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Tracking *Phytophthora infestans* with SSR markers within and between seasons – a field study in Sweden

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The dynamics of a late blight epidemic and sexual reproduction in *Phytophthora infestans* were studied in an experimental field in mid-Sweden. The field was inoculated with six isolates of *P. infestans* taken from another potato field where sexual reproduction of the pathogen was suspected. Three weeks after inoculation single-lesion leaflets were sampled and the resulting isolates characterized using microsatellites (SSRs) and mating type as markers. Among the 151 isolates analysed, the inoculum genotypes constituted more than 80% of the genotypes found, with three other genotypes making up the remainder. The following year, *P. infestans* obtained from soil samples taken from this field were analysed, and six novel genotypes were identified. Genotypes from the previous summer's population were not detected. Analysis of the genotypes recovered was consistent with them being recombinants, with the previous summer's population acting as parents. These findings are consistent with the hypothesis that oospores produced during a summer epidemic in Sweden can overwinter and cause infection the next year.

Keywords: CERVUS, late blight, oospores, parentage analysis, population genetics, soilborne inoculum

Introduction

The oomycete *Phytophthora infestans*, the cause of potato late blight, is heterothallic, with two different mating types designated A1 and A2. Before the 1980s both mating types were reported only from Mexico (Spielman *et al.*, 1991) and consequently the pathogen was believed to be restricted to vegetative reproduction in other parts of the world. Asexually reproducing *P. infestans* is only able to overwinter as mycelium surviving in blight-affected tubers. In Europe, a new, more diverse population of *P. infestans*, possessing genotypes of both mating types, replaced the old population in the 1980s (Spielman *et al.*, 1991; Fry *et al.*, 1993). The result of coexistence of both mating types may be sexual reproduction and formation of oospores. Oospores are hardy, thick-walled structures tolerant to adverse environmental conditions (Fay & Fry, 1997; Mayton *et al.*, 2000). Sexual reproduction thus provides the pathogen with the ability of long-term survival independent of its host, e.g. in soil. Also, by genetic recombination, sexual reproduction will enhance the ability of the pathogen to respond to changes such as introduction of cultivars with new types of resistance (Crow, 1992; Barton & Charlesworth, 1998). The first observation of oospores under natural field conditions was made in the 1950s in Mexico (Gallegly & Galindo, 1958). In Europe the first reports came several decades later from Germany (Götz, 1991), the Netherlands (Drenth *et al.*, 1993), the UK (Hanson & Shattock, 1998) and the Nordic countries (Andersson *et al.*, 1998). However, these were more sporadic observations and they did not give any information as to the importance of oospores as an inoculum source.

Field surveys in Norway and Sweden have shown that oospores are commonly formed in blighted potato crops (Dahlberg *et al.*, 2002). There is also strong circumstantial evidence that oospores in the Nordic countries can overwinter and cause early infections (Andersson *et al.*, 1998; Lehtinen & Hannukkala, 2004; Bødker *et al.*, 2006). However, the hypothesis that oospores can act as a primary source of inoculum in the Nordic countries still lacks full direct evidence (Hannukkala & Andersson, 2006). Results from a single field in southwest Sweden (Widmark *et al.*, 2007) strongly indicate that a substantial portion of the late blight attacks in the investigated field was derived from oospores. However, it is difficult to provide experimental evidence that clearly confirms that oospores in the soil act as a primary inoculum source during field conditions. In the Netherlands, Drenth *et al.* (1995) inoculated small field plots with two isolates of different mating types and sampled soil the next season from the plots. The single-lesion isolates obtained using a bioassay were subjected to DNA fingerprint analyses. By using RFLP with probe RG-57 as a marker they identified recombinants of the parental generation and concluded that oospores can survive the winter in the Netherlands. Turkensteen *et al.* (2000), using the same baiting technique, tested oospore survival in pot tests, and showed

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that oospores could survive for up to 48 months during field conditions in the Netherlands in sandy soil. From Mexico it was reported that oospores in naturally infested soil can survive for at least 2 years (Fernández-Pavía *et al.*, 2004).

The main objective of the work presented here was to study oospore survival during Swedish winter conditions, using the same soil bioassay technique as Drenth *et al.* (1995), but using SSRs (microsatellites) as markers instead of RFLP since they offer a much higher taxonomic resolution and thereby enable more accurate estimates of the population structure. This type of marker system is considered well suited for estimating genealogical relationships (Blouin, 2003).

Materials and methods

Field trial

A field, situated at Ultuna near Uppsala, was planted with certified potato cv. Bintje seed tubers on 1 June 2001. There were no commercial potato fields within 5 km, but an allotment garden was situated about 400 m from the experimental field. The field was usually used for growing cereals, but potato cultivar field trials were carried out in 1998 and 1999. Tuber blight was noticed in 1998, but no data is now available on whether late blight and tuber blight were observed in the 1999 trial. The experimental site was divided into four blocks (Fig. 1). Each block was inoculated on 17 July with six isolates of *P. infestans*. These isolates were collected on 21 May from six different infection foci in a field in southwest Sweden where it was suspected that sexual reproduction of *P. infestans* took place (Widmark *et al.*, 2007). On the inoculation date, mating type and genotype of the inoculum isolates were not known. However, by sampling from different foci in the field it was assumed that at least some of the isolates were of different genotypes and mating types. The six isolates were of five different genotypes, three of the A1 mating type and two of the A2 mating type (Table 1). For each isolate, one leaf in the middle part of a plant at one end of each block was inoculated with 10 drops of a sporangial suspension (~200 sporangia per drop). At the date of inoculation the plant height was 50 cm. After inoculation the four blocks were individually covered with a polypropylene fleece to reduce interference between the blocks. On 7 August about 50 single leaflets with blight lesions were collected from the crop at the uninoculated end of each block (Fig. 1). Ten days later infected leaves were collected in the field and left to partly decompose in a moist chamber. The leaf material was then carefully pressed between two microscopic slides and examined for oospores. Tuber harvest took place in the middle of September, after which the field was ploughed.

The period December–January was cold, with a mean temperature of −1·5°C and a lowest temperature of −28°C. The following spring, at the end of April, barley was sown in the field where the trial had been located.

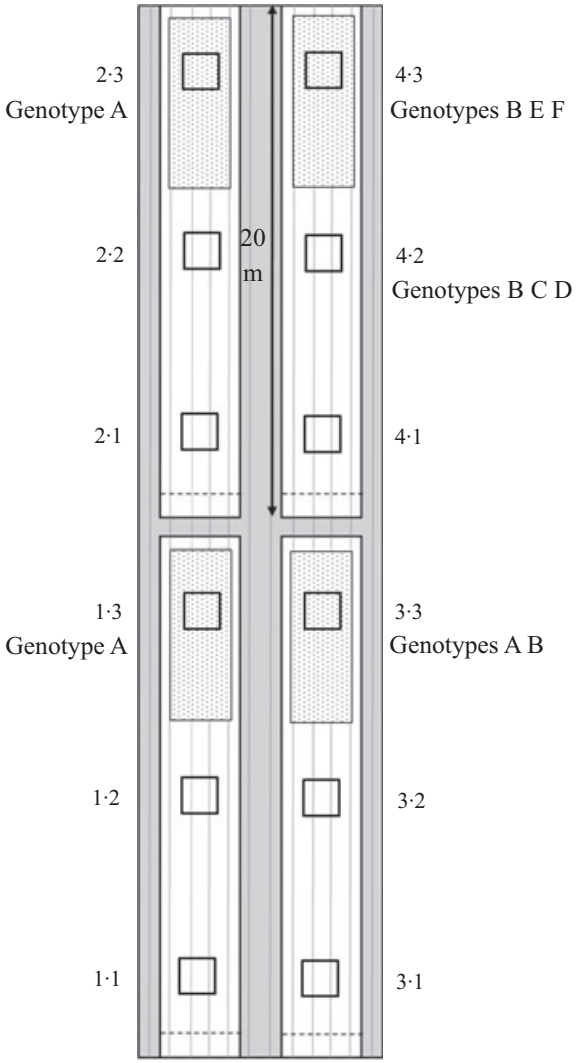

Figure 1 Site map of a field trial in Sweden in 2001 inoculated with six isolates of *Phytophthora infestans*. The numbers indicate block and soil sampling plot number. Inoculation sites are marked with a broken line. Dotted rectangles indicate sampling areas. The positions of the plots where soil samples were taken the next year are marked as squares. The letters A–F represent the six different SSR multilocus genotypes detected in *P. infestans* isolates captured from the soil samples.

The positions of the blocks from the potato experiment conducted the previous year were determined using physical markers and GPS. On 1 July 2002 soil samples were collected from the field using an auger (1·6-cm diameter), drilling to a depth of 25 cm, from three plots (1 m^2 each) per block (Fig. 1). Twelve drillings were made in each of these plots and together yielded about 0·7 L soil. The soil samples were dried for 2 weeks in a greenhouse.

Sample processing and mating type determination

Isolation, propagation, mating type determination and DNA extraction of *P. infestans* from the leaflets collected

Table 1 Number and distribution of each SSR multilocus genotype of *Phytophthora infestans* detected in collected material in four repetitive blocks in a field trial in Sweden in 2001, 3 weeks after inoculation with six isolates of the pathogen

		Distribution of the genotypes					Marker[a]					
Genotype	Mating type	Block 1	Block 2	Block 3	Block 4	% of total[b]	4B	G11	Pi16	Pi56	Pi63	Pi70
Inoculum												
1[c]	A1			1	2	2·0	213/217	160/160	178/178	176/176	148/157	192/192
2	A2	14	19	3	2	25·2	217/217	142/142	176/178	176/176	157/157	192/195
3	A1	6		1	4	7·3	217/217	162/162	178/178	176/176	157/157	192/195
4	A2	16	22	12	5	36·4	217/217	142/160	178/178	176/176	157/157	192/195
5	A1	3	1	2	10	10·6	217/217	142/160	178/178	174/174	157/157	192/195
New												
6	A2			17	7	15·9	217/217	154/206	176/178	176/176	157/157	192/195
7	A1			3		2·0	213/213	142/206	178/178	174/176	157/157	192/192
8	A2			1		0·7	205/213	206/206	176/178	176/176	157/157	192/192

[a]Allele sizes for each marker in bp.
[b]Sum of all blocks divided by total number of isolates (151).
[c]Of the six isolates used as inoculum this was inoculated twice.

in the summer were carried out as described by Widmark *et al.* (2007).

Phytophthora infestans from the soil samples was captured with a detached leaf baiting technique developed by Drenth *et al.* (1995). From each soil sample, aliquots were distributed across three trays (18 cm in diameter). The soil layer was covered with water up to 0·5 cm and detached leaflets of cv. Bintje were floated on the water surface. Over a period of 3 weeks, leaflets with visible blight symptoms were immediately taken away and replaced with a new leaflet. *Phytophthora infestans* was isolated from the infected leaflets as described earlier. Many of the soil isolates grew poorly on rye agar and because of this the mating test was conducted on pea agar (Flier *et al.*, 2003) with known reference isolates. If the isolates did not mate on this medium they were transferred to half-strength pea agar to enhance oospore production. The isolates captured from the soil were transferred to liquid pea medium, cultured for about 3 weeks, and the mycelia harvested and frozen. The mycelia were lyophilized and DNA-extractions were carried out as described earlier (Widmark *et al.*, 2007), except that the homogenization was made with glass beads in a Precellys® preparation shaker (Bertin Technologies).

SSR analysis

The isolates were characterized using SSR markers. The same set of markers as in an earlier study (Widmark *et al.*, 2007) was used: 4B and G11 (Knapova *et al.*, 2001) and Pi16, Pi56, Pi63 and Pi70 (Lees *et al.*, 2006). For DNA extracted from *P. infestans* isolates from infected leaflets collected in the summer the following conditions were used: Forward primers 4B, Pi63 and Pi70 were labelled with 6-FAM (TAC Copenhagen) and G11, Pi16 and Pi56 with NED (Applied Biosystems). The PCR reaction volume of 15 μL contained 0·04 U μL^{-1} ThermoRed *Taq* polymerase (Saveen & Werner) and corresponding buffer Y containing 2 mM $MgCl_2$ and 0·4 μM forward and reverse primers. For primers for locus 4B, 4 mM $MgCl_2$ was used. The annealing temperature for each forward and reverse primer pair was as described earlier (Widmark *et al.*, 2007), except for Pi56, where the annealing temperature was lowered by 2°C. The following PCR conditions were used: 3 min pre-denaturation at 94°C; followed by 30 cycles of denaturation at 94°C for 30 s, 30 s annealing at a temperature depending on the primer, and elongation at 72°C for 30 s; and finally an extension step of 72°C for 25 min. All PCR reactions were performed with a GeneAmp PCR System 2700 (Applied Biosystems). The amplified fragments were separated with an ABI 3730*xl* DNA Analyzer using GeneScan™ ROX-500™ (Applied Biosystems) as the internal size standard at Uppsala Genome Center, Rudbeck Laboratory, Uppsala University. The software GENEMARKER® version 1.6 (Softgenetics) was used to derive the fragment length of the fluorescently labelled fragments.

For DNA extracted from isolates captured from soil the following PCR conditions were used: The forward primers were fluorescently labelled with dye 04 (Well RED; Sigma-Proligo). The PCR reaction assay composition was as mentioned above except that the assay volume was 25 μL. The annealing temperature for each forward and reverse primer and the PCR cycles were as described earlier (Widmark *et al.*, 2007). The amplified fragments were analysed using a Beckman Coulter CEQ™8000 Genetic Analysis System using the CEQ™ DNA Size standard Kit-600 (Beckman Coulter) as an internal size standard. Since the allele sizes of the control isolates differed slightly depending on the analysis system used, the allele sizes obtained with Beckman-Coulter and ABI 3730*xl* were adjusted to the sizes obtained at the Scottish Crop Research Institute (SCRI) (Lees *et al.*, 2006) to make comparison with other studies possible. Therefore, DNA from *P. infestans* isolates representing the six genotypes used for inoculation of the field, together with reference isolates (kindly supplied by Drs Lees and Cooke, SCRI), were included in the analyses.

Determination of parentage

Parentage analysis was performed using a likelihood-based approach in CERVUS version 3.0.3 (Marshall *et al.*, 1998; Kalinowski *et al.*, 2007). CERVUS calculates the combined log-likelihood score for each parental pair/offspring combination (Trio LOD score). Empirical allele frequencies were calculated based on the complete set of genotypes sampled in the same field as the inoculum isolates (Widmark *et al.*, 2007), the summer population investigated in this study and the isolates captured from soil the next season. These data were used to run simulations for the likelihood-based parentage analysis. Positive Trio LOD score values indicate that the candidate parents are more likely parents than randomly selected individuals from the population. For statistical inference, the difference between the LOD score of the most likely and second most likely candidate pair was calculated, using a minimum value of zero for the latter. This difference, called delta, was calculated using strict and relaxed confidence levels, corresponding to 95% and 80%, respectively, as suggested by Marshall *et al.* (1998).

Results

Summer field trial

By the time of sampling of the summer population of *P. infestans*, the first inoculated plants were totally blighted. Abundant oospore formation was observed in the collected leaf material. Some isolates were lost during the isolation and DNA purification phases, and of the 190 isolates collected, 151 were analysed with SSR markers. Three of the genotypes were of mating type A1. In total, eight SSR genotypes were identified, five of which were the inoculum genotypes, in the sampled material 3 weeks after inoculation (Table 1). Of the collected isolates, 81% were of the inoculum genotypes. Not all five inoculum genotypes were found in each block, but all five were found in blocks three and four. Of the inoculum genotypes, SSR type 4 was most frequent and constituted 36% of the sampled isolates. Genotype 1 was only detected three times in the sampled material and was found only in isolates from blocks 3 and 4. In block 3, three genotypes (6, 7 and 8) that were not one of the inoculum genotypes were detected among the sampled isolates. Genotype 6 was also found in block 4 (Table 1). In these three genotypes, new alleles not present in the inoculum isolates were detected. One new allele (206 at locus G11) was found in all these three genotypes (Table 1).

Soil samples in the following year

Five of 12 soil samples collected were infective in the bioassay (Fig. 1). In these five soil samples, infected leaflets were found in only one tray of three from each sample. In total, 27 isolates were recovered. Many of these grew poorly, had low sporulation capacity and were difficult to mate compared to isolates collected during the summer epidemic. With SSR analysis, six different genotypes (A, B, C, D, E and F) were detected among the isolates captured from soil (Table 2). Within the group of isolates representing genotype B, both mating types were found. In the soil sample from plot 4·2, one new allele was detected at locus G11 (156) in one of the genotypes, SSR type D (Table 2). Allele 148 at locus Pi63 and alleles 142 and 162 at locus G11, present in the summer population, were not detected in the soil samples the following year. None of the *P. infestans* isolates baited from the soil samples had multilocus genotypes that matched the previous summer's inoculum isolates.

Table 2 Genotypic characterization using SSR markers of *Phytophthora infestans* isolates captured from soil samples collected the year after a late blight field trial in Sweden

Genotype	Mating type	Number of isolates[a]	Marker[b] 4B	G11	Pi16	Pi56	Pi63	Pi70
A	A2	8	217	160	178	174	157	192
			217	160	178	176	157	195
B	A1	10	213	160	178	176	157	192
			217	160	178	176	157	195
B	A2	1	213	160	178	176	157	192
			217	160	178	176	157	195
C	A2	2	213	160	178	174	157	192
			217	206	178	176	157	195
D	A2	1	205	156	178	176	157	192
			217	160	178	176	157	192
E	A2	3	205	160	176	174	157	192
			213	206	178	176	157	195
			217					
F	A2	2	205	160	178	174	157	192
			213	206	178	176	157	195
			217					

[a]It was considered that multiple occurrences of the same genotype would be confounded by clonal reproduction.
[b]Allele sizes for each marker in bp.

Parentage analysis of isolates captured from soil samples in the next season

Data obtained from the parentage analysis showed that SSR genotypes A and B were most likely recombinants of some of the inoculum genotypes, but that C, D, E and F were recombinants of genotypes from external inoculum sources. Genotype A was assigned as a recombinant of genotypes 4 and 5; genotype B as a recombinant of genotypes 1 and 4. For genotype C, two parental pairs gave positive LOD scores. Since genotypes E and F had three alleles at locus 4B, this locus was excluded in the parentage analyses for these genotypes, as CERVUS cannot handle triploids. Based on five loci, two parent pair combinations gave positive LOD scores for genotypes E and F, respectively (Table 3). To test the effect of a possible mutational event in locus G11 on parental assignment, an additional calculation was made for SSR genotype D,

Table 3 Parentage analysis using the software CERVUS of each SSR multilocus genotype of *Phytophthora infestans* captured from soil samples the year after a late blight field trial in Sweden in 2001

Offspring genotype	A1 parent: genotype	A2 parent: genotype	No of loci	Trio LOD score[a]	Delta[b]	Offspring and parents detected in the same block in the field
A	5	4	6	2·67	2·67[c]	Yes
B	1	4	6	1·78	1·77[c]	Yes
C	7	4	6	3·26	0·67	No
C	5	8	6	2·58	0	No
E	5	8	5	3·78	0·69	No
E	5	6	5	3·09	0	Yes
F	5	8	5	2·14	0·01	No
F	7	4	5	2·12	0	No

[a]Only combinations with positive Trio LOD score are included in the table.
[b]Critical value of delta for 80% confidence (relaxed criterion) is 0·69 for analyses with six loci and 1·18 for analyses with five loci. Corresponding values for 95% confidence are 3·96 and 4·05. See Marshall *et al.* (1998) for details.
[c]Delta significant at 80% confidence (relaxed criterion).

where allele 156 at locus G11 was changed to 154. Both analyses performed on genotype D gave negative LOD scores. Only negative LOD scores were obtained for genotypes 2 and 3 as candidate parents.

Discussion

In this study microsatellite markers were used to monitor changes in the population of *P. infestans* sampled from potato leaves during an epidemic during one growing season, as well as from the soil the following year. From these data, it was possible to see shifts in the population since new genotypes appeared in the samples. Such changes in pathogen populations can arise through mutation, migration or recombination. There have been no published reports of the mutation rate in the *P. infestans* SSR loci used in this study. Preliminary data from Nicaragua (U. Blandón-Díaz, Universidad Nacional Agraria, Nicaragua, personal communication) shows that one SSR genotype dominates in that population, indicating that the mutation rate is not very high in the SSR loci used. Migration or recombination can be responsible for the appearance of new genotypes. This will be discussed in connection with the genotyping data obtained for samples taken from the summer epidemic, as well as those isolated from the soil.

In the summer epidemic, the inoculum genotypes constituted more than 80% of the sampled isolates 3 weeks after inoculation. However, some of these isolates seem to have been more successful than others, e.g. SSR genotype 4 (Table 1). In the collected material from the field, three 'new' genotypes were detected, of which genotype 6 was the most prevalent, competing well with the inoculated genotypes in these two blocks. The appearance of allele 206 at locus G11 probably rules out recombination or mutation. Immigration is the most likely origin of these new genotypes. For this particular field, these migrants could have arisen from airborne sporangia or infected tubers (at least two, since genotype 6 was found in two separate blocks). Also, it cannot be excluded that oospores in the soil produced in previous potato crops could have resulted in genotypes 6, 7 and 8. Immigrants could also have contributed to other novel genotypes not detected in the sampled material. Even though the sampling was intensive (50 isolates from about 3 m^2/block), the sampled isolates constituted a minor part of all isolates in the sampling plots.

An assigned parent pair/offspring combination was not always found together within the same block in the sampled material (Table 3). Not only the size of the sample but also the time of sampling may affect the results because of the continuous selection during an epidemic. Genotype 1 was only detected three times in the sampled material (which only constitutes a very small portion of all blighted leaves in the field), but still succeeded in mating and giving rise to progeny. Possible explanations are that genotype 1 is a better parent or was more abundant at an earlier stage of the epidemic and succeeded to mate, but was later outcompeted by other genotypes. More abundant genotypes have a greater chance to mate than less frequent ones. Genotype 4 was successful, both during the epidemic in the field and also as a parent. However, genotypes 2 and 3 were prevalent, but apparently not involved as parents, since their respective alleles at locus G11 were not found in the progeny.

Only five of the 12 soil samples yielded infected leaflets in the bioassay. Despite the abundant oospore formation in the field the previous summer season, the baiting technique did not capture *P. infestans* from all soil samples. The low sensitivity of this technique was reported by Lehtinen & Hannukkala (2004), who only obtained infection from three of 16 soil samples using this bioassay. Moreover, the number of oospores in the soil will not be directly indicative of the inoculum potential of the soil, since only successful mating will result in the formation of oospores that can germinate and infect potato plants (Pittis & Shattock, 1994; Fay & Fry, 1997; Flier *et al.*, 2001; Knapova *et al.*, 2002).

Novel genotypes, none of which were identical to any of those found during the previous year, were isolated from the soil samples. Since they came from oospores in the soil, they could be either recombinants from individ-

uals present the previous year, or oospores that were already present in the soil. If complete control of parental individuals had been possible, simpler methods of parentage analysis, such as exclusion, could be used (Jones & Ardren, 2003). In inoculated field trials using semi-natural populations of *P. infestans*, it is impossible to exclude interference from other inoculum sources such as soil, seed tubers and airborne migrants. Numerous methods, of which CERVUS is one, have been developed to determine parentage in natural populations (Jones & Ardren, 2003), where incomplete sampling of the parents is quite common. Results from CERVUS can be interpreted as a likelihood as to whether a particular candidate individual is more or less likely to be a parent than a random individual from the background population. This study used CERVUS to determine that the inoculum isolates 4 and 5 were probably the parents of soil genotype A, and 4 and 1 the parents of genotype B. This strongly indicates that overwintering oospores produced in crossings of some of the inoculum isolates generated progeny the following season. For genotype C, there were two possible sets of parents, but the higher LOD score for 7×4 indicates that this parental combination was more likely than 5×8. Also, it can be seen from the result of the analysis that the genotypes C, E and F could be siblings (Table 3).

Likelihood-based analyses of parentage are not perfect, and are conditional on the data available. If the population being studied has a high proportion of common alleles, assigning parentage can be difficult. While the deliberate inclusion of inoculum isolates with rare alleles would increase the probability of determining parentage, this cannot be done in studies using natural or semi-natural populations. Another major shortcoming is the inability to handle triploids, which occur with *P. infestans*, although this problem is hardly unique for CERVUS. In this study, locus 4B was eliminated when triploids occurred, reducing the number of loci to five. Since the critical values of delta required for significance were bootstrapped based on allele frequencies at the various loci, elimination of locus 4B for soil genotypes E and F limited the power of CERVUS to determine the parents. However, crosses involving genotype 8 were probably the most likely parents since they had slightly higher LOD scores than others, and genotype 8 was the only genotype with allele 205 at locus G11. Genotypes E and F both contained relatively common alleles at the remaining loci, which reduced the ability to determine their parents. The parents of genotype D could not be identified, even when allele 156 was replaced with 154. Despite these drawbacks, it was concluded that a program such as CERVUS can be a valuable tool in assigning parentage.

Three alleles were amplified at locus 4B in two genotypes captured from the soil. This phenomenon for locus 4B was previously reported from surveys in the UK as a rare occurrence (Lees *et al.*, 2006). In mating experiments trisomic offspring have been found (Carter *et al.*, 1999; Lee *et al.*, 2002). Van der Lee *et al.* (2004) demonstrated the presence of trisomic linkage groups in some progeny in crossing experiments. In late blight experiments in plastic tunnels in the UK, triallelic loci were detected in the offspring generation of *P. infestans* in some isolates from the following crop (Shaw *et al.*, 2009). These reports show that triallelic loci may appear in *P. infestans* isolates, and that they seem common after sexual recombination, although they complicate genetic analyses.

The use of SSRs as markers makes it possible to compare genotypes analysed in different laboratories, but only if reference isolates are always included in the analysis. Even if SSRs are considered to be very robust markers, differences in allele sizing do appear across laboratories (Jones *et al.*, 1997; Weeks *et al.*, 2002), since the allele sizes determined depend on the analysis system used. The estimated allele size is not only dependent on the number of nucleotides, but also on the mobility of the fragment in the electrophoresis. For example, DNA fragments with a high GC content migrate differently compared with fragments of the same length having a low GC content. Exact fragment size can only be obtained by sequencing (Haberl & Tauz, 1999; Amos *et al.*, 2007).

The fact that recombinant genotypes of the summer generation of *P. infestans* in the field studied were found in the detached leaf assay the next season is consistent with the hypothesis that the oospores produced during the previous summer can survive the Swedish winter and give rise to infective progeny the next season. However, there are still several ways in which studies like this can be improved. The effects of immigration could be reduced via site selection (temporal and physical isolation from potato production) and the use of disease-free planting material. A more comprehensive survey of allele frequencies would make the determination of parentage more robust and using more markers would increase the resolving power of the analyses. Nevertheless, SSR genotype data are a useful tool to study selection of *P. infestans* through a summer epidemic, and combined with parentage analysis enable the identification of the origin of oospore-derived isolates. Experimental evidence that directly confirms the role of oospores as primary inoculum is difficult to obtain. However, this work, in combination with earlier Nordic studies, provides substantial support for the hypothesis that overwintering oospores serve as an inoculum source in the Nordic countries.

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