Population Genetic Structure of *Phytophthora infestans* in the Netherlands

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ABSTRACT

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Isolates of *Phytophthora infestans* were collected from six different regions in the Netherlands in September–October 1989 and subsequently characterized. Regions contained one to four sampling sites and yielded 186 isolates. Additionally, 19 isolates from an ongoing metalaxyl-resistance monitoring project were characterized. In total, 205 isolates were characterized in terms of allozymes (glucose phosphate isomerase [*Gpi-1*] and peptidase [*Pep-1*]), mating type, and metalaxyl resistance. The analysis revealed 17 different genotypes. Samples from some sites were highly heterogeneous, whereas samples from other sites appeared homogeneous. Three genotypes each were detected in five of the six regions and together accounted for 61% of all isolates. Metalaxyl-resistant isolates accounted for 35% of the total sample and 45% of the samples from commercial fields. Chi-square contingency analysis indicated significant differences in genotype frequencies among subpopulations from different regions of the country, between A1 and A2 individuals, and between potato and tomato isolates. In most locations the frequency of allozyme alleles differed significantly from frequencies expected according to Hardy-Weinberg equilibrium. The results were consistent with asexual reproduction, although the occurrence of a low level of sexual reproduction cannot be excluded.

Additional keywords: epidemiology, late blight.

Variability in virulence and fungicide resistance in populations of fungal plant pathogens contributes to failures in established disease management procedures. Recently, variation in sensitivity to fungicides has been especially troublesome, and for decades, diversity in specific virulence has severely limited the usefulness of cultivars with specific resistances. Certainly, *Phytophthora infestans* (Mont.) de Bary fits this general scenario (6,7,29). Resistance to the specific fungicide, metalaxyl, has limited the usefulness of that fungicide in various locations around the world, and the occurrence of many specific virulence factors in populations of *P. infestans* has eliminated the use of genes for specific resistance as an important disease management strategy. On the premise that knowledge of the population genetics of fungal plant pathogens may eventually contribute to the development of more durable disease management strategies, we have initiated such a study for *P. infestans.*

Biochemical and molecular markers contribute significantly to the abilities of scientists to gain understanding of the population biology of fungi. Spieth's study of the saprophytic fungus, *Neurospora intermedia* (26), indicated that levels of diversity are comparable to those of *Drosophila,* with most of the diversity occurring within local populations. Comparisons among collections of *Puccinia graminis* f. sp. *tritici* (causal agent of stem rust of wheat) suggested that global diversity in that pathogen population was relatively low, although levels of diversity within populations were variable (2–4). Leung and Williams (11) found little variation in allozyme genotypes within a geographically diverse collection of isolates of *Magnaporthe grisea* (causal agent of rice blast)—two electrophoretic types accounted for 90% of the samples. Diversity was higher from nonrice hosts. The recent availability of various molecular markers provides a potentially powerful tool for analyzing phylogenic relationships and population genetic structure (1,12).

Information concerning the population genetics of *P. infestans* is still minimal, but is beginning to develop (8,21–23). There was much greater diversity for allozyme genotypes in a population from central Mexico than in populations from other locations in the world. Preliminary tests indicated that alleles for glucose phosphate isomerase-1 (EC 5.3.1.9) (*Gpi-1*) in the population from central Mexico fit Hardy-Weinberg equilibrium, but such alleles from a population of isolates from the United States and Canada did not (27). The number of allozyme alleles useful in population studies now number at least 10 (six for *Gpi-1*, four for peptidase [EC 3.4.3.1] [*Pep-1*]) (24,25). Each of the *Gpi-1* and *Pep-1* alleles has been shown to function according to Mendelian expectation in classical genetics studies (25).

Discovery in the mid-1980s of the A2 mating type in Europe (10) was the first indication of significant changes in the populations of *P. infestans* there and subsequently throughout the world. Before the 1980s the A2 mating type had been found only in central Mexico (8). Further analysis of the allozymes in individuals from various locations around the world stimulated the hypothesis that a new population (apparent during the 1980s) of *P. infestans* was displacing an older (pre-1980s) population in Europe (21,23).

The major goal of our study was to determine the distribution of genetic variation in populations of *P. infestans* at several geographical levels (within a country, among regions in a country, and among sites within a region). Obviously, such a question needed to be tested in a location with numerous potato fields and where late blight could be easily found. The Netherlands fit these criteria. Results of this analysis would be helpful in several respects, but we were particularly interested in estimating the contribution of sexual reproduction in a location where both mating types had existed for a relatively short period.

MATERIALS AND METHODS

Collection of isolates. Isolates were obtained in two ways. First, several collecting trips to commercial potato fields and community gardens in various locations in the Netherlands were conducted during September and October 1989 (Table 1). All tomato and

TABLE 1. Sites sampled randomly for *Phytophthora infestans* in the Netherlands in 1989

Region	Site	Nearest town	Code number	Host	Field size	Disease severity[a]	Number of isolates	Collection date
NE	2	Ter Wisch	89140	Potato	< 2 ha	10–20% defoliation	27	7 Sept.
NE	3	Ter Wisch	89141	Potato	< 1 ha	Very mild	2	7 Sept.
NW	1	Middenmeer	89142	Potato (cv. Bintje)	10 ha	Very mild	17	7 Sept.
NW	2	Middenmeer	89143	Potato (cv. Bintje)	10 ha	Very mild	18	7 Sept.
C	1 (t)	Wageningen	89147	Tomato	Community garden	Very mild	20	11 Sept.
C	1 (p)	Wageningen	89148	Potato	Community garden	Moderate	16	11 Sept.
W	1	Leiden	89154	Potato	Community garden	1–10% defoliation	12	15 Sept.
W	2 (p)	Leiden	89155	Potato	Community garden	Very mild	6	15 Sept.
W	2(t)	Leiden	89156	Tomato	Community garden	Very mild	7	15 Sept.
SW	1	Middelburg	89153	Potato	Volunteers	Moderate	17	19 Oct.

[a]Disease severity was not measured quantitatively, so only general descriptions are given here: Very mild = one lesion per plant and infected plants are widely separated; Moderate = one to several lesions per plant; more severe disease is indicated via an estimate of defoliation.

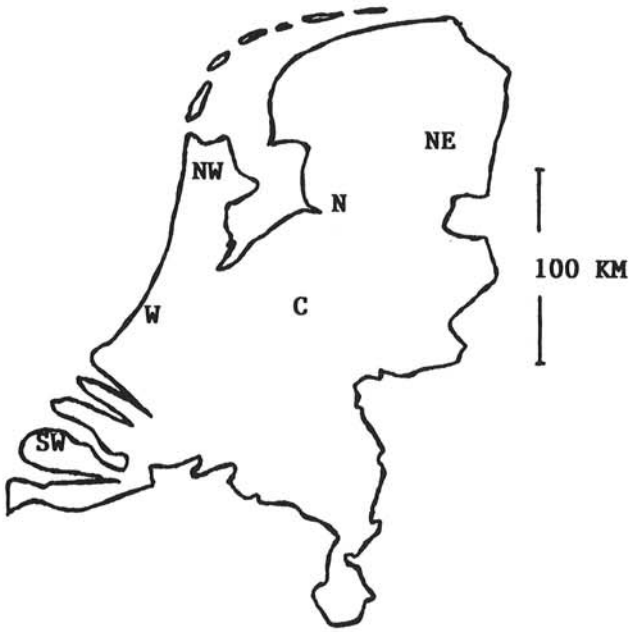

Fig. 1. Collection sites of *Phytophthora infestans* in the Netherlands in 1989. One or a few sites were sampled in each of six regions (NE, N, NW, W, C, SW) to yield 186 isolates. All sites in a region were within 25 km of a central point, and regions were separated by 75–300 km.

potato plants in community gardens were inspected and plants along a series of transects in commercial fields were inspected. Each field or contiguous group of community gardens was regarded as a single site, and sites within 40 km were regarded to be from the same region. The minimum distance between collecting regions was approximately 75 km, and the maximum distance was approximately 300 km (between Middelburg in the southwest and Ter Wisch in the northeast) (Fig. 1). Regions represented the northeast, north, northwest, west, central, and southeast (NE, N, NW, W, C, and SE) parts of the Netherlands (Fig. 1). Fields were scouted and single lesion samples were collected randomly. Where there was sufficient late blight, at least 30 single-lesion samples were obtained from a site by random collecting. Unfortunately, most sites did not enable collection of 30 samples. Individual isolates were obtained from leaves with single lesions, or from single tubers, using standard isolation techniques.

Isolates also were obtained from infected tubers and leaves sent to the Agricultural University in Wageningen during summer and fall 1989 as part of an ongoing anlysis of the frequency of metalaxyl resistance in the Netherlands. Usually only one or a few isolates were obtained from each sample.

Culture of isolates. After isolation into pure culture, isolates were kept on rye A medium (5) at 18 C in the dark. For analysis of allozymes, isolates were cultured on liquid Rye A medium (without agar) in 9-cm petri plates for approximately 14 days at 18 C in the dark. For assessment of mating type, isolates were grown on clarified Rye A agar medium in proximity to a strain of known mating type (A1 or A2). Each isolate was tested against a known A1 strain and against a known A2 strain. Oospores usually appeared within 5–10 days.

Metalaxyl resistance. The floating leaf disk test was used as described by Davidse et al (6), except that only three test concentrations of metalaxyl were used: 0.0, 1.0, and 10 μg/ml. Each of five leaf disks was inoculated with a suspension of sporangia obtained from a sporulating tuber slice or from a culture growing on Rye A medium for each metalaxyl concentration. Inoculated floating leaf disks were incubated in the light (16 h per day, intensity 75,000 lx) at 15 C for 5–7 days. Sporulation within 5–6 days on leaves floating on 1.0 or on 1.0 and 10.0 μg/ml indicated metalaxyl resistance.

Allozyme analysis. Mycelium from a single 9-cm petri plate was harvested from liquid culture by filtration and then stored at −80 C until further use. The mycelium (approximately 0.12 g wet weight) was ground to a powder in a mortar and pestle cooled with liquid nitrogen. The ground mycelium was transferred to a microcentrifuge tube and 0.4–0.6 ml of Tris-citrate gel buffer, pH 7 (ref 17 buffer system I), was added to each tube. The homogenate was not centrifuged but was stored at 4 C for use within a few days or stored at −80 C for later use.

Electrophoresis was done using standard techniques (18). For *Gpi-1*, we used a gel buffer of 0.01 M histidine-HCl, pH 6.0, and an electrode buffer of 0.135 M Trizma base, 0.04 M citric acid, pH 6.0. These gels were run for 6–8 h at approximately 150 V and approximately 50–60 mA at 4 C for resolution of the 90/100 genotypes. (Alloyzyme genotypes are described in terms of the relative mobilities of their bands of activity in an electric field in starch gels [25]. The most common allele is assigned a mobility of 100 and other alleles are assigned numbers based on their relative mobility. Thus 90/100 refers to a heterozygous genotype with two alleles, one allele being the most common type, and the other producing an enzyme that migrates 90% as far as the most common type.) For *Pep-1* we used buffer 10 of Soltis et al (20); the gel buffer was a 1:4 dilution of the electrode buffer (0.18 M Trizma base, 0.10 M boric acid, 0.001 M Na-EDTA, pH 8.7). These gels were run for 6–8 h at 290 V and approximately 40 mA at 4 C. All gels were 12% hydrolyzed potato starch (for electrophoresis, Sigma, St. Louis, MO). Enzymes were detected using agar overlays and the techniques of Micales et al (13), Shaw and Prasad (17), and Siciliano and Shaw (19). Controls to assess the relative mobilities of allozymes were from the culture collection at Cornell University.

Isoelectric focusing proved to be a useful technique for *Gpi-1* isozyme analysis. Samples were loaded on the cathode side of a 5% polyacrylamide gel (LKB Ampholine 1804-102, Piscataway, NJ), pH range 4.0–6.5, with a 3% degree of cross-linkage and ampholine concentration of 2.2% (w/v). The gel was run for 1.5 h at 4 C at a constant power of 25 W. *Gpi-1* was detected using the agar overlay as described above.

Statistical analyses. Contingency chi-square analyses were conducted to detect differences in frequencies among subpopulations

from the six regions, between A1 and A2 individuals, and between isolates obtained from potato and tomato, and to compare observed allozyme genotype frequencies with those expected at Hardy-Weinberg equilibrium. Genotypic analyses were emphasized because of the importance of asexual reproduction in this fungus. Analyses based on allele frequencies are most appropriate for sexually reproducing organisms. Groupings were done on traits thought to be neutral—no distinction was made between metalaxyl-resistant and -sensitive isolates. Groupings were constructed to achieve expected values of 5 or more, because expected values of < 5 can lead to unreliable chi-square values. When only a few expected values were below 5 (and above approximately 2.5), chi-square values were calculated, but clearly identified.

The extent of population subdivision also was assessed using Nei's gene diversity analysis (14). Gene diversity within each region or subpopulation (H_s) was calculated for each allozyme locus in each location. The mean proportion of the total gene diversity (H_t) that was due to differences among subpopulations (G_{st}) was calculated according to the methods of Nei and Chesser (15) for obtaining unbiased estimates of H_s and H_t assuming a mixed mating model.

RESULTS

Collection of isolates. A total of 205 isolates was collected (Tables 1–3). Most isolates (142) were obtained by random sampling of single lesions in commercial potato fields or community gardens during September–October 1989 (Table 1). Of the many fields and garden sites inspected by the authors, five commercial fields and three sets of gardens had late blight. Additionally 44 isolates were obtained from samples submitted from the advisory service during fall 1989 (Table 2). These 44 isolates came from four sites, but the details of sampling (random or nonrandom) are unknown and thus are identified separately (Table 2). Finally, 19 additional isolates (10 A2 and nine A1 isolates) were selected (not randomly sampled) from those received during summer 1989 for a metalaxyl-resistance monitoring project. The 10 A2 isolates represented all of the A2s received during the summer.

The sites had widely diverse characteristics (Tables 1 and 2). Host density in community gardens was dramatically less than in commercial fields. Both host genotypes and field size varied. In most sites sampled, disease was very light (widely scattered lesions), apparently caused by the unusually warm and dry 1989 growing season. However, one commercial field (NE-2) and potatoes in one set of community gardens (W-1) had disease so severe that some defoliation had occurred.

Allozyme analysis. Two alleles for *Gpi-1* and two alleles for *Pep-1* were detected. For *Gpi-1* the 90/100 genotype occurred in 84% of the isolates, whereas the 100/100 genotype occurred in 16% of the isolates. However, there were two forms of the 90/100 genotype, which differed in the relative staining intensity of the 100 homodimer. The most common type of 90/100 genotype was one in which the 100 homodimer stained with expected intensity, but in the other genotype (labeled 90/100a), the 100 band was extremely faint. Isoelectric focusing of selected samples confirmed the 90/100 designations. Controls for *Gpi-1* included isolates 575, 579, 618, 619, 807, 821, and 1118, from the collection at Cornell University with genotypes of 100/122, 100/100, 86/122, 86/100, 86/100, 86/100, and 90/100, respectively. The 86 allele for *Gpi-1* was not detected in any of the 205 isolates.

For *Pep-1*, two genotypes were detected: 83/100 and 100/100,

TABLE 2. Locations of *Phytophthora infestans* samples submitted from the advisory service during fall 1989

Region	Site	Nearest town	Code number	Host	Number of isolates	Acquisition date
NE	1	Erica	89102	Potato (cv. Ehud) (leaves)	7	7 Sept.
NE	4	Ter Wisch	89150	Potato (cv. Astarte) (leaves)	20	26 Sept.
N	1	Appelscha	89157	Potato (cv. Astarte) (tubers)	8	10 Nov.
N	2	Appelscha	89158	Potato (cv. Van Gogh) (tubers)	9	10 Nov.

TABLE 3. Genotypes of *Phytophthora infestans* isolates collected in the Netherlands in 1989

Genotype			Number of isolates in various 1989 collections[a]															
Gpi-1	*Pep-1*	MT[b]	MR[c]	NE-1	NE-2	NE-3	NE-4	N-1	N-2	NW-1	NW-2	C-1(t)	C-1(p)	W-1	W-2(p)	W-2(t)	SW-1	NL	TOTAL
100/100	100/100	1	S						1			1	2					2	6
100/100	100/100	2	S									18	1					6	25
100/100	83/100	1	R										1						1
100/100	83/100	1	S										1		2				3
100/100	83/100	2	R		1														1
100/100	83/100	2	S															1	1
90/100	100/100	1	R		2				1	1	1		1					2	8
90/100	100/100	1	S		6			5	4	12	17		2				7	3	56
90/100	100/100	2	S										1						1
90/100	83/100	1	R	7	15	1	19	1	2	4			2					1	52
90/100	83/100	1	S		1			2	1				1	12			9	1	27
90/100a[d]	100/100	1	R		2	1							3				1		7
90/100a	100/100	1	S										1		4	7			12
90/100a	100/100	2	R															1	1
90/100a	100/100	2	S									1						1	2
90/100a	83/100	1	R				1												1
90/100a	83/100	2	S															1	1
TOTALS (individuals)				7	27	2	20	8	9	17	18	20	16	12	6	7	17	19	205
(genotypes)				1	6	2	2	3	5	3	2	3	11	1	2	1	3	10	17

[a] Locations of collections (except NL) are indicated in Tables 1 and 2 and pictured in Figure 1. NL indicates the 19 isolates selected from the metalaxyl monitoring project. All A2 isolates from that project were analyzed and geographically close A1 isolates were selected for comparison.
[b] MT = mating type (A1 or A2).
[c] MR indicates metalaxyl resistance (resistant R, or sensitive S).
[d] 90/100a differs from 90/100 in the relative staining intensity of various electromorphs of *Gpi-1* as described in the text.

with frequencies of 42 and 58%, respectively. Detection of the 83 allele for *Pep-1* was unexpected, and to our knowledge it is the first report of this allele in Europe. Comparisons with the peptidase patterns of control isolates confirmed the presence of the allele. The control isolates were isolates 562, 575, 579, and 807 from the culture collection at Cornell University, with *Pep-1* patterns of 83/100, 100/100, 92/92, and 92/100, respectively. None of the 1989 isolates collected in the Netherlands had the *Pep-1* 92 allele.

Characterization of isolates. Analysis of the 205 isolates revealed 17 different genotypes (Table 3). When we eliminated metalaxyl resistance as a distinguishing character, the most commonly occurring genotype (79 individuals) was A1, 90/100 for *Gpi-1* and 83/100 for *Pep-1*. Except for the community gardens in Wageningen (C-1[p], C-1[t]), the A2 type was found only rarely.

The *Gpi-1* and *Pep-1* markers identified some collections as considerably heterogeneous, but others as apparently homogeneous. Collections NE-1, W-1, and W-2(t) were completely homogeneous, and collections NW-2 and NE-4 were dominated by a single genotype. In contrast, collections NE-2, C-1(p), N-1, and N-2 appeared heterogeneous (Table 3).

The distribution of *Gpi-1* and *Pep-1* alleles was tested via chi-square analysis for conformation to Hardy-Weinberg equilibrium. The analysis was done by grouping all 90/100 genotypes together. In general the distribution of *Gpi-1* alleles was more divergent from that expected than was the distribution of *Pep-1* alleles. In all comparisons for which the chi-square value was reliable, the observed frequency of alleles was significantly different from that expected (Table 4).

Metalaxyl resistance. The fact that fungicide resistance is a selectable marker, strongly affected by fungicide use, was clear in this collection. In the collection as a whole, metalaxyl resistance occurred in approximately 35% of the isolates (Table 3). However, the proportion of metalaxyl-resistant isolates was much higher

TABLE 4. Contingency chi-square analysis[a]: Conformation to Hardy-Weinberg equilibrium

Region or group	*Gpi*				*Pep*			
	Genotype	Obs.	Exp.	X^2	Genotype	Obs.	Exp.	X^2
NE[b]	90/90	0	13.4	13.4	83/83	0	20.2	20.2
	90/100	55	28.0	26.0	83/100	45	26.8	12.3
	100/100	1	14.6	12.7	100/100	11	9.0	0.4
				52.1**[c]				32.9**
N	90/90	0	3.7	3.7	83/83	0	0.6	0.6
	90/100	16	8.5	6.6	83/100	6	5.0	0.2
	100/100	1	4.8	3.1	100/100	11	11.4	0.0
				{13.4**}[d]				...[e]
NW	90/90	0	8.8	8.8	83/83	0	1.0	1.0
	90/100	35	17.4	17.8	83/100	4	3.8	0.1
	100/100	0	8.8	8.8	100/100	31	31.1	0.0
				35.4**				...[e]
C	90/90	0	1.2	1.2	83/83	0	0.2	0.2
	90/100	13	10.6	0.5	83/100	6	5.3	0.1
	100/100	23	24.2	0.1	100/100	30	30.5	0.0
				...[e]				...[e]
W	90/90	0	5.3	5.3	83/83	0	2.0	2.0
	90/100	23	12.4	9.1	83/100	14	10.1	1.5
	100/100	2	7.3	3.8	100/100	11	13.0	0.3
				18.2**				...[e]
SW	90/90	0	4.3	4.30	83/83	0	1.1	1.1
	90/100	17	8.4	8.80	83/100	9	6.5	1.0
	100/100	0	4.3	4.30	100/100	8	9.3	0.2
				{17.40**}[d]				...[e]
Total collection	90/90	0	34.4	34.4	83/83	0	9.5	9.5
	90/100	159	91.1	50.6	83/100	84	65	5.6
	100/100	27	60.4	18.4	100/100	102	111	.7
				103.4**				15.8**
C-1 (t)	90/90	0	0	0	83/83	0	0	0
	90/100	1	1	0	83/100	0	0	0
	100/100	19	19	0	100/100	20	20	0
				...[e]				na[f]
C-1 (p)	90/90	0	2.3	2.3	83/83	0	0.5	0.5
	90/100	12	7.5	2.7	83/100	6	4.9	0.2
	100/100	4	6.3	0.8	100/100	10	10.6	0.0
				{5.8*}[d]				...[e]

[a] Each analysis had one degree of freedom.
[b] Regions are identified in Figure 1.
[c] Probabilities of a greater chi-square due to chance were: * = < 0.05; ** = 0.005.
[d] The chi-square may be unreliable because one expected value was between 5.0 and 3.0.
[e] The chi-square value was not calculated because expected values were too low.
[f] The analysis was not applicable because there was no diversity for either *Gpi-1* or *Pep-1*.

from commercial fields (about 45%), where metalaxyl has been widely used, than from community gardens (11%), where metalaxyl is rarely used. Metalaxyl resistance was detected in all genotypes found in commercial potato fields. Interestingly, no isolate obtained from infected tomato (all obtained in community gardens) was resistant to metalaxyl.

Analysis for genetic substructure. Several types of genetic substructuring were tested. Fungicide resistance was not used as a character in these analyses because the genetic base was unclear, and because it is a strongly selected phenotype. Approximately 6% of the overall gene diversity (15) was due to differences among regions ($G_{st} = 0.061$). The unbiased mean values for total (H_t) and within region (H_s) gene diversity were 0.412 and 0.387, respectively. This indicates a low level of population subdivision.

Other substructuring was detected from analysis of genotypic frequencies. Subpopulations from different regions were significantly different from each other—whether the comparison was based on all isolates from six regions, potato isolates from six regions, or potato isolates from commercial fields (Table 5). The A1 isolates appeared to represent a different subpopulation than did the A2 isolates in most comparisons. The A1 and A2 samples were significantly different when all 186 isolates were examined or when only the central region isolates (C-1[t], C-1[p]) were examined (Table 5). Finally, isolates from potato appeared to represent a different subpopulation than did the isolates from tomato (Table 5). This result held for comparison of all potato and tomato isolates or only the potato and tomato isolates from the central region (Table 5). However, at one site (W-2) the tomato isolates had the same genotype as the majority of potato isolates (Table 3). Small sample size at this site precluded assessment of statistical significance.

Additional A1:A2 comparison. In an additional effort to assess the similarity of A1 and A2 isolates in the Netherlands, all of the A2 isolates ($n = 10$) and nine additional A1 isolates selected from those submitted to the metalaxyl-resistance monitoring project in 1989 were characterized. The A1 isolates were chosen from the same sites as the A2 isolates if possible, but if not, from a geographically close location. The vast majority of A1 isolates were 90/100 for *Gpi-1*, whereas most A2 isolates were 100/100 or 90/100a for *Gpi-1* (Table 3). Even though these isolates were submitted from all over the Netherlands, the frequencies of *Gpi-1* alleles in A1 isolates were not significantly different from the frequencies in A2 isolates. The expected number of *Pep-1* alleles was too low in some categories to enable assessment of statistical significance (Table 5).

TABLE 5. Contingency chi-square analysis for nonrandom frequencies

Groupings	Chi-square	
	Gpi-1	*Pep-1*
Regional comparisons[a]		
All isolates (six regions)	{90.5**[b] (5)[c]}[d]	58.5** (5)
Potato isolates (six regions)	...[e]	49.9** (5)
Potato isolates from commercial fields (NE, N, NW, SW)	...[e]	47.0** (3)
A1:A2 comparisons		
All isolates six regions	...[e]	16.9** (5)
19 summer isolates	{4.3* (1)}[f]	...[e]
Central region isolates {C-1(t) and C-1(p)}	15.4** (1)	...[e]
Potato:tomato host comparisons		
Central and western isolates	17.2** (1)	26.1** (1)
Central isolates	18.7** (1)	...[e]

[a] The six regions are identified in Figure 1.
[b] The probability of a great chi-square value is indicated by numbers of asterisks: * = < 0.05; ** = < 0.005.
[c] The number in parentheses indicates the degrees of freedom.
[d] This chi-square value may be unreliable because three of 12 expected values were < 5.0 (2.5, 3.6, and 2.5).
[e] The chi-square was not calculated because expected values were too low.
[f] This chi-square value may be unreliable because three of four expected values were between 4.2 and 5.0.

DISCUSSION

The present study confirmed previous studies in identifying limited diversity in *P. infestans* in the Netherlands, but differed from other studies in terms of the specific genotypes detected. The absence of the 86/100 genotype for *Gpi-1* in any of the 205 isolates was surprising because of the prevalence of this genotype in early collections (pre-1980s) from the Netherlands (22). However, these data are consistent with a recent report from the United Kingdom indicating no individuals with *Gpi-1* genotype of 86/100 in recent collections (16). The common occurrence of individuals with 90/100 genotype for *Gpi-1* in the Netherlands is not necessarily inconsistent with recent reports from the United Kingdom where only 100/100 genotypes for *Gpi-1* were detected. The 90/100 genotype is only detectable with the lower pH gels done in this study, which were not done when the United Kingdom population was characterized. Had we used pH 7.0 gels, we also would not have been able to distinguish the 90/100 from the 100/100 genotype. The 90 allele is legitimate because it segregated according to Mendelian expectation in the two crosses that we have done (L. J. Spielman, *unpublished*).

The discovery of the 83/100 genotype for *Pep-1* was unexpected but very interesting, because this is the first report of the *P. infestans* allele from western Europe. In earlier reports this allele had been erroneously recorded as 92/100 (21). The 83 allele was first detected in the late 1980s (23). However, the 83/100 genotype is sometimes difficult to distinguish from the 92/100 genotype because of poor resolution of *Pep-1* bands on gels. Many controls are needed. Therefore some isolates previously characterized as 92/100 had been misidentified. For example, isolate 1117 collected from the Netherlands in the mid-1980s was initially characterized by us as 92/100 for *Pep-1*, and only after documentation of the 83/100 genotype (23) was this isolate correctly characterized as 83/100. We have reevaluated our isolates characterized before the discovery of the 83/100 genotype and have found that all isolates (previously labeled as 92/100) collected in Europe after 1982 were actually 83/100 (L. J. Spielman, *unpublished*). However, isolates collected before 1980 and characterized as 92/100 were correctly identified as 92/100 (22). The 83 allele is apparently characteristic of the "new" (early 1980s and later) population of *P. infestans* in Europe, which has apparently displaced the "old" (pre-1980s) population (22).

The present study indicated that some genotypes are widely distributed, whereas others may be unique to a single site. Individuals that were mating type A1 and 90/100 for *Gpi-1* and 83/100 for *Pep-1* were found in all regions of the country. Each of six genotypes was found in only one site (Table 3). Some sites appeared to contain only individuals of a single genotype, whereas other sites contained individuals with different genotypes. Thus our results for *P. infestans* are similar to those for *P. g. tritici* (4) in that diversity was variable among subpopulations.

An important question in the Netherlands and in other locations where both A1 and A2 mating types occur is whether or not sexual reproduction is contributing to the epidemiology of late blight. Although data from the present study do not conclusively resolve this issue, the infrequent occurrence of A2 isolates, and the discoveries that the A2 mating type is associated with certain allozyme genotypes and that A1 mating types are associated with other allozyme genotypes, is more consistent with the notion of asexual reproduction than with significant sexual reproduction. Associations between mating type and allozyme genotypes have not been detected in central Mexico (27). If sexual reproduction were common and contributing to the diversity of the Netherlands population, one would expect A1 and A2 individuals to have similar allozyme genotypes and both to be represented in all regions of the country. Conformation or lack of conformation of allele frequencies to those expected at Hardy-Weinberg equilibrium is not at all conclusive in this issue because of the limited diversity and many generations of asexual reproduction during epidemics.

The occurrence of metalaxyl resistance in several different genotypes supports the hypothesis that this trait has developed

many times in a variety of genetic backgrounds (especially if sexual recombination is rare). The influence of selection is reflected in collections from commercial fields relative to collections from community gardens. The incidence of metalaxyl resistance in community gardens was 11% whereas the incidence of metalaxyl resistance in commercial potato fields was approximately 45%. Metalaxyl has been used rarely if at all in community gardens, but it has been used for years in commercial potato production (6).

This study also sheds some light on the question of host-plant specialization in *P. infestans*. Some reports suggest that isolates from tomato are different from those on potato (9,28), and some of our data support this suggestion. Tomato isolates from the Wageningen community gardens (C-1[t]) appeared to be quite distinct from the potato isolates (C-1[p]) collected there. The absence of metalaxyl resistance in isolates from tomato hosts may support the hypothesis of host-plant specialization. Presumably, metalaxyl has been used much less on tomatoes (community gardens) than on potatoes (commercial production). However, tomato isolates from Leiden community gardens (W-2[t]) had a genotype identical to that of the majority of potato isolates (W-2[p]) from Leiden. Thus our data support the concept of patchy population substructuring of *P. infestans* according to host plants.

Several results of this study support the hypothesis that a new population of *P. infestans* is replacing an older population in Europe (21,22). The 86/100 genotype for *Gpi-1* and the 92/100 genotype for *Pep-1* are characteristic of the older (pre-1980s) population but not of the new population. The frequency of the 86/100 genotype for *Gpi-1* continues to decrease in the Netherlands (not detectable in the 205 isolates of this study). Similarly, it was not detected in recent collections in the United Kingdom. However, collections of *P. infestans* made in the early 1980s and late 1970s in the Netherlands and United Kingdom were dominated by individuals with this genotype (R. C. Shattock, personal communication; 21). In addition, the absence of *Pep-1* 92/100 in this collection provides further evidence of population change in western Europe. Studies of earlier collections identified this genotype in western Europe (21). The discovery of the 83/100 genotype for *Pep-1* in this study provides an additional difference between the current and previous (pre-1980s) isolates of *P. infestans*. The 83/100 genotype has been found in central Mexico, but not in the United States and Canada (21).

The present study provides preliminary results concerning the population structure of *P. infestans* in the Netherlands. Analysis of gene diversity (G_{st}) was not very helpful in interpreting population substructure. The relatively low value for G_{st} (= 0.06) indicated little substructure among geographic regions. However, chi-square analysis of genotypic diversity indicated significant differences among populations in different regions (Table 5). Furthermore, conclusions are limited because of the limited number of markers and small sample sizes. Unfortunately, the number of isolates collected was limited because the unusually dry and sunny 1989 growing season limited the occurrence of late blight, even in the Netherlands. Many fields and gardens had no detectable late blight. More precise conclusions also will be available with the application of a greater number of markers. We expect to provide these results by application of restriction fragment length polymorphism analysis to this collection. With a larger number of polymorphic markers it will be possible to identify with greater certainty the degree of geographic substructuring, and to determine with greater confidence the contribution (if any) of sexual reproduction to population diversity in the Netherlands.

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