011000001010110	Pila	NW	1
PO-39	A2	44	44	10010000000011010001100110	Czestochowa	SW	1
PO-40	A1	34	45	00110010010001010001100111	Jelenia Gora	SW	1
PO-41	A1	44	55	00111010010001010001000111	Jelenia Gora	SW	1
PO-42	A1	44	45	11010000110010011001110110	Gdansk	NW	1
PO-43	A2	34	55	10011100010011010011110110	Ostroleka	NE	1
PO-44	A2	34	55	10011000010011001011100010	Ciechanow	EC	1
PO-45	A1	44	55	11111011010011010001100110	Chelm	SE	1
PO-46	A1	44	45	10011000000011011011110110	Radom	EC	1
PO-47	A2	44	44	11111011010011000001110110	Siedlce	EC	1
PO-48	A1	44	55	10010000010011010011110110	Zamosc	SE	1
PO-49	A2	44	55	11011000010011001011010110	Torun	NW	1
PO-50	A1	34	35	10111111110011010011110110	Torun	NW	1
PO-51	A1	44	35	10011000010011001011110110	Konin	WC	1
PO-52	A1	44	35	10111111110011010001100110	Warszawa	EC	1
PO-53	A1	44	45	10011000110011010001100110	Warszawa	EC	1
PO-54	A2	44	25	10011000110011010001100110	Rzeszow	SE	1
PO-55	A2	44	45	10010000010011011001100110	Rzeszow	SE	1
PO-56	A1	44	55	11111110010011010011110111	Rzeszow	SE	1
PO-57	A2	44	55	10011000010011010001100110	Rzeszow	SE	1
PO-58	A1	34	44	10011000110011010001100110	Rzeszow	SE	1
1991							
PO-4	A1	34	55	10011000110011010001100110	...	...	29
PO-11	A1	34	45	10011000110011010001100110	...	...	3
PO-28	A1	44	55	10011000110011010001100110	Bielsko Biala	SE	1
PO-29	A1	33	55	10011000110011010001100110	...	SE	2
PO-59	A1	34	55	11011000110011010001100110	...	...	2
PO-60	A1	34	45	10010100010011011011100110	Zielona Gora	WC	1
PO-61	A2	34	45	10111010110011010001100110	Gdansk	NW	1
PO-62	A1	34	35	10011000110011010001100110	Bydgoszcz	NW	1
PO-63	A1	34	55	10011111000011010011110010	Gdansk	NW	1
PO-64	A1	34	55	10111010010011000001110111	Rzeszow	SE	1
PO-65	A1	34	55	10111010110011010001100110	Rzeszow	SE	1
PO-66	A1	34	55	11111010010011000111110110	Rzeszow	SE	1
PO-67	A1	34	25	10011000110011010001100110	Katowice	SW	1
PO-68	A1	34	55	10010100010011011001100110	Torun	NW	1
PO-69	A1	44	55	11011000110011010001100110	Walbrzych	SW	1
PO-70	A1	44	45	10011000010011000001010110	Walbrzych	SW	1
PO-71	A1	34	55	11010000010011001001110110	Konin	WC	1
PO-72	A1	44	55	10011000010011010001100110	Piotrkow Tryb.	EC	1
PO-73	A2	44	45	10111110000011001010110110	Warszawa	EC	1
PO-74	H3	44	45	11111010110011010001110110	Warszawa	EC	1
PO-75	A1	34	55	11111011010011000001110110	Rzeszow	SE	1
PO-76	A1	34	45	10011000000011010001100110	Rzeszow	SE	1
PO-77	A1	34	55	10011100110011010001100110	Rzeszow	SE	1
PO-78	A1	44	45	10011000000011000011110110	Warszawa	EC	1
PO-79	A1	44	45	10011001010011010001100110	Warszawa	EC	1
PO-80	A2	44	45	11111010010011000001010110	Warszawa	EC	1
PO-81	A1	44	55	11111011010011000001110110	Chelm	SE	1

described in Fry et al (5) except that extracted samples were centrifuged at high speed for 30 s and used immediately for electrophoresis. Unused extracts were stored at −80 C for later use. Allozyme analyses of the remaining isolates were done using fresh tissue as described in Goodwin and Fry (10).

Allozyme electrophoresis for glucose-6-phosphate isomerase (GPI) was done using standard techniques described elsewhere (5,10). Peptidase (PEP) was assayed using the buffer system described by Mosa et al (16). Known standards were used as controls to determine the identity of allozyme alleles (5,10,24).

For each isolate, DNA was extracted from approximately 0.3 g of powdered, lyophilized mycelium as described by Drenth et al (4) or Goodwin et al (8). Approximately 2 μg of DNA from each isolate was digested with the restriction enzyme *Eco*RI and subjected to Southern analysis using DNA fingerprinting probe RG57 as described in Goodwin et al (8).

Statistical analyses. A multilocus genotype was constructed for each isolate by combining data for mating type and two allozyme and 26 DNA fingerprint loci. Because the exact genetic bases for mating type and some of the DNA fingerprint loci are not known, these are really multicharacter phenotypes, although they will be referred to here as multilocus genotypes for simplicity. For this analysis, the DNA fingerprint loci were only scored for the presence or absence of a band at each locus. This was done to avoid bias in the data, because it is not possible to determine the genotype accurately (whether heterozygous or homozygous for the "plus" allele) at all the DNA fingerprint loci. This approach is very conservative, and probably underestimates the level of genotypic diversity. From these data, genotypic diversity was calculated by a normalized Shannon's diversity index (23), as described in Goodwin et al (11,12).

The occurrence of geographic substructuring was assessed by Nei's gene diversity analysis (17). The two allozyme loci and a subset of 21 DNA fingerprint loci (those for which the genotype can be determined most accurately [12]) were included in the analysis. Gene diversity within each region (H_S) and the proportion of the total gene diversity (H_T) due to differentiation among regions (G_{ST}) (averaged over all 23 loci) were calculated according to the methods of Nei and Chesser (18) to remove the expected bias due to finite sampling within and among regions as described elsewhere (11,12).

Frequencies of genotypes at the two allozyme and 21 DNA fingerprint loci were tested for deviations from expected Hardy-Weinberg frequencies by the BIOSYS-1 statistical package (27). This analysis was done after removing clonal genotypes for the populations with the largest sample sizes in 1988, 1989, 1990, and 1991. The replications of a clone were removed because they represented asexual reproduction—a well-known feature of the *P. infestans* life history. The removal of a known cause of deviation from Hardy-Weinberg equilibria should improve the opportunity to detect a contribution of sexual reproduction. The amount of genetic differentiation between A1 and A2 mating-type isolates was determined by G_{ST} as described above. Measures of gametic phase disequilibrium were not calculated for these data, because there are many potential sources of disequilibrium (e.g., migration, selection, asexual reproduction) that occurred in these populations, and there were probably not enough generations of sexual reproduction to decay such disequilibrium. Therefore, disequilibrium values would be difficult to interpret and probably would not provide additional insight into the population structure of *P. infestans* in Poland.

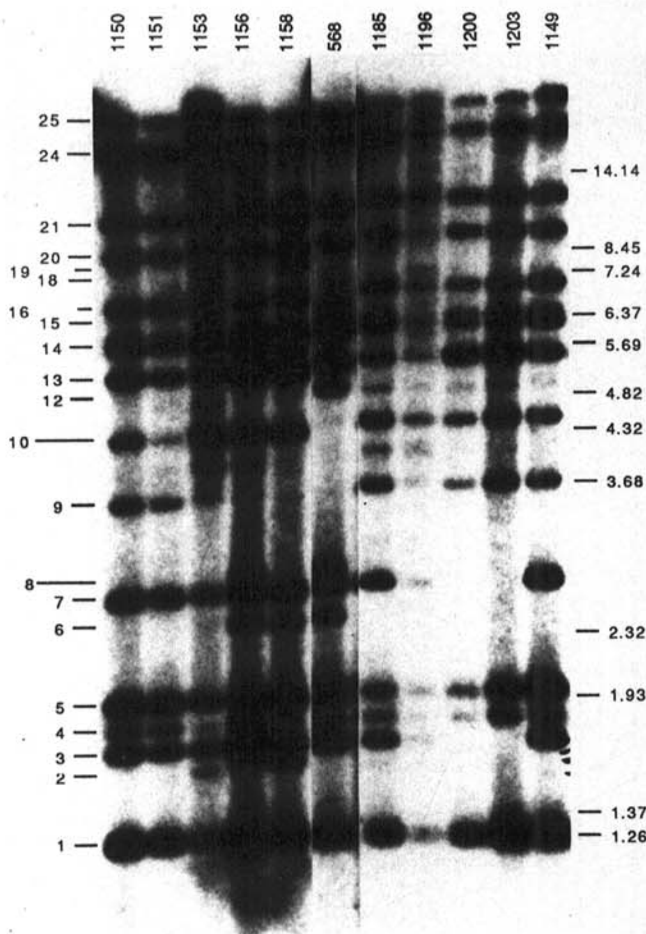

Fig. 2. DNA restriction fragment length polymorphisms in Polish isolates of *Phytophthora infestans* as revealed by the DNA fingerprint probe RG57. Isolates 1150, 1151, 1185, 1196, and 1149 had the most common "old" genotype (PO-1). Isolates 1200 and 1203 had the most common "new" genotype (PO-4). Isolate 568 is a central Mexican reference isolate that possesses bands not found in Poland. Not all of the fingerprint bands found in Poland are shown.

RESULTS

Overall diversity in Poland. There was considerable genetic diversity in the sample of 247 isolates from Polish populations of *P. infestans*. Not all isolates were analyzed for all markers, so sample sizes (indicated in parentheses below) differ for each marker. Although both mating types were present, 82% of all isolates tested ($n = 233$) were A1. Three allozyme alleles were detected at the *Gpi* locus (*86*, *90*, and *100*) ($n = 235$) and four at the *Pep* locus (*83*, *92*, *96*, and *100*) ($n = 234$), which were combined into 13 dilocus genotypes. Probe RG57 hybridized to the "present" allele at 22 of the 26 DNA fingerprint loci, and 20 loci were polymorphic, giving 62 distinct fingerprints among the 189 isolates assayed.

There were 81 multilocus genotypes among 175 isolates that were analyzed for all markers (Table 1). Only 12 genotypes were detected more than once, with one (PO-4) (Fig. 2) occurring 50 times (frequency in the total sample = 29%). The second most commonly occurring genotype (PO-1, frequency = 14%) (Fig. 2) was detected in 1985 through 1988. This genotype was identical to the genotype that dominated worldwide populations outside of Mexico prior to the 1980s (S. B. Goodwin, *unpublished data*; [9]). Five other genotypes were detected during more than one year and in more than one region (Table 1). Four genotypes were detected two or three times in one region during one year, and the remaining 69 genotypes (39% of the total sample) were unique (Table 1).

Temporal diversity. All isolates collected in Poland from 1985 through 1987 had one of three genotypes that appeared to be part of a single clonal lineage (Table 1). Except for a change from heterozygosity to homozygosity at one allozyme or DNA fingerprint locus, the two variants in the clonal lineage were identical to the more common genotype. The frequency of this genotype decreased to 54% by 1988, and it was not detected after 1988 (Table 2). Another genotype (PO-4) (Table 1; Fig. 2), first detected in 1988, increased in frequency and made up almost 50% of all isolates collected in 1991 (Table 2). This genotype also occurred in the same field from one year to the next in

the middle east and southeast regions. It was detected in three consecutive years (1989–1991) in one field in Boguchwala (southeast Poland). The frequency of unique genotypes increased dramatically after 1987 (Table 2) and reached a peak at 65% of all isolates collected in 1990. The genotypic diversity was evident within individual fields; there was typically a high frequency of unique genotypes in the small samples within each field. For example, in Mlochow in most years, most individuals were unique genotypes ($n = 3$–6).

The A2 mating type was first reported in 1988 (24), when it made up 5% of the sample (Table 2). The frequency of A2 isolates varied widely from year to year (44% in 1989 and 12% in 1991) (Table 2). The A2 isolates were first detected in southeast Poland but were found throughout the country by 1991 (Fig. 1).

Both gene diversity and Shannon's index of genotypic diversity increased markedly with the appearance of new genotypes in 1988 (Table 3). Both measures of diversity peaked in 1990 and declined somewhat in 1991 (Table 3). Analysis of regional differences as measured by G_{ST} indicated that there was virtually no genetic differentiation among regions in any year except 1988 (Table 3), when over 10% of all the genetic variation was due to differentiation among regions.

Cluster analysis of populations in the east central and southeast regions of Poland collected from 1988 through 1991 showed that most populations were similar, with a maximum Roger's genetic distance of only 0.13 (Fig. 3). The most closely related populations were those in east central Poland in 1990 and 1991 and in the southeast in 1990 and 1991, with genetic distances of 0.04 and 0.07, respectively. The east central population in 1988 was the most distantly related among all the populations (Fig. 3). All other populations were excluded from this analysis because small sample sizes precluded meaningful conclusions.

Sexual reproduction in Poland. The populations used for the cluster analysis also were analyzed for conformance to expected Hardy-Weinberg genotype frequencies at the two allozyme loci and 21 of the DNA fingerprint loci (not all were polymorphic in each location). Most (78%) of the 104 individual Hardy-Weinberg analyses were in agreement with the assumption of random mating. Most of the exceptions occurred at DNA fingerprint loci 3, 7, and 17, and virtually all of them resulted from a deficiency of heterozygotes. There was no genetic differentiation between A1 and A2 isolates ($G_{ST} = 0.01$).

DISCUSSION

There was a surprisingly high level of genetic diversity within populations of *P. infestans* in Poland regardless of how it was measured; both allelic (gene) and genotypic diversity values were high. Perhaps the most interesting result was that very few clonally related genotypes were identified: Only 12 genotypes were identified more than once, and only two of these were common during the 7-yr period of the study. This is in striking contrast to *P. infestans* populations in other parts of the world, including South America, Asia, the United States, and northern Mexico, where only three or four genotypes were present (6,7). Other than Poland, the only location with such a large number of genotypes is central Mexico, the putative center of origin for *P. infestans*, where almost every isolate has a unique genotype (12).

Migration undoubtedly played a role in increasing the genetic diversity in Polish populations of *P. infestans*. The current study not only reports DNA fingerprints on some of the same isolates for which allozyme and mating-type data were previously summarized (24), but also includes complete data on a large number of additional isolates collected in 1990 and 1991. Thus, the current study provides a more precise look at the changes associated with migration. Polish populations prior to 1988 were composed exclusively of a single clonal lineage, the same one that occurs throughout the world, possibly as a result of the initial introduction of *P. infestans* into Europe in 1845 (S. B. Goodwin, B. A. Cohen, and W. E. Fry, *unpublished data*; [9]). The first migrants were detected in 1988 when they made up almost 50% of all isolates collected. This migrant population was very diverse and contained isolates with characteristics not seen previously in Poland: A2 mating type, *Gpi90*, *Pep83*, and *Pep96* alleles, and many new DNA fingerprint bands. The *Pep96* allele was present in our previous samples (24) but was not detected until we switched to the buffer system used by Mosa et al (16). The modified procedure provided much greater resolution of peptidase genotypes. Thus, the peptidase genotypes for some isolates reported earlier (24) may have been misclassified. In 1988 a new genotype (PO-4) appeared; it apparently had high fitness in Poland because it rapidly increased in frequency until by 1991 it made up almost 50% of the isolates collected throughout the country. Beginning in 1988, there was a sharp increase in the frequency of uniquely identified genotypes as the first wave of migrants swept through the country.

This massive migration through Poland did not lead to geographic substructuring. There was very little genetic differentiation

TABLE 2. Frequencies of the A2 mating type and of particular genotypes of *Phytophthora infestans* in Poland over time

Year(s)	Frequency of A2 mating type[a]	Frequency of old clonal lineage[b]	Frequency of PO-4 genotype[c]	Frequency of unique genotypes
1985–1987	0	1.00	0	0.06 (14)[d]
1988	0.05	0.54	0.11	0.29 (28)
1989	0.44	0	0.35	0.46 (26)
1990	0.26	0	0.20	0.65 (46)
1991	0.12	0	0.49	0.39 (59)
Total sample	0.18	0.18	0.29	0.39 (175)

[a] Mating type was determined from a sample size of 233 isolates—including some for which allozyme and DNA data were unavailable. Sample size for isolates for which complete data for all markers were available was 175.
[b] This clonal lineage was composed of PO-1, PO-2, PO-3 (Table 1; Fig. 2).
[c] Table 1; Figure 2.
[d] Sample size is given in parentheses.

TABLE 3. Genetic diversity and genetic differentiation of *Phytophthora infestans* isolates among regions by year

Year(s)	Gene diversity[a]	Genotype diversity[b]	G_{ST} among regions[c]
1985–1987	0.114	0.254	0.000
1988	0.185	0.578	0.101
1989	0.178	0.711	0.030
1990	0.223	0.853	0.028
1991	0.174	0.569	0.039

[a] Gene diversity was determined according to the method of Nei (17).
[b] Genotype diversity was calculated using a normalized Shannon's diversity index (23) according to Goodwin et al (12) to minimize the sample size dependence of the usual (nonnormalized) Shannon's diversity index.
[c] Among-region diversity (G_{ST}) was calculated according to the methods of Nei and Chesser (18).

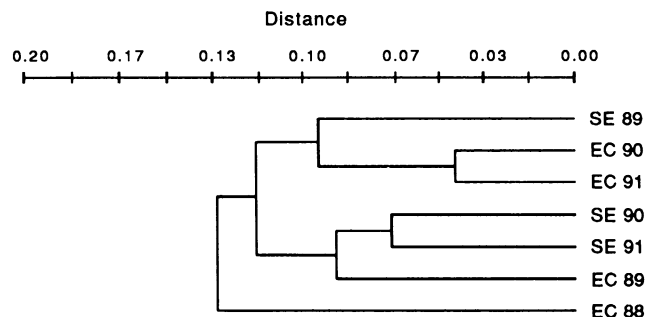

Fig. 3. Dendrogram of Roger's genetic distance values among populations of *Phytophthora infestans* in the east central (EC) and southeast (SE) regions in Poland from 1988 through 1991. Populations in other regions and in other years were excluded from this analysis due to small sample sizes.

(as measured by G_{ST}) among isolates collected from different regions in Poland. The largest G_{ST} value occurred in 1988, which probably resulted from an uneven spread of new genotypes. During the initial phases of the migration, differences in the frequency of new genotypes versus the old clonal lineage in the southeast region probably caused the slightly higher G_{ST}. This elevated G_{ST} disappeared in subsequent years as migration continued and the old clonal lineage effectively became extinct. Although there were no great differences among regions within Poland, cluster analysis indicated that the most closely related populations were those collected in the same locations (east central and southeast) in 1990 and 1991. Thus, the populations from these two regions in 1991 were probably derived mostly from those in the same locations in the previous year rather than from new migrants.

In an attempt to determine the probable source of the migrants, genotypes in Polish populations were compared with those in other European populations. Populations in Poland from 1985 through 1991 were very different from a sample of 153 isolates collected in the Netherlands in 1989 (4). Although many of the allozyme alleles and DNA fingerprint bands were common to both locations, some fingerprint alleles that were common in Poland were rare or absent in the Netherlands and vice versa. There were no matches between any of the genotypes found in Poland and those in the Netherlands. In contrast, isolates collected in Russia were closely related to those in Polish populations (S. B. Goodwin, B. A. Cohen, and W. E. Fry, *unpublished data*). A small sample of isolates collected from 1979 to 1990 in the Moscow area had the old predominating clonal lineage as well as newer genotypes, whereas isolates collected in 1990 in the Ural region represented only the "old" clonal lineage (S. B. Goodwin, *unpublished data*). Isolates collected in 1983 in Estonia and in 1990 in Belarus had the same allozyme and DNA fingerprint genotypes as the most common Polish genotype (PO-4). In Germany there were both "old" and "new" genotypes (as determined by allozymes and mating type) (3); however, isolates representing the old genotype were only found prior to 1978 (3). Later detection of the "new" genotypes in the Ural region and their earlier detection in Germany may indicate that the founder clones of current Polish populations migrated with the prevailing winds from west to east across Europe.

There are four lines of evidence that indicate that sexual reproduction is probably occurring within *P. infestans* populations in Poland. First, genotype frequencies at most loci were in Hardy-Weinberg equilibrium. Most of the exceptions were at three DNA fingerprint loci and were caused by a deficiency of heterozygotes. At least two of these loci, 3 and 7, are very tightly linked (8), so the deviations really only occurred at two independent loci. These loci may be more difficult to score genetically, or, possibly, they (or a gene linked to them) cause a reduction in fitness when they are heterozygous. Second, there was no genetic differentiation between A1 and A2 isolates; G_{ST} values between the mating types were less than the G_{ST} values among regions in each of the years tested. Genetic recombination through the sexual cycle is the most likely explanation for this lack of genetic differentiation between mating types. Third, there was a high frequency of unique genotypes in the samples collected from 1989 through 1991; this would be unexpected in a population that reproduced completely asexually. Finally, the *Gpi 90/90* genotype was found for the first time in Poland in 1989. Previous collections indicated that the *Gpi90* allele was probably introduced to Europe in the heterozygous state (as a *90/100* genotype) in the late 1970s or early 1980s (5,24). Inability to detect *90/90* homozygotes was one reason Fry et al (5) concluded that sexual reproduction might not be occurring in the Netherlands; however, the most common A1 genotype in that study was *90/100*, and the most common A2 genotype was *100/100*. Sexual recombination between these genotypes would generate initially only *90/100* and *100/100* genotypes. It would require additional time for sexual recombination and selection to generate *90/100* A2 isolates that could recombine with *90/100* A1 isolates to generate *90/90* homozygotes. Therefore, it is not surprising that *90/90* homozyogotes were not detected until a decade or more after the introduction of the *90* allele to Europe. The *90/90* genotype was detected in western France in 1990 (D. Andrivon, *personal communication*). These may have arisen by sexual recombination in France, rather than by migration from Poland.

Sexual reproduction may complicate disease-control measures, particularly if oospores in the soil become a source of inoculum. If oospores survive in soil, then rotation as a late blight-control measure may need to be introduced. Depending on the length of oospore survival, rotations might need to be longer than 1 yr. Sexual recombination also would generate new genotypes, some of which may have greater fitness in Poland. Thus, it may become increasingly difficult for plant breeders to develop resistant cultivars. An example of what may occur can be seen by recent changes in the resistance of the cultivar Bronka. During the early and mid-1980s, Bronka was highly resistant to *P. infestans* (typically less than 20% foliage blighted by the end of the season), but by the late 1980s, it was severely blighted (approximately 90% foliage blight by the end of the season) (L. S. Sujkowski, *unpublished data*). Bronka contains at least some specific resistance genes (26) that are overcome by "new" genotypes of *P. infestans* (25). Sexual recombination may have played a role in the rapid breakdown of resistance in Bronka, and it may play an increasing role in the breakdown of late blight resistance in Poland in the future.

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