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Phenotypic and genotypic diversity in *Phytophthora infestans* on potato in Great Britain, 1995–98

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A total of 2691 single-lesion isolates of *Phytophthora infestans* was established from samples of late-blight disease from 354 commercial and garden/allotment sites in Scotland, England and Wales over four growing seasons, 1995–98. The A2 mating type was rare (3·0% of isolates) and was detected at only 34 sites. *In vitro* tests of sensitivity to the phenylamide fungicide metalaxyl showed that 316 sites yielded isolates with some insensitivity (resistant and/or intermediate); these were more often commercial sites than garden/allotment sites. Over the four seasons, the frequency of isolates with intermediate fungicide sensitivity increased, while the frequency of resistant phenotypes decreased. Resistant isolates were always of A1 mating type. A subset of 1459 isolates from 326 sites was analysed for molecular diversity. Mitochondrial DNA (mtDNA) haplotype Ia predominated (91·0% of isolates); haplotype IIa was present at 54 sites and both haplotypes at 33 sites. The multilocus RFLP probe RG57 detected 30 fingerprints. Four fingerprints were particularly common (RF002, RF006, RF039 and RF040) and 10 were unique to a single site in a single year. The three commonest fingerprints (RF039 > RF002 > RF006) were of A1 mating type and the fourth (RF040) was A2. RF002 isolates were resistant to the phenylamide metalaxyl and were more common in Scotland than in England and Wales. Small sample sizes limited the usefulness of estimates of diversity. Although approximately half of all sites appeared to be colonized by RF039 genotypes, some sites (both commercial and garden/allotment) showed a higher diversity, having both common and unique genotypes. The genotypic diversity within isolates collected from commercial sites and those from garden/allotment sites were similar. The contributions of sexual reproduction and alternatives to sex to the generation of variation are discussed.

Keywords: mating type, metalaxyl, mtDNA, oospores, potato late-blight disease, RG57 fingerprinting

Introduction

Analyses of populations of *Phytophthora infestans* from many countries throughout the world have detected increases in diversity following recent migrations of the pathogen from Mexico, its apparent centre of diversity (e.g. Goodwin *et al*., 1994; Shaw & Wattier, 2003). In Europe, an essentially monomorphic population of A1 mating type in the 1970s has been replaced by variable, new populations having the potential for sexual reproduction between A1 and A2 mating types (Fry *et al*., 1993). Low diversity was detected in some populations such as those on seed-tuber potato crops in north-west France (Lebreton & Andrivon, 1998), whereas higher diversity has been reported from Poland and the Netherlands (Sujkowski *et al*., 1994; Zwankhuizen *et al*., 2000). In previous surveys of *P. infestans* from England and Wales up to 1995, both mating types, a new homozygous allozyme variant with alleles 100/100 for glucose phosphate isomerase and new mitochondrial (mt) DNA haplotypes (Ia and IIa) were detected from 1981 onwards, providing evidence for the replacement of the old population with a new one (Shattock *et al*., 1990; Day & Shattock, 1997). Here, an analysis of phenotypic variation (mating type and sensitivity to the fungicide metalaxyl) and molecular variation (mtDNA haplotype and RG57 fingerprint) in *P. infestans* from a large number of commercial crops of potato and from crops grown in gardens and allotments, collected between 1995 and 1998, is presented to explore the extent of variation and to learn more about the recent evolution of the pathogen throughout Great Britain

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(England, Scotland and Wales). Preliminary reports of this work have been published (Day *et al*., 2001; Shattock, 2002).

Materials and methods

Sampling

Samples of diseased potato were collected from commercial fields and from gardens/allotments in 1995, 1996, 1997 and 1998 (Fig. 1), of which the latter two years, and especially 1997, were acknowledged in the industry as being 'blight years'. Each field or garden or allotment is referred to as a 'site'. Samples of diseased leaves, stems or occasionally tubers, collected by agronomists and growers, both commercial and amateur, as well as by academic colleagues, were sent to Bangor for *in vitro* isolation of the pathogen. The geographical distribution of sites varied greatly from year to year and not all areas were sampled (Fig. 1). Most sites were in England and Wales, particularly

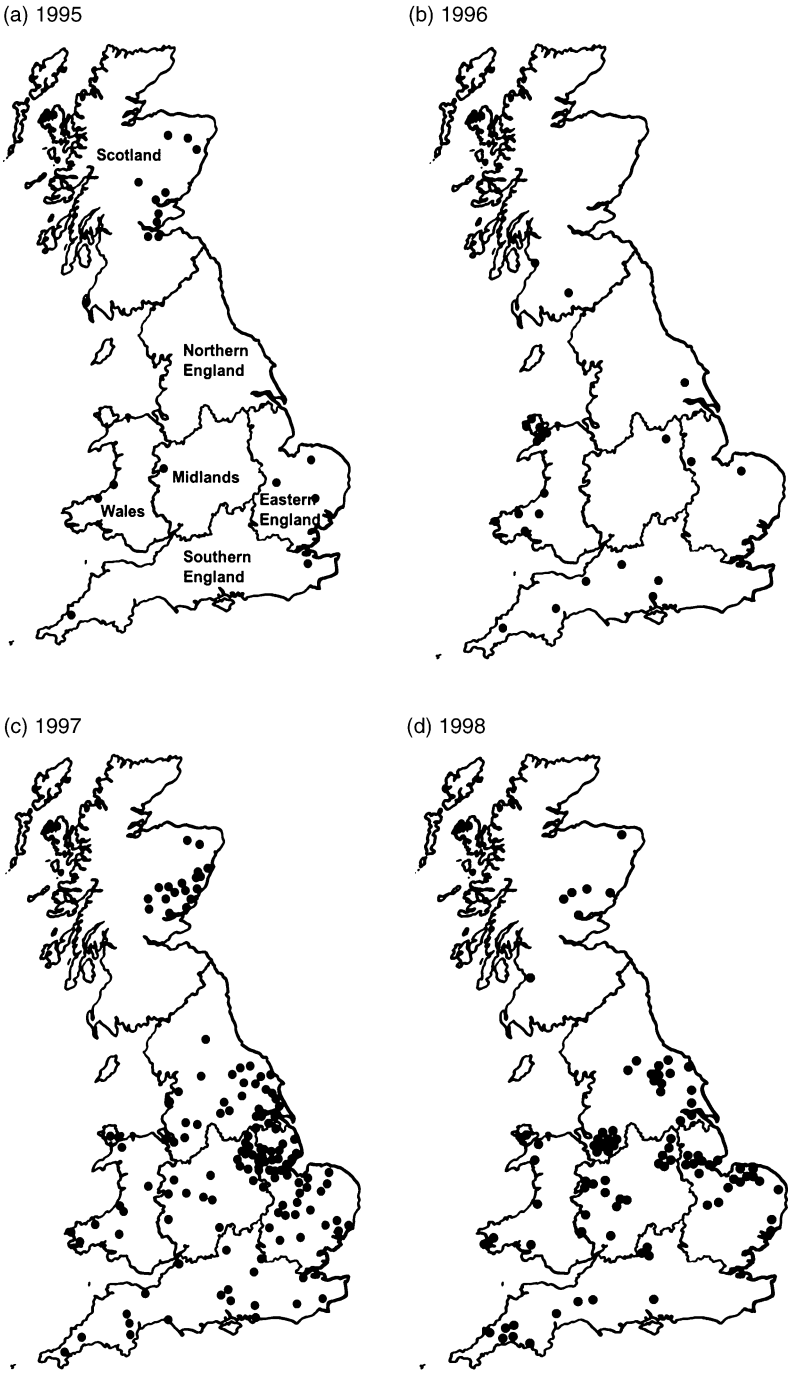

Figure 1 Sites of origin of isolates of *Phytophthora infestans* from potato (a–d, 1995–98, respectively).

Table 1 Percentages of sites (actual numbers in parentheses) where A1 only, A2 only or mixtures of both A1 and A2 mating types were detected in isolates of *Phytophthora infestans* from potato in commercial or trial crops (C) or garden/allotment sites (GA), 1995–98

Site type	Mating type	1995[a]	1996	1997	1998	Total (1995–98)
C	A1 only	92 (22)	100 (17)	93 (145)	86 (81)	91 (265)
	A2 only	4 (1)	0 (0)	3 (5)	2 (2)	3 (8)
	A1 and A2	4 (1)	0 (0)	4 (6)	12 (11)	6 (18)
GA	A1 only	50 (4)	85 (11)	97 (34)	86 (6)	87 (55)
	A2 only	12 (1)	0 (0)	0 (0)	0 (0)	2 (1)
	A1 and A2	38 (3)	15 (2)	3 (1)	14 (1)	11 (7)
Total (no.)		(32)	(30)	(191)	(101)	(354)

[a]Contains data previously published by Day & Shattock (1997) with additional data for the Scottish isolates provided by SCRI (Cooke *et al.*, 2003a).

the main commercial potato-growing areas in eastern England. The mean number of isolates characterized per site over the 4 years 1995–98 was 4·4 (variance 15·5). Single-isolate samples were obtained from 74 sites (22·8%), two isolates from 51 sites (15·7%), and three isolates from 33 sites (10·2%). Twenty-seven cultures from England and Wales, isolated in 1978, 1981, 1982 and 1985 and stored in liquid nitrogen, were revived and characterized for comparative purposes.

Isolation and characterization

Single-lesion isolates were obtained by transferring fragments of diseased tissue from the edge of single lesions on rye A agar containing antibiotics (Day & Shattock, 1997). The mating type of isolates was determined (Day & Shattock, 1997); isolates which formed abundant oospores in single culture were classified as self-fertile. There is evidence that such self-fertile cultures were mixtures of two distinct clones, one of A1 and the other of A2 mating type (Fyfe & Shaw, 1992; Pipe *et al.*, 2000). In this study self-fertility was considered to indicate the presence of strains of both mating type at a site (Table 1) but molecular fingerprints (RG57 genotypes) of self-fertile isolates were not characterized. *In vitro* sensitivity to metalaxyl was also determined by assessing growth on 10 μg mL^{-1} metalaxyl: isolates with ≥ 60% of control growth were designated resistant (R); those with ≥ 5% and < 60% of control growth as intermediate (I); and those with < 5% of control growth as sensitive (S) (Day & Shattock, 1997). The 164 isolates from England & Wales in 1995 were characterized previously for mating type and metalaxyl sensitivity (Day & Shattock, 1997), while a further 81 isolates collected in Scotland in 1995 were kindly supplied by D. E. L. Cooke, Scottish Crop Research Institute, Dundee, DD2 5DA, UK, and formed part of data presented by Cooke *et al.* (2003a). For the years 1996–98, 2446 isolates from Wales, England and Scotland were collected for the present study and characterized for mating type and sensitivity to metalaxyl. Mitochondrial (mt) DNA haplotype (Carter *et al.*, 1990) was determined using the PCR-based method of Griffith & Shaw (1998). DNA fingerprinting was carried out using the multilocus nuclear DNA probe RG57 (Goodwin *et al.*, 1992) and employing nonradioactive methods developed by Pipe & Shaw (1997). Nuclear DNA fingerprints and mt DNA haplotypes were obtained for a subset of 1459 isolates composed of six isolates (where available) from each site, except where both mating types were detected when all available isolates were characterized.

Data analysis

Contingency tables were constructed to compare frequencies of genotypes among types of site and/or years. Geographical comparisons were also made by subdividing the area studied into six regions (Fig. 2). Isolates from all four sampling years were pooled to obtain a sufficient number of isolates for analysis. All contingency tables were analysed using a Fisher exact test, analogous to a chi-squared test but applicable to extended contingency tables (Rousset & Raymond, 1995). The STRUC program, included in the GENEPOP 3·3 software package, was used to compute the exact *P*-value of the probability test (Raymond & Rousset, 1995), with $P < 0{\cdot}05$ indicating significance for all tests.

The Shannon index (Bowman *et al.*, 1971) was used to quantify diversity at different types of site (Goodwin *et al.*, 1992). The significance of different Shannon indices was assessed with the *t*-test of Hutcheson (1970).

Results

Characterization of isolates

Mating type

Over the period 1995–98, of 2691 isolates established, 96·4% were of A1 mating type, 3·0% were of A2 mating type and 0·6% were self-fertile. Of the 354 sites studied, the A2 mating type was detected alone at nine sites and in combination with A1 at 25 sites (Table 1).

Sensitivity to metalaxyl

Over the same period and with the same number of isolates, 33·9% were resistant, 44·2% were intermediate and 21·9% were sensitive to metalaxyl. Of the 354 sites sampled, 316 included isolates showing some insensitivity (intermediate and/or resistant). Resistance was detected at 187 sites, intermediate resistance at 236 sites and all three sensitivities at 41 sites (Table 2).

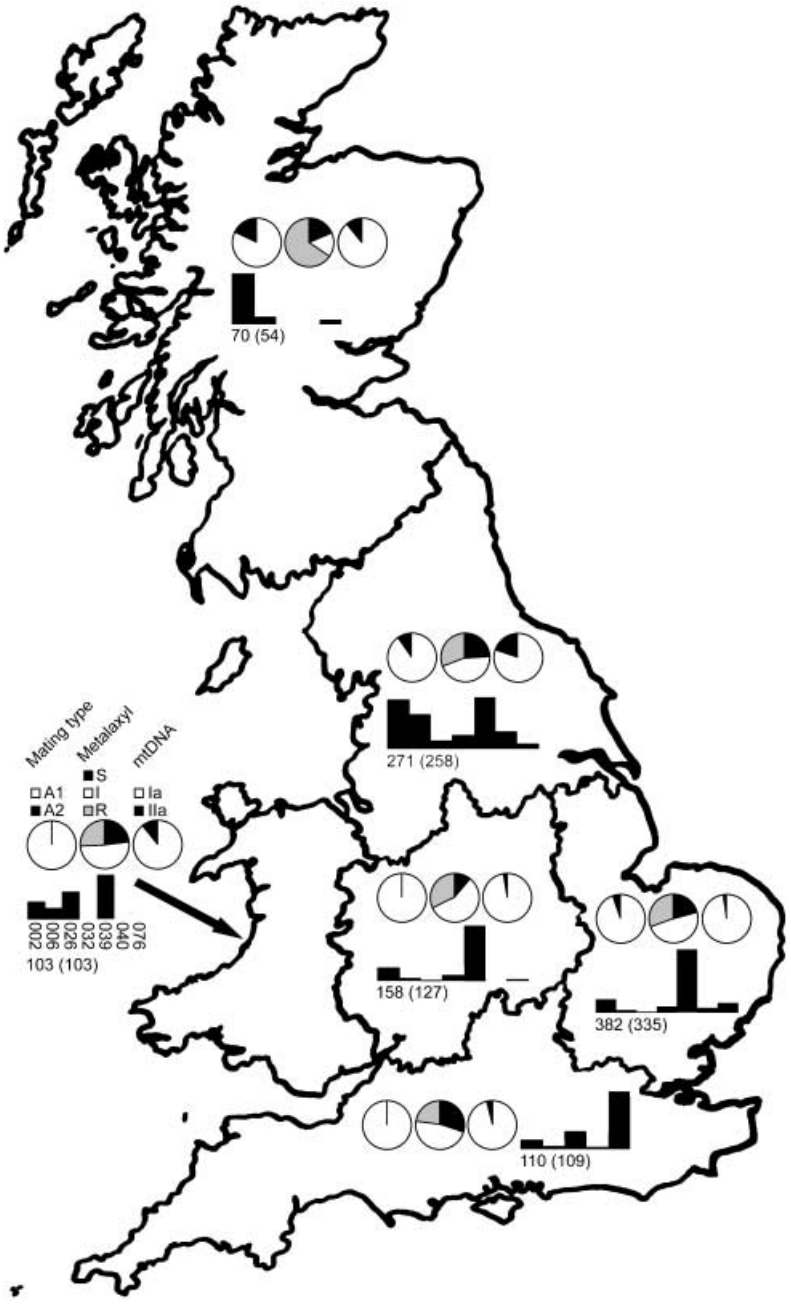

Figure 2 Frequency of different mating types (A1 or A2), responses to metalaxyl [sensitive (S), intermediate (I) or resistant (R)] and mtDNA haplotypes (Ia or IIa) among potato isolates of *Phytophthora infestans* collected from commercial sites in England, Wales and Scotland for the years 1995–98. Frequencies of the common RG57 fingerprints RF002, RF006, RF026, RF032, RF039, RF040 and RF076 are indicated in the histograms. Numbers below histograms are numbers of isolates characterized from each region and (in parentheses) numbers of isolates which showed one of the above six common fingerprints.

Mitochondrial DNA haplotype

Of the four mtDNA haplotypes which are known to be detected by the method used here, only two were found over the 4 years 1995–98; 1328 (91%) of the subset of 1459 isolates were of haplotype Ia and 131 (9%) were IIa. Of the 326 sites from which isolates were characterized, 272 (83%) had haplotype Ia only, 21 had IIa only and 33 had both haplotypes (Table 3).

Fingerprints detected with RG57

Based on a worldwide collection of isolates, the RG57 probe can detect (presence/absence) a total of 25 restriction fragments/bands (Goodwin *et al.*, 1992; Forbes *et al.*, 1998). In the subset of 1459 isolates characterized in 1995–98, seven bands were not detected (bands 4, 11, 12, 15, 17, 18 and 23) and five occurred in all isolates (bands 1, 14, 20, 21 and 25); band 14 was always found as a 'doublet' with band 14a. The remaining 13 bands were polymorphic (bands 2, 3, 5, 6, 7, 8, 9, 10, 13, 16, 19, 22 and 24). Two additional polymorphic bands were detected and designated '5a' and '24a'; the latter previously reported by Forbes *et al.* (1998). Among the 1459 isolates from 326 sites, a total of 30 different fingerprints were detected (Table 4a). Four fingerprints were frequently detected and widespread: RF039 (46·4%; observed at 180 sites), RF002 (17·4%; 101 sites), RF006 (8·5%; 53 sites) and RF040 (4·3%; 29 sites). Sixteen fingerprints occurred

Table 2 Percentages of sites (actual numbers in parentheses) at which metalaxyl-sensitive (S), resistant (R) and intermediate (I) phenotype isolates, and mixtures of phenotypes, were detected in isolates of *Phytophthora infestans* from potato in commercial or trial crops (C) or garden/allotment sites (GA), 1995–98

Site type	Phenotype(s)	1995[a]	1996	1997	1998	Total
C	S only	25 (6)	12 (2)	11 (17)	4 (4)	10 (29)
	I only	4 (1)	24 (4)	16 (25)	33 (31)	21 (61)
	R only	38 (9)	18 (3)	28 (44)	10 (9)	22 (65)
	S + I	13 (3)	18 (3)	6 (10)	17 (16)	12 (32)
	S + R	0 (0)	6 (1)	3 (4)	3 (3)	3 (8)
	I + R	13 (3)	18 (3)	26 (41)	16 (15)	21 (62)
	S + I + R	8 (2)	6 (1)	10 (15)	17 (16)	12 (34)
GA	S only	50 (4)	8 (1)	11 (4)	0 (0)	14 (9)
	I only	13 (1)	54 (7)	29 (10)	57 (4)	35 (22)
	R only	13 (1)	0 (0)	11 (4)	0 (0)	8 (5)
	S + I	0 (0)	23 (3)	23 (8)	43 (3)	22 (14)
	S + R	13 (1)	0 (0)	3 (1)	0 (0)	3 (2)
	I + R	0 (0)	8 (1)	9 (3)	0 (0)	6 (4)
	S + I + R	13 (1)	8 (1)	14 (5)	0 (0)	11 (7)
Total (no.)		(32)	(30)	(191)	(101)	(354)

[a]Contains data previously published by Day & Shattock (1997) with additional data for the Scottish isolates provided by SCRI (Cooke *et al.*, 2003a).

Table 3 Percentages of sites (actual numbers in parentheses) at which mtDNA haplotype Ia or IIa, or both, were detected in isolates of *Phytophthora infestans* from potato in commercial or trial crops (C) or garden/allotment sites (GA), 1995–98

Site type	MtDNA	1995[a]	1996	1997	1998	Total
C	Ia only	90 (19)	82 (14)	90 (128)	74 (63)	84 (224)
	IIa only	10 (2)	12 (2)	4 (6)	6 (5)	6 (15)
	Ia and IIa	0 (0)	6 (1)	6 (9)	20 (17)	10 (27)
GA	Ia only	75 (6)	69 (9)	81 (26)	100 (7)	80 (48)
	IIa only	13 (1)	23 (3)	6 (2)	0 (0)	10 (6)
	Ia and IIa	13 (1)	8 (1)	13 (4)	0 (0)	10 (6)
Total (no.)		(29)	(30)	(175)	(92)	(326)

[a]Contains data previously published by Day & Shattock (1997) with additional data for the Scottish isolates provided by SCRI (Cooke *et al.*, 2003a). Not all of the sites characterized for mating type and metalaxyl phenotype were also characterized for mtDNA haplotype.

at between two and 19 sites and 10 fingerprints were detected at a single site only, in one season, and are referred to as 'unique' fingerprints. A single fingerprint was detected at 56·5% of sites, two fingerprints at 32·4% of sites, three fingerprints at 9·1% of sites and four or more at 2·0% of sites (Table 5).

Of the 10 unique fingerprints occurring individually at one site only (Table 4a), RF041, RF045 and RF109 were each found along with one or more of the four common fingerprint types. The other seven unique fingerprints were from sites at which no other genotypes were detected.

Comparison of isolates and their diversity at commercial and garden/allotment sites

Mating type

For all four years pooled, there was no significant difference between the detection of A2 isolates at commercial sites and that at garden/allotment sites (Table 1). The frequency of commercial sites where A2 was detected did not change significantly over the four sampling years. However, the frequency of garden/allotment sites with A2 isolates was significantly heterogeneous; more sites with A2 were detected in 1995 (four out of eight) than in subsequent years (two out of 13; one out of 35; one out of seven).

Sensitivity to metalaxyl

Significant differences ($P < 0{\cdot}05$) in the frequency of sites with each of the metalaxyl phenotypes in 1998 compared with the preceding years were indicated for commercial sites, but not for garden/allotment sites. In 1998 there was a higher frequency of commercial sites with intermediate resistance (83·0% of sites, compared with 58·3% in 1997) and a lower frequency of sites with resistant isolates (45·7% of sites, compared with 66·7% in 1997). Over the 4-year period 1995–98 the proportion of commercial sites yielding resistant isolates (58·1%) was significantly higher than that of garden/allotment sites (28·6%). However, there was no significant difference when the data for 1998 (when there were only seven garden/allotment samples) were excluded.

Mitochondrial DNA haplotype

The proportions of commercial vs. garden/allotment sites with Ia only (84·2 and 80·0%, respectively), with IIa only (5·6% and 10·0%) and with both Ia and IIa haplotypes (10·2% and 10·0%) were not significantly different. There was no significant change in the proportions of Ia and IIa haplotypes detected over the four sampling years. However, in 1998 the occurrence of commercial sites containing both Ia and IIa (20%) was significantly higher than in the previous years (0, 6 and 6% in 1995, 1996 and 1997, respectively) (Table 3).

RG57 fingerprints

No significant differences were detected in either the frequency of sites overall with more than one RG57

Table 4 Genotypes, phenotypes and locations of British isolates of *Phytophthora infestans* from potato

RG57 fingerprint[a]		Mating type[b]	mtDNA haplotype	Metalaxyl[c] sensitivity	Number of isolates	Number of sites[d]	Type of site[e]	Year found	Location[f]
(a) Isolates from England, Wales and Scotland, 1995–8									
RF002	110-10000100110100011101l	A1	Ia	R (S, I)	252	100	C/DV/GA	1995–98	W, S, E, M, N, Sc
RF002		A1	IIa	I, R	2	2	C	1998	W, Sc
RF005	100-10000100110100011101l	A1	Ia	R	3	3	C/DV/GA	1995	Sc
RF006	100-10001100110100011001l	A1	Ia	I, R	8	5	C/DV/GA	1996,98	W, S, N
RF006		A1	IIa	S, I (R)	116	50	C/DV/GA	1995–98	W, S, E, M, N, Sc
RF008	100-1000010011010001100l1	A1	Ia	R	4	2	C	1997	M, N
RF008		A1	IIa	S, I	4	2	C	1997–98	N, Sc
RF026	111-11101100110100111101l	A1	Ia	S, I	89	18	C/GA	1995–98	W, S, E, M, N
RF026		A2	Ia	S	1	1	GA	1995	Sc
RF032	111-11111100110100111101l	A1	Ia	S (I, R)	54	19	C/GA	1996–98	W, S, E, M, N
RF035	111-11110100110100111101l	A1	Ia	S, I	15	10	C/GA	1997–98	S, E, M, N
RF037	101-11101100110100111101l	A1	Ia	I, R	8	5	C/DV	1995–97	S, E
RF039	101-11111100110100111101l	A1	Ia	S, I, R	672	179	C/DV/GA	1995–98	W, S, E, M, N, Sc
RF039		A1	IIa	I	5	3	C	1996–98	M, N
RF040	111-10100100110100111101l	A1	Ia	S, I	3	2	C/GA	1998	S, N
RF040		A2	Ia	S, I	59	29	C/GA	1995–98	W, E, N, Sc
RF040		A2	IIa	I	1	1	C	1998	N
RF041	111-10000100110100111101l	A2	Ia	S	1	1	C	1997	S
RF042	111-10100100110100011101l	A1	Ia	I	7	2	C	1997–98	M, N
RF045	101-10110100110100111101l	A1	Ia	S	5	1	GA	1997	M
RF049	111-10110100110100011101l	A1	Ia	S	1	1	C	1997	E
RF058	110-10000000110100111101l	A1	Ia	R	2	1	C	1996	E
RF060	101-10100100110100011001l	A1	Ia	S	1	1	GA	1995	Sc
RF061	110-11101100110100011101l	A1	Ia	R	1	1	C	1995	Sc
RF065	101-10100100110100111101l	A2	Ia	S, I	3	2	C	1995, 97	E
RF069	110-10001100110100111101l	A1	Ia	S	2	1	C	1995	Sc
RF076	111-11110100110100011101l	A1	Ia	S, I	43	15	C/GA	1997–98	S, E, M, N
RF078	101-10111100110100111101l	A1	Ia	I	2	2	C/GA	1997	M
RF079	101-11110100110100011101l	A1	Ia	S, I	5	4	C/GA	1997	E, N
RF082	101-11111100110000111101l	A1	Ia	S, R	3	2	GA	1997	S, N
RF083	101-11101100110000111101l	A1	Ia	S, I	2	2	C/GA	1997	S, N
RF088	101-11110100110100111101l	A1	Ia	S, I, R	27	11	C/GA	1997–98	S, E, M, N, Sc
RF094[g]	100-10000100110100011101l	A1	Ia	R	29	9	C	1997–98	E, M
RF097	111-11110100110100011001l	A1	Ia	S	9	1	GA	1997	S
RF105[h]	111-00100100110100011101l	A1	Ia	S	3	1	C	1998	M
RF109[h]	100-10010100110100111001	A2	Ia	I	1	1	GA	1998	S
RF112	101-10100100010000011101l	A2	Ia	S, I	3	2	C	1997	Sc
(b) Isolates from England and Wales, 1978–85									
RF015[i]	101-10101100110100011001l	A1	Ib	S, I	2	2	?	1978	W, S
RF015	101-10101100110100011001l	A1	Ib	S	1	1	?	1981	W
RF040	111-10100100110100111101l	A2	Ia	S	1	1	?	1981	W
RF008	100-10000100110100011001l	A2	IIa	S	2	1	C	1982[j]	W
RF015	101-10101100110100011001l	A1	Ib	S (I)	11	1	C	1982	W
RF018	101-11101100110100011001l	A1	Ib	S	1	1	C	1982	W
RF063	111-10100100110000111101l	A1	Ia	S	1	1	C	1982	W
RF063		A2	Ia	S	2	1	C	1982	W
RF064	111-10100100110100011001l	A2	Ia	S	1	1	C	1982	W
RF026	111-11101100110100111101l	A1	Ia	S	1	1	–	1985	N
RF032	111-11111100110100111101l	A1	Ia	S	1	1	–	1985	W
RF039	101-11111100110100111101l	A1	Ia	S	1	1	–	1985	S
RF063	111-10100100110000111101l	A2	Ia	R	1	1	–	1985	E
RF072	101-11101100110100011101l	A1	Ia	S	1	1	–	1985	W

[a]RG57 fingerprint is denoted using '1' and '0' to indicate presence or absence, respectively, of bands 1–25 recognized by the RG57 probe (Goodwin *et al.*, 1992). The '–' represents band 4, which is not recognized by the probe used here, and was excluded from the analysis.

[b]Self-fertile isolates, assumed to be mixtures of different phenotypes, are not included on this or other tables and are discussed separately.

[c]S, sensitive; I, intermediate; R, resistant. Parentheses indicate that the enclosed phenotype was extremely rare relative to the others.

[d]Individual sites are listed repeatedly for the occurrence of each RG57 fingerprint.

[e]C, commercial crop (farm or trial); DV, commercial dump or volunteer; GA, garden or allotment; –, unknown.

[f]Regions illustrated in Fig. 1. E, eastern England; M, Midlands; N, northern England; S, southern England; Sc, Scotland; W, Wales.

[g]A new band intermediate in size between bands 5 and 6 (designated '5a') was also present.

[h]A new band intermediate in size between bands 24 and 25 (designated '24a') was also present.

[i]Corresponds to the common 'old' lineage US-1, described by Goodwin *et al.* (1994).

[j]All 1982 isolates were from a single site.

Table 5 Percentage of monomorphic sites (containing one RG57 fingerprint only) and polymorphic sites (containing two, three or more fingerprints). Actual number of sites in parentheses. Excludes sites where only one isolate of *Phytophthora infestans* was characterized

Site type[a]	Fingerprints detected	1995	1996	1997	1998	Total
C	1	56 (5)	63 (10)	58 (66)	50 (36)	55 (117)
	2	22 (2)	31 (5)	37 (42)	32 (23)	34 (72)
	3	22 (2)	6 (1)	3 (3)	15 (11)	8 (17)
	> 3	0 (0)	0 (0)	3 (3)	3 (2)	2 (5)
	Mean sample size ± SE[b]	5·6 ± 5·3	8·2 ± 6·0	5·1 ± 2·9	4·6 ± 2·3	
GA	1	25 (1)	60 (6)	71 (17)	50 (2)	62 (26)
	2	50 (2)	20 (2)	17 (4)	50 (2)	24 (10)
	3	25 (1)	20 (2)	13 (3)	0 (0)	14 (6)
	> 3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Mean sample size ± SE[b]	3·0 ± 1·4	7·3 ± 3·7	4·7 ± 2·4	3·5 ± 1·7	
Total (no.)		(13)	(26)	(138)	(74)	(253)

[a]C, commercial or trial crops; GA, garden/allotment sites.
[b]Excluding single-isolate samples.

genotype in different years or that frequency in commercial vs. garden/allotment sites.

Diversity

The diversity of isolates collected each year was estimated from the Shannon index, calculated from RG57 fingerprint, mtDNA haplotype and mating-type markers. Diversity was highest in 1996 and 1997, significantly lower in 1998 and significantly lower again in 1995 (Table 6). Sufficient isolates for comparison between commercial and garden/allotment sites were obtained only in 1996 and 1997; in each year, there was no significant difference in diversity between the two types of site.

Geographical analysis for each marker

Fisher analysis was only carried out for isolates pooled across the years 1995–98 from commercial sites, since too few isolates from the other types of site were available from the different locations.

Significant differences in the distribution of mating type, metalaxyl phenotypes, mtDNA haplotypes and the commoner RG57 fingerprints (RF002, RF006, RF026, RF032, RF039, RF040 and RF076) were observed between isolates from four different English regions, from Scotland and from Wales ($P < 0{\cdot}005$; Fig. 2). A2 isolates were most common in eastern England, northern England and Scotland (Fig. 2). Metalaxyl-resistant (R) phenotypes made up 65% of the Scottish isolates, compared with 23–32% elsewhere (Fig. 2). When Scottish isolates were removed from the analysis, there were still significant differences ($P < 0{\cdot}05$), with resistant isolates being relatively less common in Wales and southern England than elsewhere. Isolates with mtDNA haplotype IIa were relatively rare in eastern and southern England and the Midlands (2–4%), but common in northern England (20%) (Fig. 2). The commonest RG57 fingerprint overall, RF039, was most common in southern England (63·6% of isolates) and declined in frequency northwards. It accounted for only 5·7% of fingerprinted isolates from commercial sites in Scotland. Conversely, RF002 was the commonest fingerprint among isolates from the Scottish sites (62·9%) and decreased in frequency at the more southerly sites, accounting for only 10·0% of the isolates from southern England (Fig. 2). RF006 was most common (18·1%) in northern England and present in Wales, while RF040 was only found in northern and eastern England.

Associations among markers

Common RG57 genotypes showed strong associations with mating type and mtDNA haplotype. With rare exceptions, RF039 and RF002 were associated with A1 mating type and mtDNA type Ia, RF006 with mating type A1 and haplotype IIa, and RF040 with mating type A2 and haplotype Ia (Table 4a). Fingerprints closely similar to these common types, i.e. with one band difference, tended to have the same mating type and mtDNA haplotype as the common genotype. RF006 and RF039 were found with all three sensitivities to metalaxyl, although intermediate (I) phenotypes were most common in each case (78·2 and 67·6%, respectively) and only 4% of RF006 isolates were resistant. RF002 isolates were predominantly resistant (96·4%) and RF040 A2 isolates were never fully resistant to the fungicide. Twenty-five fingerprints were associated wholly or predominantly with the A1 mating type and five fingerprints with the A2 mating type.

Diversity at Norfolk and Cheshire sites

Following the discovery of both mating types at a commercial site in Norfolk (eastern England) in 1997, more samples were taken two more times that year and again in 1998 at an adjacent site. Within 25 isolates recovered in

Table 6 Genetic diversity of isolates of *Phytophthora infestans* at different types of site

	No. of sites	No. of isolates	No. of genotypes[a]	Shannon index[b]
Sampling years (England & Wales)				
1995[c]	9	41	7	1·26
1996	28	246	10	1·93 a
1997	164	712	32	1·97 a
1998	96	368	20	1·73
Type of site[d]				
Commercial sites (1996)	15	128	8	1·36 a
Garden/allotment sites (1996)	12	76	5	1·31 a
Commercial sites (1997)	126	570	26	1·93 b
Garden/allotment sites (1997)	35	129	16	1·72 b
Commercial sites (1998)	85	334	19	1·76 b
'Mixed' and 'A1 only' sites[e]				
'A1 only' sites (1997)	95	463	16	1·73 a
'Mixed' sites (1997)	6	54	16	2·23
'A1 only' sites (1998)	63	209	12	1·44
'Mixed' sites (1998)	12	80	11	1·81 a
'Mixed' and 'A1 only' sites[f] (diversity of A1 isolates only)				
'A1 only' sites (1997)	95	463	16	1·73 b
'Mixed' sites (1997)	6	41	9	1·54 ab
'A1 only' sites (1998)	63	209	12	1·44 a
'Mixed' sites (1998)	12	50	8	1·61 ab

[a]Genotypes were defined using a combination of RG57 fingerprint, mtDNA type and mating type. Self-fertile isolates were included in this analysis.
[b]Shannon indices within the sections of the table followed by a common letter are not significantly different at $P < 0{\cdot}05$ according to the *t*-test of Hutcheson (1970) for pairwise comparisons.
[c]Too few isolates were characterized for 1995 for any further within-year comparisons to be carried out.
[d]Comparison carried out only for isolates from England and Wales, since sampling from both types of site was less balanced elsewhere. The number of isolates obtained from garden/allotment sites in 1998 was not sufficient for valid analysis.
[e]'Mixed' sites were those where A2 and/or self-fertile isolates were found together with A1 isolates. Sites where A2/self-fertile isolates were found alone were excluded from analysis (as they were so few in number). Sites where fewer than three isolates were sampled were also excluded from analysis. Sufficient numbers of isolates to allow statistically valid comparison were only obtained for commercial sites, in 1997 and 1998 (England and Wales only).
[f]Analysis was carried out as above, but data for A2 and self-fertile isolates were excluded (see text).

1997, 12 fingerprints were characterized, four of which were unique to this site (three of these being self-fertile isolates) and three of which were common fingerprints of both mating types; in total five isolates were self-fertile. The fingerprints found in 1998 were quite distinct from those from the previous year. A site in Cheshire (northern England) close to the border with Wales with both mating types in 1998 stimulated more intensive sampling at eight other commercial fields in the area. Self-fertile or A2 isolates were obtained at all nine sites, but of the seven fingerprints identified, five were common types (of both mating types) and none were unique. Unusual associations of common fingerprints with mating types and mtDNA haplotypes were found at one of the sites, where RF040;IIa;A2 and RF040;Ia;A1 were present as well as the more usual combination RF040;Ia;A2. In addition, RF006;IIa;A1 was also present at this site.

Variation detected in isolates collected between 1978 and 1985

For comparative purposes, 27 isolates from 1978, 1981, 1982 and 1985 from 10 sites, stored in liquid nitrogen, were revived and characterized (Table 4b). Both isolates collected in 1978 and one from 1981 were A1 and genotype RF015;haplotype Ib, identical to the old clonal lineage, US-1. The A2 isolates from 1981 had a genotype (RF040;haplotype Ia) identical to the majority of the A2 isolates in the 1995–98 collection. Eighteen isolates from one site on the Isle of Anglesey, North Wales, sampled in 1982, showed high diversity. Most isolates (11) conformed to US-1 clonal lineage with haplotype Ib and one other isolate had just one extra band (RF018). Four genotypes had new RG57 bands not present in the US-1 fingerprint. Both mating types and both of the new mtDNA haplotypes, Ia and IIa, were present at this site. The five isolates from 1985 were from five different sites and each had a fingerprint different from RF015 (US-1) and had mtDNA haplotype Ia. The only isolate which showed full resistance to metalaxyl was an A2 isolate of RG57 phenotype RF063 from 1985.

Discussion

Phenotypic and genotypic characterization of British cultures isolated in 1978, 1981 and 1982 has confirmed the presence of the old clonal lineage, US-1 (RF015). The analysis

of isolates from 354 sites throughout Great Britain during 1995–98 failed to detect US-1 and instead detected a range of genotypes of both A1 and A2 mating types. This confirms the displacement of US-1 indicated in earlier work (Spielman *et al*., 1991; Day & Shattock, 1997).

Mating types

Previous estimates of the frequency of the A2 mating type in England and Wales have fluctuated but have rarely been more than a few percent of all isolates (Day & Shattock, 1997). Only 3·0% of the 2691 isolates established over the 4 years 1995–98 were of A2 mating type; these were detected at 10% of the 354 sites. This proportion of sites with A2 must be an underestimate, as sample sizes at each site were small and rare A2 strains would not have been sampled. The frequency of garden and allotment sites having the A2 mating type was not significantly different from that of commercial crops. This contrasts with findings of higher A2 frequencies at garden/allotment sites than at commercial sites in Scotland (Cooke *et al*., 2003a) and in southern Flevoland (Netherlands) (Zwankhuizen *et al*., 2000). In the latter study, the frequency of A2 isolates in commercial sites in southern Flevoland, more accurately estimated in larger samples, was higher (up to 25%) than that found at British commercial sites. On a regional basis, however, a higher proportion of UK commercial sites with A2 mating type was found in the present study in eastern and northern England and in Scotland than in other regions. There is some evidence that the frequency of A2 in Ireland has declined from a maximum of 10% of isolates in the early 1990s (O'Sullivan *et al*., 1995); A2 has not been detected there in recent years (Carlisle *et al*., 2001; Griffin *et al*., 2002). At most sites where A2 occurred in Great Britain, A1 isolates were also found and self-fertile isolates were not uncommon. This close proximity of sexually compatible strains indicates that oospores would be produced in potato foliage (e.g. Turkensteen *et al*., 2000). The potential for sexual reproduction was thus present at such sites.

Metalaxyl sensitivity

Over the 4 years 1995–98, metalaxyl insensitivity (full or intermediate resistance) was detected at approximately 90% of sites; the other sites yielded isolates which were sensitive to the fungicide. As might be expected, insensitivity was more often found at commercial sites, where metalaxyl was commonly applied, than at garden/allotment sites where fungicides were rarely, if ever, used. An earlier survey in England & Wales in 1993–95 (Day & Shattock, 1997) indicated a lower frequency of sites with some insensitivity (61%), but the larger number of garden/allotment sites in that survey offers at least a partial explanation for the difference. The frequency of sites with intermediate resistance increased over two seasons (1997–98) and the frequency of sites with full resistance decreased over this time. A similar phenomenon was reported in Scotland in 1995–97, where it was interpreted to indicate an increase in mating between resistant and sensitive strains to generate intermediate offspring (Cooke *et al*., 2003a). A lower frequency of resistance was detected in Ireland in 1995 and 1996: 14% of isolates were resistant and none were intermediate in Northern Ireland (Carlisle *et al*., 2001); 19% were resistant and 2% intermediate in the Republic of Ireland (Griffin *et al*., 2002).

In the present study, all isolates from 1995 to 98 that were resistant to metalaxyl were A1 mating type. Similar results, with rare exceptions, were obtained in earlier surveys in England and Wales (Day & Shattock, 1997), Scotland (Cooke *et al*., 2003a) and continental Europe (Gisi & Cohen, 1996; Knapova & Gisi, 2002). It has been argued (Day *et al*., 2001; Cooke *et al*., 2003a) that the continued low frequency of the A2 mating type is a result of the use of metalaxyl and selection against metalaxyl-sensitive phenotypes. It is not known why metalaxyl-resistant A2s (e.g. the single isolate RF063, Table 4b), several of which were detected in previous surveys (Shattock *et al*., 1990; Day & Shattock, 1997), are not more successful in the UK, as the most widespread clone in North America in recent years, US-8, is A2 and resistant (Goodwin *et al*., 1998; Shattock, 2002). Recent migrations of exotic late-blight isolates in Europe and North America have been separate events. Subsequently, differences in crops and cropping, alternative hosts among indigenous weeds, and crop protection practices in each continent may influence evolution of *P. infestans* populations.

MtDNA

MtDNA haplotype Ib, which is typical of the old clonal lineage US-1, was not present among the 1459 isolates characterized from the 4 years 1995–98, but was detected in the small collection of stored isolates up to 1982. On potato, haplotype Ib was last detected at a single site in 1986 (Day & Shattock, 1997). (On tomato, it was found as recently as 1998, on an allotment site where it occurred with two different, unique fingerprints, not US-1, and with A2 mating type; JPD, unpublished data). This result is consistent with the elimination of the US-1 clonal lineage throughout Great Britain. The large majority of sites (305 out of 326) had isolates of haplotype Ia, whereas haplotype IIa was detected at only 54 sites and 33 sites had both haplotypes. Predominance of Ia and a low frequency of IIa have also been found in other recent surveys in Europe (Griffith & Shaw, 1998; Lebreton *et al*., 1998), although studies of isolates from Northern Ireland (Carlisle *et al*., 2001) and from the Republic of Ireland (Griffin *et al*., 2002) indicated that haplotype IIa was more frequent than Ia. Haplotype IIb, previously found in western North America, was not detected in the British isolates in the present study, but was a rare haplotype in surveys in Northern Ireland (Carlisle *et al*., 2001) and the Netherlands (W. G. Flier, PRI, 6700 AA Wageningen, Netherlands, personal communication).

Fingerprints

The replacement of US-1 by new genotypes, formerly detected by mating type, allozymes and mtDNA haplotype

(Shattock *et al*., 1990; Spielman *et al*., 1991; Day & Shattock, 1997) was confirmed by absence of the US-1 fingerprint RF015 after 1982. Five new RG57 bands not present in US-1 were found in isolates from 1981 to 85 (Table 4a) and allowed nine new fingerprints to be distinguished. These same five bands occurred in the 1995–98 isolates and only two more bands (5a found in one fingerprint and 24a found in two fingerprints) were detected among the 1459 isolates characterized. A band similar or identical to 24a was found in Europe, Asia and S. America (Forbes *et al*., 1998; Brurberg *et al*., 1999; Zwankhuizen *et al*., 2000), but band 5a has not been reported in other studies. Within the variable population from the Isle of Anglesey, Wales, in 1982, the US-1 genotype was accompanied by four other fingerprints, one of which was identical to US-1 except for the presence of a new band 6; this is evidence of introgression of genes from US-1 into the new population of the pathogen (additional evidence for this was the finding, mentioned above, of mtDNA haplotype Ib in combination with non-US-1 fingerprints in tomato isolates). The detection of RF040 in 1981 and RF039 in 1985 suggests that these genotypes, which were the commonest genotypes of the A2 and A1 mating types, respectively, in the 1995–98 survey, were already established by the early 1980s.

A total of 30 fingerprints was distinguished in the 1995–98 population using the genotypes at the 13 loci which were polymorphic. The four commonest fingerprints (RF039 > RF002 > RF006 > RF040) were quite distinct, differing by three to seven bands, were found in all four years and were widely distributed. Construction of a dendrogram (not illustrated) based on RG57 fingerprints, albeit with low bootstrap values, indicated that each of these common fingerprints is part of a cluster of three or four other, similar fingerprints with a lower but still substantial frequency of occurrence. A possible hypothesis is that a cluster of similar genotypes of higher frequency represents a clonal lineage. Previously, Purvis *et al*. (2001) presented dendrograms constructed for 98 isolates selected from a core of the 1995–97 isolates which included three of the four most common RG57 fingerprints described in this study. In some cases, isolates with the same RG57 fingerprint (e.g. RF006 and RF040) had very similar or identical amplified fragment length polymorphism (AFLP) fingerprints, and isolates with very similar, but not identical, RG57 fingerprints often had identical AFLP profiles; this supports a clonal-lineage hypothesis for clusters of similar fingerprints. Exceptionally, isolates with common RG57 fingerprints (e.g. RF039) displayed higher levels of variablity for AFLPs, suggesting convergent evolution of some RG57 fingerprint genotypes. Each member of a cluster of fingerprints had, with rare exceptions, the same mating type and the same mtDNA haplotype, a finding consistent with a clonal origin. For example, almost all RF040 isolates were A2 and mtDNA Ia, whilst most RF006 isolates were A1 and haplotype IIa.

There were significant differences in the distribution of the four commonest fingerprints (Fig. 2). RF002 was the commonest fingerprint in the combined regions of Scotland and northern England, but was less common further south. It is possible that this clone migrated southwards within Scottish seed tubers to the main English potato-growing regions. The commonest fingerprint in the south, RF039, had a lower frequency further north. Perhaps seed tubers from continental Europe (mainly the Netherlands) might facilitate migration of perennial clones northwards. Another possibility is that RF002 and RF039 represent, respectively, northern and southern ecotypic variants selected by environmental and/or agroecological factors and spread more locally among ware crops, gardens/allotments and dumps.

Clonal lineages

Although all four of the commonest clonal lineages have been detected in small samples from Ireland, frequencies were different from those in Great Britain (Carlisle *et al*., 2001; Griffin *et al*., 2002; JPD, unpublished data). In the Republic of Ireland, RF006;A1;IIa predominated in 1996 (in a sample of 21 isolates from 10 sites), RF040;A2;Ia, (the only A2 detected), was present at only two sites, while RF039;A1 was detected for the first time at one site in 1999. The genotype RF006;A1;IIa probably also dominated the pathogen population in Northern Ireland in 1995–96 because mtDNA IIa was detected at all of 29 sites and the commonest fingerprint in a small sample of the isolates was RF006 (Carlisle *et al*., 2001); mating type A2 was not detected, but low frequencies were found in earlier samplings (O'Sullivan *et al*., 1995). Evidence from both the Republic (Griffin *et al*., 2002) and from Northern Ireland (Cooke *et al*., 2003b) suggests that the predominant fingerprint RF006 has declined in frequency in recent years. The clear differences in the frequencies of common clonal lineages in Ireland and Great Britain in the mid-1990s show a differentiation of the population of *P. infestans*, perhaps mediated by restricted migration (e.g. strict regulations permitting importation of only high-grade seed tubers into Ireland as a whole from the rest of Europe) and/or by climate.

In mainland Britain, the widespread occurrence of these common genotypes and their persistence at the same or adjacent sites for several years strongly indicate that they were perennial, asexually reproducing clones which survived within tubers over the winter period within seed stores, dumps or in the field as volunteers. In a contemporary study, four particularly common RG57 genotypes were found in southern Flevoland in the Netherlands in 1994 and 1995 (Zwankhuizen *et al*., 2000). In this Dutch study, the commonest clonal lineage, NL-41, was identical to RF039, and likewise NL-75 was identical to RF026, a fairly common genotype in Great Britain and analysed more comprehensively (as RF26) by Purvis *et al*. (2001). In the Netherlands, common genotypes were most frequently found within refuse piles and there was evidence of spread of these genotypes to adjacent, commercial fields. The detection of identical RG57 genotypes in many other parts of Europe, e.g. France (Lebreton *et al*., 1998), Scandinavia (Brurberg *et al*., 1999) and Poland (Sujkowski *et al*., 1994),

is evidence of long-distance migration of clonal lineages, most probably within tubers for planting or consumption. In the latter two studies, phenotypes N-1 and F2 (from Norway and Finland, respectively) and P04 from Poland have the RG57 fingerprint of RF006. More direct evidence is provided by the detection of a range of genotypes within ware tubers and tomato fruits imported into Great Britain in 1996–98 (JPD, unpublished data). For example, the common genotype RF039;A1;Ia was found in tubers imported from Egypt; RF039;A1;Ia and two unique fingerprints of A2 mating type were found in tubers/fruits imported from Israel. More detailed tracking studies are required to reveal how strains migrate into, within and out of the British Isles.

Unique genotypes

Of the 10 RG57 fingerprints detected in one year only and at individual sites, some were closely similar to more frequent fingerprints and might have belonged to a rarer clonal lineage, while others were distinct fingerprints that may have arisen by sexual recombination but were unable to survive into the next season within tubers. Sexual recombination could have taken place between compatible common clonal lineages, between a clonal lineage and an annual, unique genotype, or between two unique genotypes. As many as 138 unique genotypes out of a total of 170 genotypes were detected in the larger samples taken in southern Flevoland (Zwankhuizen *et al*., 2000). These were most frequently found in allotments and organic commercial sites and were often found in 1996, a year when common genotypes were less frequent. An analysis of large samples from populations in Wales (N. D. Pipe, School of Biological Sciences, University of Wales, Bangor, UK, unpublished data) has shown that several unique genotypes occurring at one site and assumed to be generated sexually were replaced the following year at the same site by a new set of annual genotypes.

Diversity

When the RG57 fingerprint was combined with mtDNA haplotype and mating type, a total of 51 variants was distinguished. The overall diversity of British isolates over the 4 years was lower than that of the collections from southern Flevoland. This was at least partially caused by the more efficient detection of rare genotypes in the larger number of isolates in samples from a small area in the Dutch study.

In the latter study, diversity in the wetter year of 1994 was lower than in the dry year of 1996, when migration from refuse piles was minimal; it was thought that oospore inoculum initiated infection in 1996 to produce diverse strains, more easily detected when common clonal lineages were less frequent (Zwankhuizen *et al*., 2000). Diversity in British samples also differed significantly from year to year (Table 6; these data include mtDNA haplotype as an additional measure of diversity), but high diversity was not correlated with a dry year as diversity was highest in 1997, one of the wettest years, and lowest in 1995, a drier year.

If diversity were increased by mating of A1 and A2 strains, then sites with both mating types (mixed sites in Table 6) should have been more diverse than sites with A1 only. There was some evidence in 1997, and again in 1998, that mixed sites (Table 6) were more diverse, but the difference was not sustained when the comparison was restricted to the A1 isolates at each type of site. This last comparison removes the diversity within the A2 mating type. However, variation at A1 sites was probably inflated as some of these sites could have been mixed where A2 remained undetected in the small samples taken. In addition, a high diversity might be dependent on the presence of both mating types and oospores at a site in previous years rather than in the year of sampling, although there is some evidence that sexual recombination may generate variation within one growing season (Zwankhuizen *et al*., 2000).

Diversity at single sites

There was evidence of unusually high diversity at some commercial sites (Cheshire and Norfolk) where both mating types were detected. The presence of unique fingerprints there suggested that sexual mating had taken place and the occurrence of new associations of fingerprint and mating type or mtDNA haplotype at some of these sites also indicated sexual reproduction. Alternatively, the new associations could have been generated by reassortment of mitochondria and nuclei present within heterokaryons/heteroplasmons. Pipe *et al*. (2000) recorded mating-type reversals in asexual offspring from heterokaryons. Characterization of these isolates with sensitive and reliable molecular markers such as AFLPs and microsatellites (e.g. Purvis *et al*., 2001; Knapova *et al*., 2002; Abu-El Samen *et al*., 2003; Cooke *et al*., 2003a) should show whether nuclear genotypes of the rare variants were recombinant, and therefore a product of sexual mating, or were identical to the supposed parental genotypes. There is some evidence that common clonal lineages of opposite mating type can generate recombinants within a growing crop of potatoes. An experimental population of common clonal lineages RF039;A1 and RF040;A2 established within a polyethylene-tunnel-protected crop, yielded several new genotypes in the following season; these could have been recombinants of the parental multilocus genotypes (JPD, unpublished data). Samplings made in the third season failed to detect any recombinants, but showed only the persistence of the RF039;A1 clonal lineage. This is consistent with the hypothesis that unique, annual genotypes are the product of sexual reproduction.

From this study it is not possible to determine the extent of sexual reproduction within the populations sampled. The predominance of asexual reproduction is beyond doubt, but whether, and by how much, sex has contributed to the evolving gene pool is not clear. If recombination between A1 and A2 strains is common, then mating type should assort freely with most other markers. Most A2 isolates from Scotland (Cooke *et al*., 2003a) and from England and Wales (Purvis *et al*., 2001) had similar AFLP genotypes and were grouped separately from the A1

isolates. These studies indicate that associations between mating type and other markers were limited and that, if sex occurred, it was not frequent. A range of new, single-locus, codominant markers is now becoming available (e.g. microsatellites, single nucleotide polymorphisms) which will allow quantification of homozygosis/heterozygosis in populations and thus estimation of the frequency of sexual reproduction (Shaw & Wattier, 2003).

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