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Phenotypic and genotypic characterisation of Northern Ireland isolates of *Phytophthora infestans*

Diane J. Carlisle[1], Louise R. Cooke[1,2] and Averil E. Brown[1]
[1]*Department of Applied Plant Science, Faculty of Science and Agriculture, The Queen's University of Belfast;*
[2]*Applied Plant Science Division, Department of Agriculture and Rural Development, Newforge Lane, Belfast BT9 5PX, UK (Fax: + 4402890668375)*

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Abstract

Genetic and phenotypic diversity of the population of the late blight pathogen *Phytophthora infestans* in Northern Ireland was assessed from 223 single-lesion isolates collected from 29 sites in 1995 and 1996. The proportion of metalaxyl-resistant isolates was approximately 14%. The A2 mating type was not detected. Allozyme analyses revealed that the Northern Ireland isolates were monomorphic and homozygous at the loci coding for allozymes of glucose-6-phosphate isomerase and peptidase (*Gpi 100/100, Pep 100/100*). The majority of isolates tested (156 out of 162) were mitochondrial DNA haplotype IIa. Among the remaining isolates, four were haplotype Ia, two were the rare IIb haplotype and none was of the old Ib type. RAPD-PCR analysis of selected isolates revealed relatively little diversity among the Northern Ireland isolates, which clustered separately from isolates from GB and the European mainland.

Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, remains the most important constraint to the economic production of potatoes in Northern Ireland. Copeland et al. (1993) estimated that, on average, 8% of the crop is lost annually to late blight, whilst blight fungicides are applied each year to a value of approximately £0.67 million (G. Little, pers. comm.). Current control strategies for late blight are usually combined into an integrated disease management system designed to reduce population size and growth rate of the pathogen, hence delaying the onset of disease and reducing its subsequent severity. Fry et al. (1993), therefore, wrote that 'to establish the most effective control strategies for late blight, we need to know much more about dispersal and genetic variation in local populations of *P. infestans*'.

Given that *P. infestans* is widely accepted as having originated and evolved in the central highlands of Mexico (Niederhauser, 1991) and is now found in all regions of the world where potatoes and tomatoes are grown, it is apparent that migration has played an essential role in the history of late blight (Fry et al., 1993). The discovery, for the first time outside Mexico, of the A2 mating type, initially in a 1981 isolate in a Swiss culture collection (Hohl and Iselin, 1984), and subsequently in other European countries and thence world-wide (reviewed by Drenth et al., 1993a), along with investigation of the genetic make-up of European populations using glucose-6-phosphate isomerase (*Gpi)* and peptidase (*Pep)* allozyme loci, led Spielman et al. (1991) to propose that a second world-wide migration of *P. infestans* occurred during the 1970s. Spielman's hypothesis was strongly supported by Goodwin and Drenth (1997) in a recent review of

all the available data. The *90* allele for *Gpi* and the *83* allele for *Pep* were only detected in Europe for the first time after detection of the A2 mating type: the changes in allozyme alleles occurred concomitant with the change in mating type structure (Spielman et al., 1991). Isolates of the A2 mating type and new allozyme genotypes were discovered in the Netherlands and East Germany as early as 1980 (Daggett et al., 1993; Drenth et al., 1994). In addition, this migration introduced new A1 mating type genotypes, many more DNA fingerprint bands (Goodwin et al., 1992; Drenth et al., 1993b; Goodwin et al., 1994; Sujkowski et al., 1994; Lebreton and Andrivon, 1998), additional mtDNA haplotypes (Drenth et al., 1993b; Day and Shattock, 1997; Lebreton et al., 1998) and additional allozyme allele combinations (Lebreton et al., 1998). Spielman et al. (1991) proposed that this 'new' population had displaced or was in the process of displacing the original or 'old' (US-1, *sensu* Goodwin et al., 1994) population in Europe, suggesting that it was 'fitter' in some way, given that it had rapidly increased in frequency after its introduction. Subsequently, it was suggested that this might have resulted from the import into the Netherlands in 1976 of potatoes from Mexico (Niederhauser, 1991).

The increased severity of blight outbreaks in 1980 coincided with the first discovery of *P. infestans* isolates resistant to the then recently-introduced and previously very effective systemic fungicide metalaxyl in the Republic of Ireland (Dowley and O'Sullivan, 1981) and the Netherlands (Davidse et al., 1981). Gisi and Cohen (1996) suggested that, in Europe, this resistance occurred either as a result of the immigration of resistant genotypes or from the simultaneous occurrence of mutations at different sites. Daggett et al. (1993) reported the occurrence of metalaxyl-resistant isolates, presumably from the old population, north of Berlin as early as 1977 and RG-57 analysis (Goodwin et al., 1992) of one of the first metalaxyl-resistant isolates found in the Republic of Ireland indicated that it had the old US-1 genotype (Goodwin et al., 1996), whereas a similar analysis of one of the first resistant Northern Ireland isolates from 1980 (Cooke, 1981) revealed that it belonged to the new population (D.J. Carlisle, unpubl.).

A secondary result of this migration was the potential for sexual reproduction in the European *P. infestans* population as this now comprised both A1 and A2 strains. A dramatic increase in genetic diversity, detected using virulence markers and RG-57 DNA fingerprinting, led Drenth et al. (1994) to conclude that sexual reproduction of *P. infestans* had taken place in the Netherlands. Genotypes at most of the genetic loci examined in a study of Polish isolates were in Hardy–Weinberg equilibrium. This, coupled with a lack of genetic differentiation between A1 and A2 isolates, a large proportion of genetically unique individuals and a new *Gpi* genotype (*90/90*) led Sujkowski et al. (1994) to conclude that sexual reproduction had probably occurred in Poland during the late 1980s and early 1990s. Both studies suggested that this recombination had occurred exclusively between new genotypes and not between new and old.

The first indication of major changes within the *P. infestans* populations of Northern Ireland occurred in the early 1980s. Metalaxyl resistance was initially detected in the Republic of Ireland in 1980 (Dowley and O'Sullivan, 1981) and in Northern Ireland in 1981 in isolates from tubers obtained from the 1980 crop (Cooke, 1981). The A2 mating type was first found in Northern Ireland in 1987 (Cooke et al., 1995) and in the Republic of Ireland in 1988 (O'Sullivan and Dowley, 1991). In 1989, the incidence of A2 was c. 3% in Northern Ireland (Cooke et al., 1995) which is in contrast to the c. 30% of A2 isolates collected in the Irish Republic in 1991 (O'Sullivan and Dowley, 1991). Self-fertile isolates have not been found within the Northern Ireland population (Cooke et al., 1995), although they were found in the Republic of Ireland in 1988 (O'Sullivan and Dowley, 1991). In contrast to the extensive characterisation of the Irish population carried out using biologically significant markers, relatively few data have been obtained using genetic markers. Studies of isolates collected in the Republic of Ireland in 1988 and 1989 showed that they belonged to the new population: the allozyme genotypes *Gpi 90/100 Pep 83/100; Gpi 90/100 Pep 100/100; Gpi 100/100 Pep 83/100* and *Gpi 100/100 Pep 100/100* were detected which are characteristic of the new population (Tooley et al., 1993). Nuclear DNA content measurements revealed the presence of putative diploid, triploid and tetraploid individuals in the population, again indicative of the new population (Tooley et al., 1993). No comparable studies have been carried out to analyse the genetic variation within the Northern Ireland population, nor to investigate how it related to those in other European countries. Hence, the purpose of this study was to analyse components of the 1995 and 1996 Northern Ireland *P. infestans* population using both biological and genetic markers.

For comparative purposes, a small number of isolates from other potato-growing regions of Europe was also included.

Materials and methods

Collection of fungal isolates

Blighted potato material was collected in the major production areas of Northern Ireland (Figure 1) during the growing seasons of 1995 and 1996 with the assistance of members of the Department of Agriculture and Rural Development Potato Inspection Service. The sample size during 1995 was much smaller than in 1996 due to the atypically hot, dry summer. In 1995, 59 isolates were obtained from only nine of the intended 20 crop sites and an additional 28 isolates were obtained from blighted daughter tubers from five of these crops. Samples were collected in Counties Antrim (three sites), Londonderry (four sites) and from one site in each of Counties Armagh and Tyrone; no samples were obtained from Co. Down, where blight was not reported in any field crop. In 1996, 146 isolates were obtained from each of the 20 selected sites in the three counties of Antrim (seven sites), Down (ten sites) and Londonderry (three sites). Isolates from the Republic of Ireland ($n = 16$) were supplied by E. O'Sullivan (Teagasc, Carlow, Ireland). Isolates from elsewhere in Europe ($n = 23$) were obtained from Biotransfer (Montreuil, France).

P. infestans isolates were obtained from small pieces of leaflet excised from the leading edges of single sporulating leaf lesions and from tuber lesions and transferred to rye agar (Caten and Jinks, 1968) amended with the antibiotics rifampicin (25 mg l^{-1}) and natamycin (25 mg l^{-1}). Following incubation at 15 °C with 12 h light for several days to encourage sporulation, culture fragments were transferred to either unamended rye agar, antibiotic rye agar or pea agar (Hollomon, 1965) amended with vancomycin (50 mg l^{-1}) and incubated in the dark at 15–18 °C for routine maintenance.

Mating type determination

Isolates were grown on unamended rye agar with reference isolates of the A1 (T38B/94) and A2 (15/94)

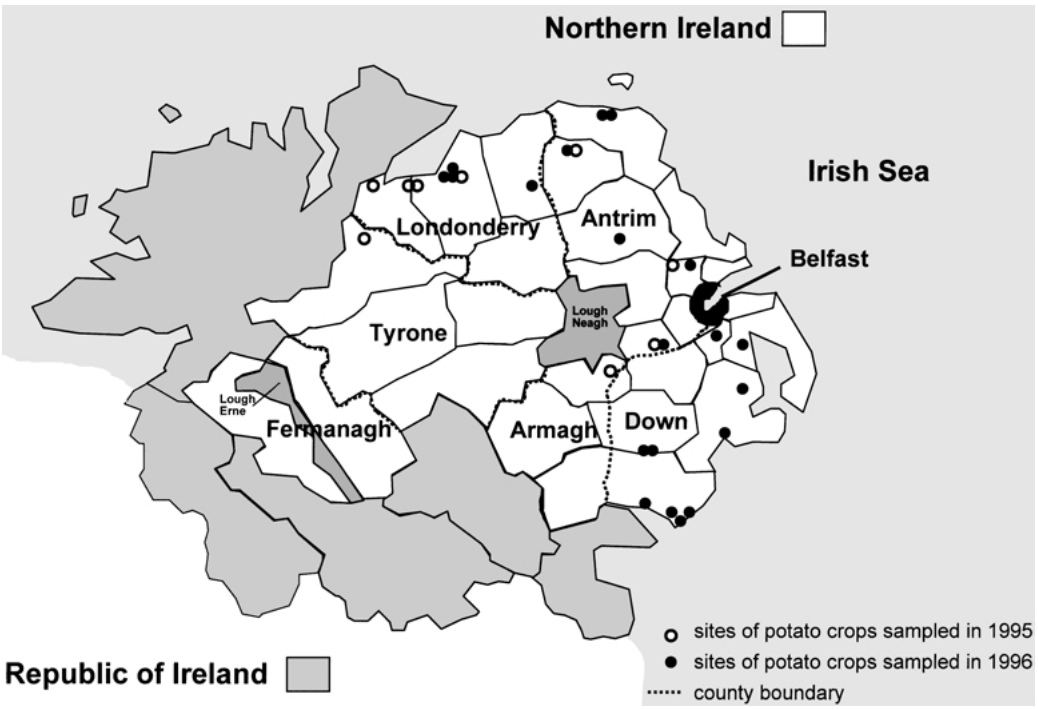

Figure 1. Map of Northern Ireland showing locations of potato crops from which samples of potato blight were collected.

mating types (Cooke et al., 1995). The dual cultures were incubated at 15 °C in darkness for 5–10 days, then examined microscopically for the presence of oospores where the two colonies interacted.

Metalaxyl sensitivity assessment

The floating leaf disc method described by Cooke (1986) was used to determine metalaxyl sensitivity. Leaf discs (10 mm diameter) of a blight-susceptible potato cultivar were floated abaxial side up in Petri dishes (six discs per dish) containing 0 (control), 2, or 100 mg of metalaxyl per ml of 0.1% aqueous acetone. The discs were inoculated (six per treatment per isolate) with sporangial/zoospore suspensions of the test isolates, incubated (15 °C, daylight) and sporulation assessed after 5 and 7 days (Cooke, 1986). Each isolate was tested on at least two separate occasions and results were discarded if the isolate failed to sporulate on at least four out of six control discs within a test. Isolates unable to sporulate on leaf discs floated on either concentration of metalaxyl solution were designated sensitive; isolates sporulating on discs floated on aqueous acetone and on 2 and 100 mg metalaxyl per litre were designated resistant and isolates sporulating on discs floated on aqueous acetone and 2 mg metalaxyl l^{-1} but not on 100 mg l^{-1} were designated intermediate.

Allozyme assays

Genotypes at two polymorphic allozyme loci, *Gpi-1* (glucose-6-phosphate isomerase, GPI, E.C. 5.3.1.9.) and *Pep-1* (peptidase, PEP, E.C. 3.4.3.1), were determined using the protocols of Goodwin et al. (1995). Chilled supernatants containing protein released from mycelial fragments in sterile distilled water were loaded onto cellulose acetate plates equilibrated in the appropriate buffer: Tris-glycine (TG) buffer (25 mM Tris–HCl, 192 mM glycine, pH 8.5) was initially used for both GPI and PEP. Tris-borate-EDTA (TBE) buffer (130 mM Tris, 2.2 mM EDTA, 6 mM sodium hydroxide, 713 mM boric acid, pH 8.9) (Spielman et al., 1990; Richardson et al., 1986) was used as necessary for improved resolution of the *Pep 83*, *92* and *100* alleles (Carlisle, 2000). Enzymatic activities were revealed following electrophoresis and staining with the appropriate agar overlays (Goodwin et al., 1995). The genotypes of unknown isolates were determined by comparing their banding patterns with those of reference isolates kindly provided by R. Corbière (INRA, Le Rheu, France; isolate 50.96, *Gpi 90/100 Pep 83/100*) and K.L. Deahl (USDA, Beltsville, MD, USA; US-1 isolate WV-63, *Gpi 86/100 Pep 92/100*; US-6 isolate CAL 7-5 *Gpi 100/100 Pep 92/100*; US-7 isolate KKK-W4B, *Gpi 100/111 Pep 100/100* and US-8 isolate NY-01, *Gpi 100/111/122 Pep 100/100*).

Identification of mitochondrial DNA haplotypes

Mitochondrial DNA (mtDNA) haplotypes of isolates were determined by PCR-RFLP using a modification of the method of Griffith and Shaw (1998). DNA was extracted from one-week-old fungal mycelium grown on antibiotic rye agar using the method of Raeder and Broda (1985) but with an additional chloroform extraction to ensure removal of any residual phenol. DNA quality and concentration was estimated by comparison with a known quantity of phage λ DNA (Promega, Wisconsin, USA) on 1% agarose gels in 1× TBE buffer stained with ethidium bromide (0.5 μg ml^{-1}) on a UV transilluminator (254 nm). DNA was amplified with two pairs of oligonucleotide primers (F2/R2 and F4/R4), synthesised by GibcoBRL® Life Technologies (Paisley, Scotland) according to the sequences given by Griffith and Shaw (1998). Amplification reactions were performed in a Perkin Elmer DNA Thermocycler 480 (Perkin-Elmer, Connecticut, USA). Each reaction mixture comprised 200 μM of each dNTP, 2.75 mM $MgCl_2$, oligonucleotide primers (0.325 μM each), 1 × buffer (20 mM Tris–HCl (pH 8.4), 50 mM KCl), 160 μg ml^{-1} BSA, 1 U *Taq* DNA polymerase (GibcoBRL® Life Technologies, Paisley, Scotland) and 10 ng genomic DNA; volumes were made up using milliQ™ water and mixtures overlaid with sterile mineral oil. The amplification programme consisted of one denaturation cycle of 94 °C for 90 s followed by 35 cycles of 94 °C for 40 s; 60 °C (primers F2/R2) or 55 °C (primers F4/R4) for 60 s and 72 °C for 90 s. A 10 μl aliquot of each amplified DNA was digested with 1 U of *Msp*I (for primer pair F2/R2 products) or 1 U *Eco*RI (for primer pair F4/R4 products) in a 30 μl final volume at 37 °C in a water bath for 60 min. Restriction patterns were revealed after electrophoresis of the digested DNA through a 2.5% agarose gel in a 1× TBE buffer stained with ethidium bromide (0.5 μg ml^{-1}) and visualised by UV transillumination (254 nm). A 100 bp DNA ladder (Promega, Wisconsin, USA) was used as a size marker. DNA haplotype of isolates was

assigned by determining the molecular weight of the individual restriction fragments of each PCR-RFLP profile (by comparison with the molecular size markers) and referring to the published literature (Griffith and Shaw, 1998).

RAPD-PCR analysis

Thirty-one *P. infestans* isolates were selected for further characterisation using RAPD-PCR analysis. Twenty of these isolates were from Northern Ireland (one isolate from each of the twenty sites sampled in 1996), five were from the Republic of Ireland and six from elsewhere in Europe (Table 2). The latter 11 isolates were chosen randomly from the selection of European isolates available in the laboratory. RAPD-PCR analysis of isolates was performed using a modified version of the method described by Maufrand et al. (1995). Genomic DNA (5 μg ml^{-1}) was amplified initially using 20 10-mer primers obtained from GibcoBRL® Life Technologies. Among these, the primers A03 (AGTCAGCCAC), A04 (AATCGGGCTG), A13 (CAGCACCCAC), B7 (GGTGACGCAG) and F1 (ACGGATCCTG) were chosen because they reliably and reproducibly detected polymorphisms among the selected isolates (Table 2). Amplification reactions were performed in 25 μl volumes using a GeneAmp PCR System 2400 (Perkin Elmer Corporation, California, USA). Reaction mixtures contained 100 μM of each dNTP, 2.5 mM $MgCl_2$, oligonucleotide primer (5 pM), 1 × buffer (20 mM Tris–HCl (pH 8.4), 50 mM KCl), 160 μg ml^{-1} BSA, 0.75 U *Taq* DNA polymerase (1 U was used with primer A13) (GibcoBRL™ Life Technologies, Paisley, Scotland), 0.5% Tween 20 and 1 ng genomic DNA; volumes were made up using milliQ™ water in 0.2 ml thin-walled amplification tubes (Axygen Incorporated, California, USA). A 5 μl aliquot of milliQ™ water was used instead of target DNA as the negative control. The amplification programme comprised a denaturation cycle of 94 °C for 30 s, three further denaturation cycles of 94 °C for 1 min; annealing at 35 °C for 1 min and extension at 72 °C for 2 min followed by 32 cycles of 94 °C for 15 s; 35 °C for 30 s, 72 °C for 1 min and a final extension step of 72 °C for 4 min. Reaction products were revealed after electrophoresis through 2% agarose gels in 1× TBE buffer and subsequent UV transillumination at 254 nm. pGem® DNA (Promega, Wisconsin, USA) was used as the molecular size marker. The image was captured for phylogenetic analysis using Grab-IT Version 2.5 (UVP Ltd., Cambridge, England). For analysis of the data, individual fragments were considered as separate characters and scored as present or absent in the Phoretix 1D Advance image analysis programme (Phoretix International Ltd., Newcastle-upon-Tyne, England). The data were combined using Excel Version 5.0 (Microsoft Corporation, Reading, England) and analysed using PAUP Version 4 (Swofford, 1998). Mean character difference measures were computed, Unweighted Pair Group Method with Arithmetic Average (UPGMA) analysis was performed and a UPGMA dendrogram constructed. Bootstrapping analysis (500 replicates employing the 50% majority rule) of the data was performed again using PAUP Version 4 (Swofford, 1998).

Results

Mating type and metalaxyl resistance

All of the *P. infestans* isolates from Northern Ireland and most of those acquired from elsewhere in Europe were of the A1 mating type. Only one of eight isolates from the Republic of Ireland and two from England were of the A2 mating type (Table 1). Most of the Northern Ireland isolates tested were sensitive to metalaxyl (Table 1); c. 19% were resistant in 1995 and c. 11% were resistant in 1996. The small number of isolates from GB and mainland Europe showed either full or intermediate sensitivity to this fungicide, with the exception of two isolates, one each from England and Wales, which were resistant (Table 1).

Allozyme genotype and mtDNA haplotype

All isolates tested were homozygous (*100/100*) at the *Gpi* locus (Table 1). At the *Pep* locus, the isolates from Northern Ireland, the Republic of Ireland, The Netherlands and Germany were homozygous (*100/100*), whereas the *Pep 83/100* genotype was found among isolates from GB, France and Belgium (Table 1).

Amplification of total DNA using the P2 and P4 primer pairs and subsequent digestion of the products with *Msp*I and *Eco*RI, respectively, revealed four haplotypes among the isolates analysed. All of the Northern Ireland isolates analysed from 1995 and 122 of the 128 isolates analysed from 1996 were mtDNA haplotype IIa; four isolates from 1996 were haplotype

Table 1. Mating type, metalaxyl resistance, allozyme genotype and mt DNA haplotype of *Phytophthora infestans* isolates collected in Northern Ireland and the Republic of Ireland in 1995 and 1996 and isolates from elsewhere in Europe collected between 1996 and 1998

Character		Number of isolates tested										
		Northern Ireland[1]		Republic of Ireland[2]		England[3]	Scotland	Wales	France	Belgium	Netherlands	Germany
		1995	1996	1995	1996	1996–98	1997	1996	1996–97	1996	1996–97	1996–97
Mating type	A1	77	146	8	7	3	2	1	5	1	5	4
	A2	0	0	0	1	2	0	0	0	0	0	0
Metalaxyl sensitivity	Sensitive	62	130	5	6	3	1	0	4	0	4[4]	3
	Resistant	15	16	3	2	1	0	1	0	0	0	0
	Intermediate	0	0	0	0	1	1	0	1	1	0	1
Allozyme genotype	*Gpi 100/100 Pep83/100*	0	0	0	0	3	2	1	2	1	0	0
	Gpi 100/100 Pep100/100	77	146	8	8	3	0	0	3	0	5	4
Mt DNA haplotype	Ia	0	4	5	3	6	2	2	4	1	3	4
	Ib	0	0	0	0	0	0	0	0	0	1	0
	IIa	34	122	3	5	0	0	0	1	0	1	0
	IIb	0	2	0	0	0	0	0	0	0	0	0

[1]Single lesion isolates
[2]Mating type and metalaxyl sensitivity were predetermined by Dr E. O'Sullivan (Teagasc, Carlow, Ireland)
[3]Includes three tomato isolates
[4]One Dutch isolate was not metalaxyl tested as it was no longer pathogenic on potato leaves.

Ia and two were haplotype IIb (Table 1). Haplotype IIb was not found among the small number of isolates from the Republic of Ireland or elsewhere in Europe. Approximately equal numbers of isolates from the Republic of Ireland were haplotype Ia and IIa. Most of the isolates from GB and elsewhere in Europe were haplotype Ia. One isolate of the old Ib haplotype was found among the five isolates from the Netherlands (Table 1).

RAPD-PCR analysis

From the 31 *P. infestans* isolates characterised, a total of 99 distinct amplification products, of which 88 were polymorphic, were consistently produced, using the five 10-base primers. The UPGMA dendrogram constructed from the data (Figure 2) divided the isolates into three main clusters. Cluster 1 comprised all the Northern Ireland isolates and three isolates from the Republic of Ireland. Of these isolates, only three from Co. Down (Cluster 1A) showed significant difference from the others (Cluster 1B). Cluster 2 comprised the isolates from Great Britain and mainland Europe. Within Cluster 2, the two isolates from France and Germany (Cluster 2B) were significantly different from the isolates from Great Britain and the Netherlands (Cluster 2A). The two remaining isolates from the Republic of Ireland formed Cluster 3.

Discussion

Isolates of the *P. infestans* A2 mating type were first discovered in Northern Ireland in 1987 and were found annually until 1992 with an overall frequency of *c.* 3% (Cooke et al., 1995). This is similar to the frequencies reported in the late 1980s in England and Wales (Shattock et al., 1990) and the Netherlands (Therrien et al., 1989). However, by 1995, only one A2 isolate was detected within Northern Ireland and none has been found since (Cooke et al., 2000). The present study confirmed this apparent decline in the occurrence of the A2 mating type in Northern Ireland. The frequency of the A2 mating type similarly declined in the Republic of Ireland from 9% to 10% of the population in 1993–1994 (O'Sullivan et al., 1995) to none being detected after 1997 (E. O'Sullivan, pers. comm.). This situation is quite different from that in some other parts of Europe.

Table 2. Details of *Phytophthora infestans* isolates used in RAPD-PCR analysis

Isolate code	Country or County	Mating type	R/S (metalaxyl)	*Gpi* genotype	*Pep* genotype	mt DNA haplotype	RAPD-PCR cluster
	Northern Ireland						
1/F/96	Down	A1	S	*100/100*	*100/100*	IIa	1A
2/F/96	Down	A1	S	*100/100*	*100/100*	IIa	1B
3/B/96	Down	A1	S	*100/100*	*100/100*	IIa	1B
4/F/96	Down	A1	S	*100/100*	*100/100*	IIa	1B
5/F/96	Down	A1	S	*100/100*	*100/100*	IIa	1A
6/D/96	Antrim	A1	S	*100/100*	*100/100*	IIa	1A
7/F/96	Antrim	A1	S	*100/100*	*100/100*	IIa	1A
8/B/96	Londonderry	A1	S	*100/100*	*100/100*	IIa	1A
9/B/96	Antrim	A1	S	*100/100*	*100/100*	IIa	1A
10/C/96	Antrim	A1	R	*100/100*	*100/100*	IIb	1A
11/D/96	Antrim	A1	S	*100/100*	*100/100*	IIa	1A
12/E/96	Antrim	A1	S	*100/100*	*100/100*	IIa	1A
13/D/96	Londonderry	A1	S	*100/100*	*100/100*	IIa	1A
14/D/96	Londonderry	A1	S	*100/100*	*100/100*	IIa	1A
15/C/96	Londonderry	A1	R	*100/100*	*100/100*	IIa	1A
16/F/96	Down	A1	R	*100/100*	*100/100*	IIb	1A
17/B/96	Down	A1	S	*100/100*	*100/100*	IIa	1A
18/A/96	Down	A1	S	*100/100*	*100/100*	IIa	1A
19/C/96	Down	A1	S	*100/100*	*100/100*	IIa	1A
20/B/96	Down	A1	S	*100/100*	*100/100*	IIa	1A
	Republic of Ireland						
96/11/1	Wexford	A1	R	*100/100*	*100/100*	Ia	1A
96/26/4	Dublin	A1	S	*100/100*	*100/100*	IIa	1A
96/47/1	Kilkenny	A1	S	*100/100*	*100/100*	IIa	3
96/50/4	Kildare	A1	S	*100/100*	*100/100*	IIa	1A
96/54/1	Carlow	A2	S	*100/100*	*100/100*	Ia	3
	Other						
97-UKA	UK-England	A1	R	*100/100*	*100/100*	Ia	2A
97-UKC	UK-Scotland	A1	S	*100/100*	*83/100*	Ia	2A
96-UKA	UK-Wales	A1	R	*100/100*	*83/100*	Ia	2A
96-GA	Germany	A1	I,S	*100/100*	*100/100*	Ia	2B
96-NLA	Netherlands	A1	NT*	*100/100*	*100/100*	Ia	2A
97-FB	France	A1	I	*100/100*	*83/100*	Ia	2B

*No longer pathogenic on potato leaves.

In the Netherlands and Poland, the occurrence of large proportions of the A2 mating type and an increase in the levels of genetic diversity exhibited by isolates provided circumstantial evidence of sexual reproduction (Drenth et al., 1994; Sujkowski et al., 1994). Recently, the finding of oospores produced under field conditions has provided more direct evidence for sexual reproduction of the pathogen in Germany (Schöber-Butin, 1999), the Netherlands (Flier and Turkensteen, 2000; Zwankhuizen et al., 2000), Poland (Zarzycka and Sobkowiak, 1999) and Sweden (Andersson et al., 1998). Both mating types have been found on the same infected plants in Finland (Hannukkala, 1999), and in Sweden there is evidence of oospores acting as an initial inoculum source (Andersson et al., 1998). In contrast, the overall lack of diversity for mating type within the Irish *P. infestans* population implies that sexual recombination takes place rarely if at all in both Northern Ireland and the Republic of Ireland (Griffin et al., 1998).

A relatively low incidence of metalaxyl resistance was found among the Northern Ireland isolates examined in this study (c. 14% of isolates contained metalaxyl-resistant strains). In Northern Ireland, formulations containing phenylamide fungicides (mainly metalaxyl and oxadixyl) were applied to 30–40% of crops at least once per season during the period of

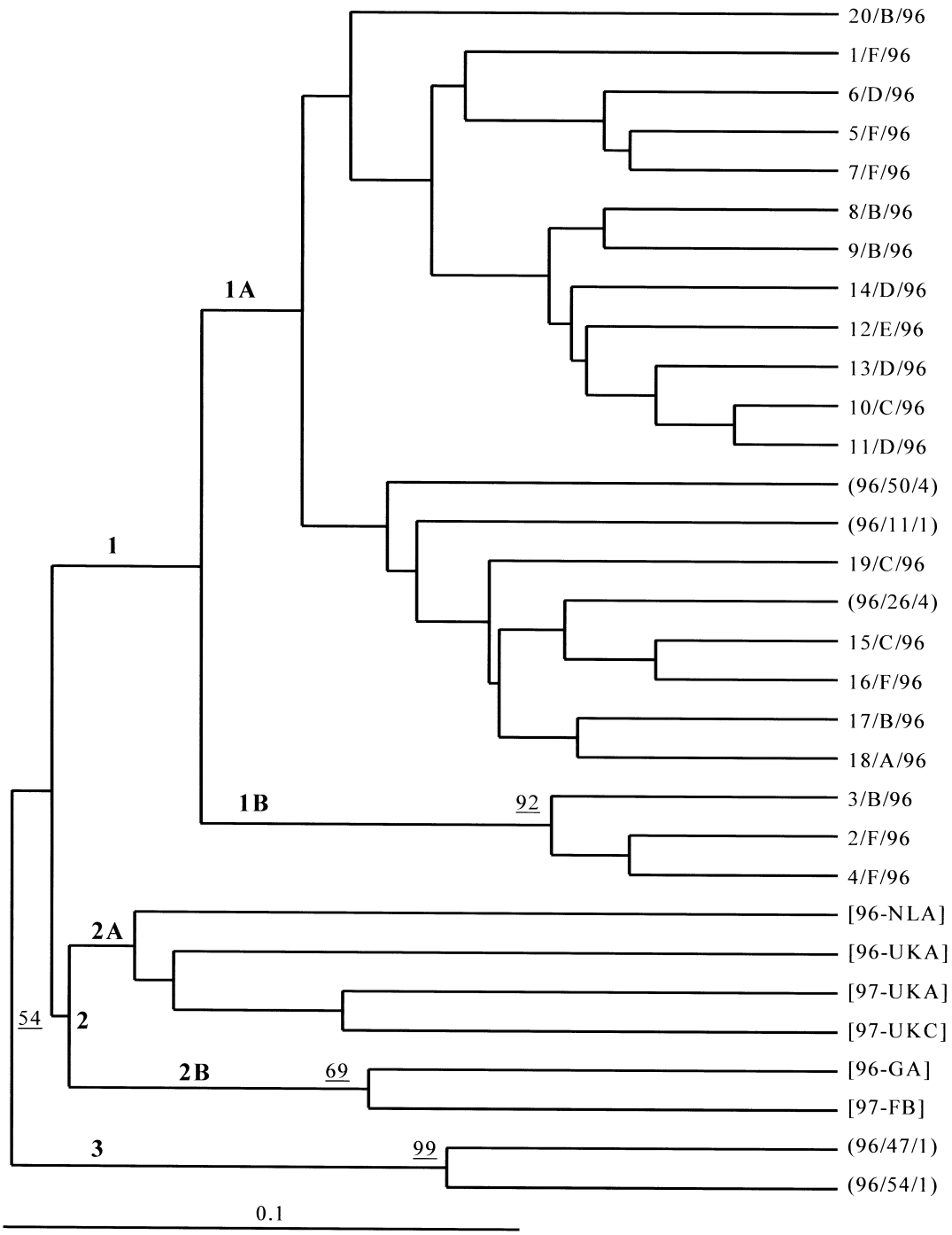

Figure 2. UPGMA dendrogram of RAPD-PCR data from *Phytophthora infestans* isolates. Genetic distance values were calculated using the Sneath and Sokal coefficient and the RAPDistance statistical package. Clusters are arbitrarily numbered (bold typeface). Bootstrap values (underlined) are shown where these are 50% or greater. Isolate designations in round brackets () indicate isolates from the Republic of Ireland, those in square brackets [] are from Great Britain or mainland Europe; all other isolates are from Northern Ireland.

the study (Cooke et al., 2000). However, the majority of growers adhered to the anti-resistance strategy endorsed by the Department of Agriculture, using no more than three sprays of this type of fungicide applied only during the early part of the season, and switching to other products for the remainder of the spray programme, and this has proved effective in preventing the build-up of metalaxyl-resistant strains (Cooke et al., 2000). In the Republic of Ireland, where an anti-resistance strategy was also adopted, this has also limited the occurrence of metalaxyl-resistant strains (Dowley, 2000).

Spielman et al. (1991) designated *Gpi 100/100*, *Pep 83/100* and *Pep 100/100* as new European genotypes, hence the results from the present study show that Northern Ireland *P. infestans* isolates, in common with those from elsewhere in Europe, belong to the new population. None of the old dilocus genotypes, *Gpi 86/100 Pep 92/100*, *Gpi 86/100 Pep 100/100* and *Gpi 100/100 Pep 92/−−* (Spielman et al., 1991), was found amongst these isolates. All the European isolates appeared to be monomorphic at the *Gpi* locus under the conditions employed. However, CAE using TG buffer (pH 7.8) (Goodwin et al., 1995) appears unable to resolve the cryptic *Gpi 90* allele (Carlisle, 2000; Erselius et al., 2000) found in European and South American isolates of the *Gpi 90/100* genotype. This genotype was first reported in isolates from the Netherlands in the early 1980s (Spielman et al., 1991) and was considered as being uniquely Mexican and European (Spielman, 1991). Tooley et al. (1993), using starch gel electrophoresis, revealed that this genotype was present in 17 out of 30 isolates collected in 1989 in the Republic of Ireland, the remainder being homozygous (*Gpi 100/100*). This genotype might thus still be present within the Irish *P. infestans* populations.

The *Pep 83* and *100* alleles were found among the isolates tested in the present study but all of the Irish isolates were homozygous (*Pep 100/100*). In 1989, Tooley et al. (1993) also found that the majority of Irish isolates studied were *Pep 100/100* with a small number then being *Pep 83/100*. The study by Shattock et al. (1990) identified only the *92/100* genotype among the GB isolates, although it is possible that, given the experimental conditions employed, some or all were actually *Pep 83/100* (Fry et al., 1991). The *Pep 83/100* genotype has been reported in East Germany (Daggett et al., 1993) and, more recently, from France where it was the most common *Pep* genotype among potato isolates (Lebreton and Andrivon, 1998).

The three mtDNA haplotypes, Ia, IIa and IIb, encountered among the Northern Ireland *P. infestans* isolates are all characteristic of the new population (Griffith and Shaw, 1998). It is of interest that a small proportion of the Northern Ireland isolates were of the relatively rare IIb haplotype (Griffith and Shaw, 1998), since this haplotype has not been detected recently in GB or France (Day and Shattock, 1997; Lebreton and Andrivon, 1998; Lebreton et al., 1998). Indeed, it appears that the IIb haplotype has previously been reported only from north-west Mexico, the United States and Canada (Gavino, 1999; Griffith and Shaw, 1998), although it has recently been detected in the Netherlands (W.G. Flier, pers. comm.). The Northern Ireland isolates collected from the limited number of blighted potato crops in 1995 were all haplotype IIa. The more detailed sampling carried out in 1996 revealed mixtures of the three different haplotypes at three different sites, including one at which a mixture of metalaxyl sensitivities had been found, although there was no association between these markers. At each of these three sites, at least two different potato cultivars were growing in close proximity. This would suggest that multiple infections had occurred within these crops. Day and Shattock (1997) also reported finding mixtures of haplotypes within fields. The mtDNA haplotypes of the isolates from the Republic of Ireland (IIa and Ia) were similarly indicative of the new population.

The predominance of mtDNA haplotype IIa in Northern Ireland appears unique when compared with previously published studies of isolates from other European countries. Drenth et al. (1993b) reported that only ten out of 153 Dutch isolates from 1989 studied were type B (II). Day and Shattock (1997) found that the largest proportion of English and Welsh isolates collected in the years 1993–1995 were haplotype Ia; haplotype IIa did not exceed 8% in these years. Studies of French *P. infestans* populations also indicated that haplotype Ia predominated in the French population (Lebreton and Andrivon, 1998; Lebreton et al., 1998). One Dutch isolate, obtained in 1997 and characterised in the present study, was found to possess the old Ib haplotype but had an allozyme genotype *Gpi 100/100 Pep 100/100,* representative of the new population. Similarly, Shattock and Day (1996) reported a 1995 tomato isolate from England with a Ib haplotype but a new nuclear DNA fingerprint; they considered this to be a 'relict' and suggested that it provided evidence that old and new isolates had recombined in the field.

The dominance of the IIa haplotype in Northern Ireland is difficult to explain. It may simply be a founder effect, or may perhaps have occurred as a result of other factors. Day and Shattock (1997) reported that isolates of haplotype Ia were 'fitter' than those of IIa, in terms of their relative ability to produce lesions and the degree of sporulation on foliage under optimum conditions. However, this may not necessarily indicate an advantage for long-term survival under field conditions. Cox and Large (1960) postulated that the rate of foliar destruction was the most important factor in tuber infection; there were more opportunities for this during 'slower' epidemics, presumably such as those caused by the less aggressive isolates. The ability of a *P. infestans* isolate to initiate tuber infection and overwinter is one of the most important factors in its long-term parasitic fitness (Shattock, 1977). Additionally, Flier et al. (1998) found that components of foliar aggression were not associated with the rate or success of tuber invasion. Possibly the climatic conditions within Northern Ireland, which are often optimal for rapid foliar infection, mitigate in favour of tuber infection by this haplotype and hence its long-term survival.

There are few published reports of the use of RAPD-PCR analysis of *P. infestans*. Maufrand et al. (1995) used RAPD-PCR analysis to fingerprint individual *P. infestans* isolates, with Punja et al. (1998) confirming that RAPD primers could be used to distinguish both US and novel genotypes of the pathogen. On the basis of these studies, the potential of RAPD-PCR to reveal diversity among isolates of the Northern Ireland *P. infestans* population was investigated. Recently, Mahuku et al. (2000) reported RAPD analysis of 141 Canadian isolates of *P. infestans*; using six primers, 84 reproducible bands were produced, of which 77 were polymorphic. This is a similar level of polymorphism to that found in the present study where five primers produced 99 reproducible amplification products of which 88 were polymorphic. However, Mahuku et al. (2000) observed no correlation between RAPD groups and geographical origin of Canadian isolates, whereas, in the present study, isolates from Northern Ireland and the Republic of Ireland clustered distinctly from the isolates from both GB and mainland Europe and bootstrap values supported the hypothesis that isolates from Great Britain and mainland Europe were genetically different from the Irish isolates. These clustering patterns may have occurred simply as a result of founder effects within the *P. infestans* metapopulation (Fry et al., 1992) in Europe. However, these clustering patterns could also imply that there has been little movement of the pathogen between GB and Ireland, or indeed, between mainland Europe and Ireland in recent years. Infected potato tubers are the most common means for long-distance transport of *P. infestans* (Fry and Goodwin, 1997; Peters et al., 1999) and because only high-grade seed tubers from elsewhere in the EU may be imported into Northern Ireland, this may have limited the transmission of *P. infestans.* However, given that even high-grade seed tubers may carry low levels of *P. infestans* inoculum and ware potatoes are also imported into Northern Ireland, the introduction of other European or even recombinant genotypes cannot be excluded.

The structure of the Northern Ireland population elucidated, as defined by the isolates employed in the present study, probably consists of closely-related clonal lineages. Clonality produces a clear pattern in populations; for a series of loci known to be polymorphic, repeated recovery of the same multilocus genotype, especially over long distances or periods of time, can be taken as a strong indicator of clonal reproduction (Anderson and Kohn, 1995), i.e. that all isolates with the same multi-locus genotype have arisen through asexual propagation from a common origin. The existing RAPD-PCR data suggest that some divergence in the pathogen has occurred asexually within Ireland. A similar level of genetic diversity was detected by Mahuku et al. (2000) in their RAPD analysis of Canadian isolates of *P. infestans* and they suggested that this might be due to the occurrence of single mutations or to the sensitivity of the RAPD assay detecting minor genetic variations within existing clonal lineages as defined by mating type, metalaxyl sensitivity and molecular markers. The actual extent of the diversity within the Northern Ireland *P. infestans* population could be further assessed by continuing the RAPD-PCR analyses or by employing RG-57 or AFLP analyses.

RG-57 analysis has been conducted on a limited number (1995, $n = 5$; 1996, $n = 5$) of the Northern Ireland isolates from this study by D. Griffin, University College, Dublin. All of these isolates were pre-characterised by the present authors as being A1, *Gpi 100/100 Pep 100/100* and mtDNA haplotype IIa. Three different RG-57 genotypes were identified, the commonest of which (100 010 001 100 110 100 011 001 1; $n = 6$) was also the most frequent within isolates from the Republic of Ireland (D. Griffin, pers. comm.).

Isolates with this fingerprint appear to be widespread within *P. infestans* isolates from Great Britain (D.S. Shaw pers. comm.) and this pattern has recently been reported as being the most frequent in Norway (Brurberg et al., 1999). Although the use of RG-57 fingerprinting has effectively distinguished clonal lineages in N. American *P. infestans* populations (e.g. Goodwin et al., 1998), the assumption that isolates with the same RG-57 fingerprint belong to a single clonal lineage was questioned by Purvis et al. (1998), who found different AFLP patterns among UK isolates with the same RG-57 fingerprint. Given that the precision of inference of population genetic structure is closely associated with the availability of precise and abundant markers (Fry et al., 1992), an integrated approach using different markers provides the most suitable means of characterising populations since some markers detect higher levels of variation than others.

The selection on clones with varying fitness reduces genotypic diversity in a population because all loci are effectively linked in clones; this also results in loss of allelic diversity for the entire genome (Milgroom, 1996). The loss of the A2 mating type, and hence homogenisation of the population, may have been accelerated by the effect of 'hitch-hiking' selection (Kojima and Schaffer, 1967) on some property or allele closely associated within isolates of the A1 mating type, resulting in the predominance of isolates which are A1, *Gpi 100/100 Pep 100/100* and mtDNA haplotype IIa. A similarly close association was previously found between the mtDNA haplotype IIa and the *Gpi 100/100 Pep 100/100* genotype (Lebreton and Andrivon, 1998). However, in contrast to the Northern Ireland population, this genotype occurs only rarely on potatoes in France, where the population shares many features of those reported from areas such as the Netherlands (Fry et al., 1991; Drenth et al., 1993b; 1994). The difference between the Northern Ireland population and those of other west European countries is probably reflective of its relative geographical isolation from the European mainland and its status as a high grade seed potato-producing region, which prohibits the importation of seed potatoes except from other such designated regions.

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