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Genetic analysis of *Phytophthora infestans* populations in the Nordic European countries reveals high genetic variability

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ABSTRACT

Late blight, caused by the oomycete *Phytophthora infestans*, is the most important disease of potato (*Solanum tuberosum*). The pathogen is highly adaptable and to get an overview of the genetic variation in the Nordic countries, Denmark, Finland, Norway and Sweden we have analyzed 200 isolates from different fields using nine simple-sequence repeat (SSR) markers. Forty-nine alleles were detected among the nine SSR loci and isolates from all four Nordic countries shared the most common alleles across the loci. In total 169 multilocus genotypes (based on seven loci) were identified among 191 isolates. The genotypic diversities, quantified by a normalized Shannon's diversity index (H_s), were 0.95 for the four Nordic countries. The low F_{ST} value of 0.04 indicates that the majority of variation is found within the four Nordic countries. The large number of genotypes and the frequency distribution of mating types (60 % A1) support the hypothesis that sexual reproduction is contributing notably to the genetic variation of *P. infestans* in the Nordic countries.

Introduction

The oomycete *Phytophthora infestans* that causes late blight in potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*) is considered one of the world's most devastating plant pathogens. Late blight in potatoes led to the Irish potato famine in the mid-1840s, which resulted in the death and displacement of millions of people, and this disease is still among the worst crop diseases of the world despite much research efforts over the years (recently reviewed by Fry 2008).

P. infestans is hemibiotrophic and the pathogen generally survives between crop seasons in potato tubers. *P. infestans* spreads asexually *via* sporangia, which are dispersed by water or wind, hence having the potential to spread over longer distances (Aylor 2003). *P. infestans* is diploid and heterothallic with two known mating types, A1 and A2. Interaction between hyphae of opposite mating type induces the formation of antheridia and oogonia that may associate and fuse to form an oospore, which means that the pathogen has the potential to reproduce sexually. In contrast to sporangia, oospores are

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tolerant of adverse conditions and can survive in soil between growing seasons (Drenth et al. 1995).

Until the mid-1970s, isolates of *P. infestans* found outside North America were generally considered to belong to the US-1 clonal lineage (mating type A1; Goodwin et al. 1994), but during the 1980s, a novel mating type, A2, was detected in Europe (Hohl & Iselin 1984). The appearance of mating type A2 could, in principle, allow *P. infestans* to reproduce sexually, with subsequent effects on disease epidemiology and control. Firstly, oospores represent a stable resting phase that is independent of the host. Secondly, sexual reproduction is likely to increase the adaptability of the organism. Interestingly, in the last two decades, new more virulent strains and increased frequencies of fungicide resistance have been observed (Fry 2008). There are now several studies showing that the pathogen population in Europe is becoming increasingly diverse (Drenth et al. 1994; Sujkowski et al. 1994; Andersson et al. 1998; Brurberg et al. 1999; Hermansen et al. 2000; Turkensteen et al. 2000; Flier et al. 2007; Widmark et al. 2007).

Over the years, a range of markers, both phenotypic and genotypic, have been used for studying genetic variation in *P. infestans* (reviewed by Cooke & Lees 2004). Standardized, validated simple-sequence repeat (SSR) protocols for improved comparison between *P. infestans* isolates, across labs and countries, as well as a database with genotypic data have made SSR-based techniques the tool of choice for studying genetic variation in *P. infestans* (Lees et al. 2006; www.eucablight.org).

Since *P. infestans* remains a major pathogen showing increasing adaptability, it is interesting to monitor its diversity, especially in areas where this diversity seems to be high. Previous studies on Nordic potato crops have revealed considerable diversity (Andersson et al. 1998; Brurberg et al. 1999; Hermansen et al. 2000; Flier et al. 2007; Widmark et al. 2007) leading to the hypothesis that sexual reproduction has been going on for more than a decade already, at least in the regions that were analyzed in previous studies. To get better insight into Nordic diversity and to analyze the consequences of more than one decade of (presumed) sexual reproduction, we have now conducted a large study on genetic diversity of *P. infestans*, covering all important potato growing regions in the Nordic countries. We have used the highly reproducible and high-throughput SSR-based technique for analyzing genetic variation in a large collection of recent isolates spanning over four Northern European countries, Denmark, Finland, Norway, and Sweden.

Materials and methods

Collection and culturing of isolates of Phytophthora infestans

During summer 2003, 743 isolates of *P. infestans* were collected from 320 potato fields, both conventional and organic, in the four Nordic countries (Lehtinen et al. 2008). Isolates were obtained from leaves with single lesions, and were mainly collected when approximately 10 % of the leaf area was affected by blight. Details regarding isolations and phenotypic characterizations, including determination of mating types, have been described by Lehtinen et al. (2008). Axenic isolates were kept on different agar media (Lehtinen et al. 2008) at 18 °C in the dark and were transferred every 4–6 weeks until characterization or storage. To get an overview of the genetic variation in the Nordic countries, 50 isolates from each country were selected for genetic analysis. A hierarchical sampling strategy was used at three macrospatial scales including countries, districts and fields. The selection of isolates covered all important potato growing regions in the Nordic countries, as well as some areas with a more extensive production (Fig 1). All selected isolates were from separate fields to avoid analyzing clones within a field, but a few fields were located close to each other. Mating type analysis of a larger number of isolates from the fields covered in this study showed that approximately 60 % of the isolates were A1 mating type in each country. Furthermore, it was shown that both mating types were present in 40 % of the fields where more than one isolate was tested, indicating strong potential for sexual reproduction (Lehtinen et al. 2008). Around a half (54 %) of the isolates selected for genetic analysis were A1. Thus, the mating type distribution among the tested isolates reflected the mating type distribution in the total collection of isolates.

DNA extraction and SSR analysis

Mycelium for DNA extraction was grown on plates of pea agar or a mixture of pea and rye B agar (Lehtinen et al. 2008), for 2–4

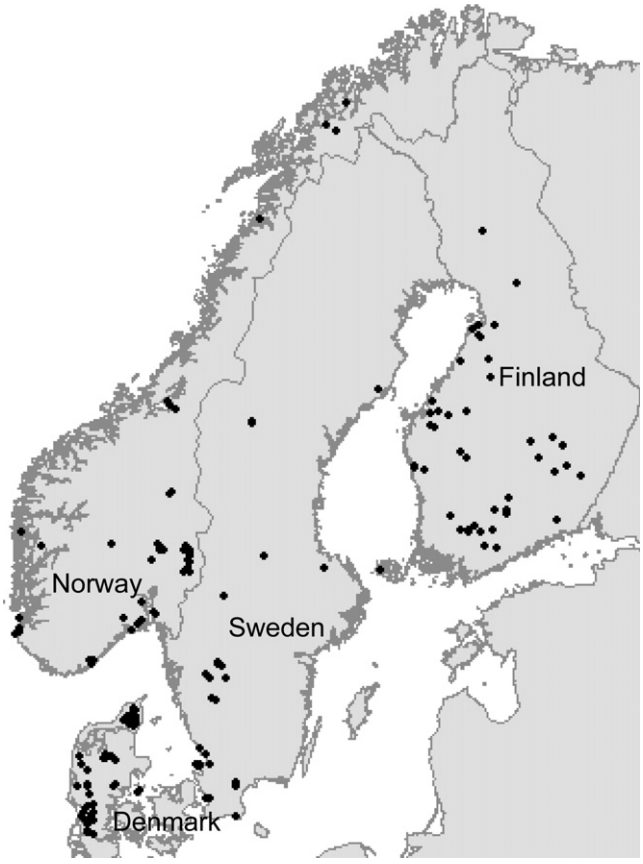

Fig 1 – Location of the potato crops from which the 200 *Phytophthora infestans* isolates were sampled.

weeks at 18 °C in the dark. Mycelium from plates was scraped off with a scalpel blade and blotted dry on filter paper. The mycelium was frozen in liquid nitrogen and disrupted using a grinding mill (model MM301; Retsch, Haan, Germany) for 1.5 min at a vibration frequency of 30 oscillations per second. DNA was extracted using the DNeasy® Plant Mini Kit (Qiagen), according to the manufacturer's instructions.

Nine polymorphic SSR regions were amplified using PCR with primers from previous studies: Pi02, Pi04, Pi16, Pi26, Pi33 (Lees *et al.* 2006); 4B, 4G, G11, D13 (Knapova & Gisi 2002). The primers were obtained from Applied Biosystems. For automated fragment analysis, one primer of each locus was labelled with a fluorescent dye (6-FAM, NED, PET, or VIC). Dyes were assigned to loci in such a way that loci with the same dye had nonoverlapping ranges of allele sizes. This allowed simultaneous loading of several loci amplification reactions from one isolate, onto the capillary system (see below). Amplifications were performed in 10-μl reaction volumes with 1.0 unit Taq DNA polymerase (Applied Biosystems), 200 μM dNTP, 10 pmol each of forward and reverse primer, 1.5 mM $MgCl_2$, and 1 μl of 10× AmpliTaq buffer (500 mM KCl, 100 mM Tris–HCl pH 8.3 and 15 mM $MgCl_2$). A total of 1 μl from the DNA preparations was used as a template in each reaction. The thermal cycling was carried out as follows: 95 °C for 4 min, followed by 35 cycles of 95 °C for 30 s, 59 °C for 30 s, 72 °C for 30 s, and a final extension of 7 min at 72 °C, before cooling to 4 °C. All PCR reactions were performed with a GenAmp PCR System 9700 (PE Applied Biosystems).

The fluorescently labelled PCR products were analyzed using an ABI3730 DNA Analyzer with 48 36 cm capillaries, using Performance Optimized Polymer for 3730 DNA Analyzers (POP-7; Applied Biosystems). 1 μl of 12.5 − 71-fold (depending on the different markers) diluted PCR products were added to a loading buffer containing 8.8 μl Hi-Di™ formamide (Applied Biosystems), and 0.2 μl of GeneScan 500 LIZ size standard (Applied Biosystems). Electrophoresis of the samples was carried out at 66 °C and at 15 kV, for 20 min. The data was collected using the software Data Collection v 2.0 (Applied Biosystems), while GeneMapper v 4.0 (Applied Biosystems) was used to derive the fragment length of the labelled DNA-fragments using the known fragment lengths of the LIZ-labelled marker peaks.

Allele size was determined by using the marker and by comparison with ten reference isolates (C1–10) kindly supplied by Drs Lees and Cooke (Lees *et al.* 2006). For some isolates, three or four alleles were consistently identified at one or more loci. This was mainly for locus Pi26, where as many as 43 and 31 isolates gave three or four alleles respectively. In addition, markers Pi02, Pi04, and G11 gave three alleles for ten, three and one of the isolates respectively. This phenomenon has also been reported by Lees *et al.* (2006), including for five of the ten reference isolates that were used in this study. Following the strategy described by Holmes *et al.* (2009), the individuals with more than two alleles at a locus were included in the present analysis after systematically excluding the largest allele(s). While exclusion of one or two alleles leads to an underestimation of genetic variation at the loci in question, it allowed us to consider more isolates in our analysis.

Multilocus genotypes were compiled for all isolates using the seven loci Pi02, Pi04, Pi16, Pi26, Pi33, 4B, G11. If data were missing in more than one of the seven loci for an isolate, it was discarded from the genotype calculations. If an isolate contained one missing locus it was considered to be identical to another if the other six loci were identical. This could potentially lead to an underestimation of genotypic diversity.

Data analysis

Deviations from Hardy–Weinberg equilibrium at all loci were tested with a chi-square test using POPGENE version 1.32 (Yeh *et al.* 2000). In addition we calculated the index of association (I_A) to test for random recombination between pairs of all the loci. This test quantifies the degree of recombination within these loci through the formula: $I_A = V_O/V_E - 1$ (where V_O is the variation observed in the number of loci in which two individuals differ and V_E is the expected variation). $I_A = 0$ indicates frequent recombination events and I_A values significantly larger than 0 indicates increasing association between loci, and possibly also increasing clonality. Monte Carlo simulations and parametric method were used to test the significance of any difference between the calculated variance (V_D) and the variance expected at linkage equilibrium (V_E). These tests were carried out using the software LIAN (Haubold & Hudson 2000).

Genotypic diversity was calculated by a normalized Shannon's diversity index (H_s): $H_s = -\sum P_i \ln P_i / \ln N$, where P_i is the frequency of the *i*th multilocus genotype and *N* is the sample size. This diversity index corrects for differences in sample size (Sheldon 1969). Values for H_s may range from 0 (single genotype present) to 1 (each isolate in the sample has a different genotype). The Shannon index could in principle lead to incorrect conclusions, particularly when diversity is low or sample sizes differ as pointed out by Grünwald *et al.* (2003). However in our study the diversity is high and the sample size is in principle the same for all the four countries included.

Nei's genetic distance (Nei 1978) was calculated using POPGENE (Yeh *et al.* 2000). We measured the F_{ST} values to estimate the genetic differentiation among all the four countries, also using the POPGENE software (Yeh *et al.* 2000). The level of divergence for each country was calculated as the mean value of pairwise F_{ST} for each country against the remaining countries using the Arlequin software package, version 2000 (Schneider *et al.* 2000). The significance of F_{ST} values was tested by 1023 permutations.

A Principal Coordinate (PCO) analysis was carried out to perform ordination analysis and to classify and detect structure in the relationships between *Phytophthora infestans* isolates using NTSYS-pc software, version 2.0 (Exeter Biological Software, Setauket, NY).

Results

All the nine tested SSR loci were polymorphic for the selected 200 Nordic *Phytophthora infestans* isolates. Whereas seven of the nine loci were detected in most of the isolates, the SSR analysis failed to give results for loci D13 and 4G in 31 and 28 % of the isolates, respectively. The failure to produce

results for these loci occurred with approximately the same frequencies in isolates from all the four Nordic countries. These two loci were therefore omitted from the genotype calculations.

The number of alleles detected at nine SSR loci in the 200 isolates analyzed was in total 49, ranging from two (at locus Pi33) to nine (at locus D13 and G11) (Table 1). Isolates from all four Nordic countries shared the most common alleles across the analyzed SSR loci. For four of the nine loci (Pi02, Pi33, 4G, D13), one specific allele was detected in 70–80 % of all the isolates, while for the other five loci (Pi04, Pi16, Pi26, 4B, G11) two specific alleles were detected in 62–95 % of all the isolates. For locus Pi02, the most frequent allele (162) was considerably less frequent in Denmark than the overall

Table 1 – Allele frequencies for SSR markers in 192 *P. infestans* isolates from the Nordic countries collected in 2003.

SSR locus	Allele	Allele frequency					Gene diversity (H)[a]
		Denmark ($n = 49$)	Finland ($n = 50$)	Norway ($n = 50$)	Sweden ($n = 50$)	Overall ($n = 199$)	
Pi02	152	0.22	0.06	0.01	0.08	0.09	0.48
	158	0.01	0.00	0.01	0.01	0.01	
	160	0.26	0.08	0.14	0.10	0.14	
	162	0.51	0.86	0.65	0.79	0.70	
	164	0.00	0.00	0.02	0.00	0.01	
	166	0.00	0.00	0.01	0.02	0.01	
	174	0.00	0.00	0.07	0.00	0.02	
	176	0.00	0.00	0.09	0.00	0.02	
Pi04	160	0.07	0.05	0.09	0.07	0.07	0.68
	166	0.33	0.29	0.35	0.30	0.32	
	168	0.18	0.20	0.09	0.26	0.18	
	170	0.42	0.46	0.47	0.37	0.43	
Pi16	158	0.00	0.00	0.07	0.00	0.02	0.47
	160	0.00	0.00	0.14	0.00	0.03	
	174	0.17	0.42	0.31	0.22	0.28	
	176	0.83	0.58	0.49	0.78	0.67	
Pi26	171	0.11	0.00	0.03	0.01	0.04	0.69
	173	0.11	0.00	0.03	0.01	0.04	
	177	0.37	0.28	0.25	0.35	0.31	
	179	0.07	0.08	0.13	0.14	0.11	
	181	0.28	0.60	0.50	0.39	0.45	
	183	0.05	0.04	0.06	0.09	0.06	
	185	0.01	0.00	0.00	0.01	0.01	
Pi33	203	0.97	0.77	0.70	0.78	0.80	0.31
	206	0.03	0.23	0.30	0.22	0.20	
4B	205	0.09	0.20	0.24	0.22	0.19	0.63
	213	0.55	0.45	0.29	0.50	0.45	
	217	0.36	0.35	0.47	0.28	0.36	
4G	161	0.04	0.07	0.09	0.07	0.07	0.32
	163	0.93	0.77	0.77	0.78	0.80	
	165	0.04	0.16	0.14	0.16	0.13	
D13	118	0.01	0.02	0.03	0.04	0.03	0.47
	132	0.01	0.00	0.00	0.03	0.01	
	134	0.01	0.02	0.00	0.08	0.03	
	136	0.74	0.64	0.86	0.65	0.72	
	138	0.03	0.00	0.00	0.04	0.02	
	140	0.06	0.17	0.02	0.03	0.06	
	152	0.00	0.00	0.02	0.05	0.02	
	154	0.06	0.08	0.06	0.09	0.07	
	156	0.07	0.09	0.02	0.01	0.05	
G11	142	0.07	0.18	0.09	0.11	0.11	0.78
	148	0.09	0.00	0.04	0.05	0.05	
	152	0.00	0.00	0.02	0.01	0.01	
	154	0.14	0.08	0.03	0.02	0.07	
	156	0.11	0.37	0.42	0.25	0.30	
	158	0.00	0.00	0.01	0.01	0.01	
	160	0.32	0.11	0.04	0.11	0.14	
	162	0.26	0.26	0.34	0.40	0.32	
	164	0.00	0.00	0.00	0.04	0.01	

a $H = 1 - \sum x_j^2$, where x_j is the frequency of the jth allele at the locus (Nei 1978).

population. While the most frequent alleles of loci Pi33 and 4G, were noticeably more frequent in Denmark than for all the Nordic countries together. Of the rarer alleles, five were detected only in Norway, at loci Pi02 and Pi16, where they were detected with relative low frequencies (0.01–0.14). The five 'Norwegian alleles' were observed in 14 isolates, originating from different regions of Norway, meaning that each of these isolates possessed at least two of such alleles. One low frequency private allele at locus G11 was detected in Sweden.

The allele frequencies were processed further to consider the combination of different alleles at each separate SSR locus, and gene diversities for each locus were calculated. The gene diversity for single loci for all countries together (overall) ranged from 0.31 to 0.78 (Table 1), and the average for all loci was 0.54 which is 71 % of the maximum gene diversity based on the number of observed alleles in all Nordic countries (results not shown). There was some variation in gene diversity for each locus in each of the countries, but the average gene diversity for each country was approximately the same (0.48–0.54) (results not shown).

The 191 isolates in which we were able to score six loci or more, produced in total 169 distinct multilocus genotypes, meaning that most genotypes were detected only once. Five genotypes occurred twice, one genotype was detected four times (three in Sweden and one in Denmark) and two genotypes occurred eight times. Of the two most frequent genotypes, one occurred only in Denmark (seven of the eight occurrences in one region) and the other was detected twice in Finland (in the same region) and six times in four different regions in Norway. The genotypic diversities, quantified by a normalized Shannon's diversity index (H_s), were larger than 0.9 for all the four individual countries and for the Nordic countries together 0.95 (Table 2).

The chi-square test (result not shown) failed to reject the null hypothesis indicating that all loci were under Hardy–Weinberg equilibrium. Furthermore, the frequency of recombination was estimated by calculating the standardized index of association (I_A^S) between loci. The I_A^S value for the 191 isolates analyzed was 0.0478, which is not significantly different from zero ($P < 0.001$) indicating that the isolates were in linkage equilibrium and hence showing evidence of a high rate of sexual reproduction among the isolates.

Genetic differentiation within the Nordic countries as measured by F_{ST} was 0.04. Pairwise measures of genetic distance ranged from 0.025 to 0.126 (Table 3). Pairwise F_{ST} was calculated for each pair of countries over seven loci. The results showed very low differentiation between all pairs of countries (Table 3). The PCO analysis indicated no association between the SSR fingerprint (or genetic diversity) and geographical origin (Fig 2).

Discussion

Using nine SSR markers, including several newly developed ones (Lees *et al.* 2006), we detected in total 49 alleles in the *Phytophthora infestans* populations from Denmark, Finland, Norway, and Sweden. Most of these alleles have previously been detected in other *P. infestans* populations (www.eucablight.org). The frequency distribution of the alleles varied somewhat between the Nordic countries and was also slightly different from what has been observed previously in other countries. The low F_{ST} value of 0.04 indicates that the majority of variation is found within, and not among, the four Nordic countries. Individuals within countries are likely to be genetically different, but each country contains the same complement of alleles in similar frequencies. The overall picture emerging from the results of the allele frequencies is that the isolates sampled in the four Nordic countries come from a common population of

Table 2 – Genotypic diversity (single and multilocus) of *P. infestans* isolates from the Nordic countries collected in 2003.

SSR locus	Number of genotypes	*N*	Genotypic diversity[a]				
			Denmark ($n = 49$)	Finland ($n = 50$)	Norway ($n = 50$)	Sweden ($n = 50$)	Overall ($n = 199$)
Pi02	12	198	0.416	0.207	0.355	0.291	0.275
Pi04	6	191	0.262	0.311	0.211	0.299	0.211
Pi16	6	186	0.182	0.261	0.331	0.223	0.212
Pi26	15	195	0.445	0.379	0.445	0.507	0.375
Pi33	3	194	0.062	0.199	0.214	0.175	0.138
4B	6	199	0.362	0.425	0.460	0.373	0.301
4G	5	144	0.123	0.266	0.303	0.206	0.184
D13	16	139	0.341	0.403	0.224	0.479	0.321
G11	25	168	0.647	0.591	0.499	0.624	0.491
Multilocus[d]	169		0.903	1.000	0.936	0.966	0.954
			3.437[b]	3.912[b]	3.644[b]	3.721[b]	5.013[b]
			0.945[c]	1.000[c]	0.969[c]	0.989[c]	0.977[c]

a Normalized Shannon index for all single loci and mulitilocus genotypes. The number of isolates analyzed from each country is given below each country name.
b Non-normalized Shannon index for multilocus genotypes in each country.
c Shannon index divided by number of genotypes.
d The multilocus genotype is based on the seven markers Pi02, Pi04, Pi16, Pi26, Pi33, 4B, G11 from 45, 50, 49 and 47 isolates from Denmark, Finland, Norway, and Sweden respectively.

Table 3 – Matrix of genetic distance and population differentiation for *Phytophthora infestans* populations from the Nordic countries, based on analysis of seven polymorphic loci. Values below the diagonal are Nei's genetic distance; values above the diagonal are estimated F_{ST} values for each pairwise comparison of isolates, sampled from Norway, Denmark, Finland, and Sweden.

Regions	Norway	Sweden	Finland	Denmark
Norway		0.03819[a]	0.03189[a]	0.04091[a]
Sweden	0.051		0.04819[a]	0.04364[a]
Finland	0.025	0.026		0.04574[a]
Denmark	0.126	0.053	0.101	

a Significant at $P \leq 0.001$.

P. infestans. However, there is in general no exchange of seed potatoes between the Nordic countries.

The PCO analysis showed that there was no distinguishable pattern of clustering of isolates from a certain country. The lack of geographic clustering was also supported by the low F_{ST} values found among the countries (Table 3). The absence of a geographic structure suggests a relatively recent expansion of a single diverse population.

The SSR analysis of *P. infestans* isolates from the Nordic countries, Denmark, Finland, Norway, and Sweden, revealed a high level of variation with respect to genotypes. Most isolates had a distinct genetic fingerprint with 169 genotypes detected among the 191 isolates in which we were able to score six SSR loci or more. No particular genotypes dominated in any of the four countries, but one genotype occurred seven times in the same district in Denmark. Our data shows that genotypic variation in *P. infestans* anno 2003 is even larger than the variation found in most previous studies. A study of isolates collected in Finland and Norway in the period 1992–1996 using RFLP (RG57) fingerprinting yielded genotypic diversities (H_s) for the Norwegian and Finnish isolates of 0.75 and 0.83, respectively (Brurberg *et al.* 1999). A study of early infection in a single field in southwest Sweden using six SSR loci including 4B, G11, and Pi16 used in the current study revealed unique genotypes for five out of the six discrete disease foci studied, and the genotypic diversity (H_s) was 0.54 (Widmark *et al.* 2007). A comparison of the genetic variation of *P. infestans* isolates from organic potato crops in Norway, France, Switzerland and the United Kingdom that was conducted in 2001 using AFLP showed that genetic variation in Norway was higher than in the other countries (Flier *et al.* 2007). While it should be noted that observed genetic diversities to some extent depend on markers used and the geographic scales of the analyses, the overall picture provided by the data referred to above indicates a relatively high genetic diversity in the Nordic countries.

High levels of genotypic diversity such as those found in the present study have previously been described for *P. infestans* populations in other North European countries (Sujkowski *et al.* 1994; Drenth *et al.* 1994) as well as in the two regions that are currently considered as possible origins of *P. infestans*, central Mexico (Goodwin *et al.* 1992) and the South American Andes (Goméz-Alpizar *et al.* 2007). In contrast, *P. infestans* populations in other parts of the world, including north-western Mexico (Goodwin *et al.* 1992), Ecuador (Forbes *et al.* 1997), Asia (Koh *et al.* 1994; Le *et al.* 2008), North America (Goodwin *et al.* 1995) and other parts of Europe (Lebreton & Andrivon 1998; Day *et al.* 2004; Cooke *et al.* 2006; Flier *et al.* 2007; Montarry *et al.* 2008, 2010) are dominated by clonal lineages. In some of these latter countries, most local populations consist of a single clonal lineage (either A1 or A2), which restricts sexual reproduction.

So far, there are few published studies that employ the same large set of markers used here. In a recent study aimed at testing the utility of SSR markers for analysis of *P. infestans*

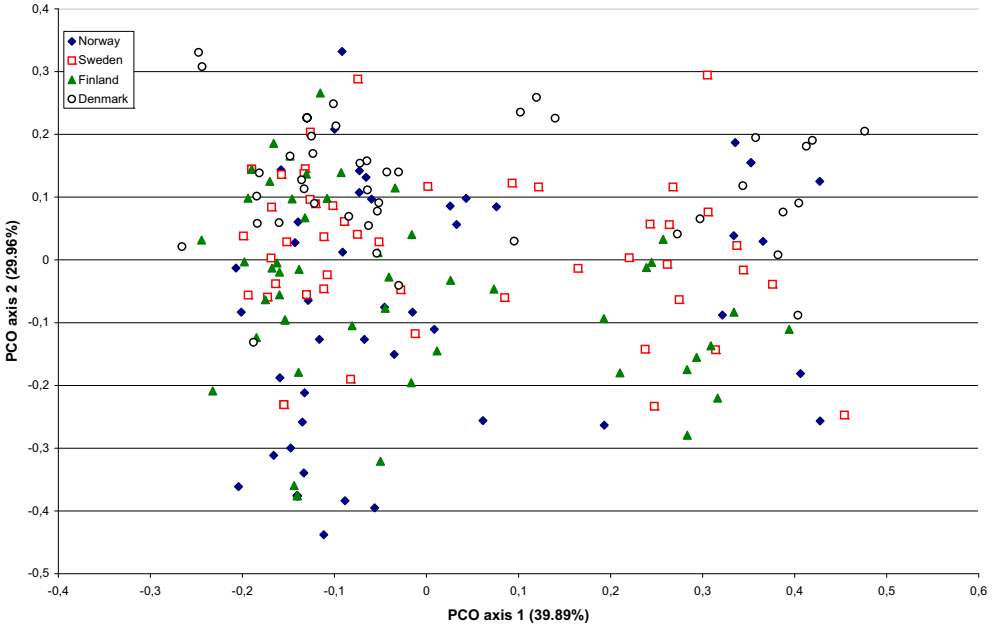

Fig 2 – PCO analysis of 200 *Phytophthora infestans* isolates from Norway, Sweden, Finland, and Denmark based on SSR diversity of seven polymorphic loci with totally 37 alleles.

populations, 77 isolates from the UK and 13 from the rest of the world were analyzed for genetic variation using 12 SSR markers (Lees *et al.* 2006), including all markers used in the present study, except 4G. Even though these isolates were selected in anticipation of a high level of diversity and were scored with up to 12 markers, only 68 genotypes were detected among the 90 isolates (Lees *et al.* 2006). Increasing the number of markers normally increases the number of genotypes, and in the study by Lees *et al.* (2006), maximum resolution was achieved by using ten loci. Increasing the number of markers from seven to ten increased the number of genotypes by approximately 30 % in their selected populations (Lees *et al.* 2006). This shows that the Nordic *P. infestans* populations have an extremely high genotypic diversity compared to other populations studied, when using the same tools for analysis. It is possible that our findings to a certain degree reflect our sampling strategy (one isolate from each field), but it still seems apparent from our study that the Nordic *P. infestans* populations are genetically highly variable.

What is less clear is the cause of the high level of genetic variation compared to many other countries. Judging from the recent mating type distributions, which showed that approximately 60 % of the isolates were of mating type A1 in each country and that both mating types were often found in the same fields (Lehtinen *et al.* 2008) there is clearly a potential for frequent sexual reproduction. Previous studies indicate that both mating types have been present in the Nordic countries since around 1992. The mating type frequencies have been monitored most carefully in Finland, in which A2 increased gradually from approximately 20 % in 1992 to 50 % in 2000, so the potential for sexual recombination is not entirely new. It is well known that sexual recombination increases genotype diversity in populations, since it creates novel recombinants. A high level of recombination is suggested by the low index of association and the fact that no loci are in linkage disequilibrium. The hypothesis that sexual reproduction greatly contributes to the variation in the population is also supported by the fact that the number of alleles at each locus and their frequencies are not very different from what we see in for example UK where the population is regarded as mainly clonal (Lees *et al.* 2006).

In the first report concerning the high level of genetic variability in Norway and Finland compared to some other European countries it was speculated that the increased rate of sexual reproduction could be caused by climatic differences between the Nordic countries and other European countries (Brurberg *et al.* 1999). Comparatively cool summers slow the infection process and the deterioration of tissue (Erwin & Ribeiro 1996) and such conditions may promote mating and oospore formation. Romero-Montes *et al.* (2008) concluded from an experiment in Mexico that oospore formation is more dependent on environmental factors (rain) and on the induction of slow epidemics (disease management), than on the genetic makeup of the host. In a climate with cold winters, inoculum from volunteers and dump piles are less important since survival of *P. infestans* on tubers is largely dependent on the viability of the tuber tissue as well as the pathogen's physiological capability of surviving low temperatures or freezing conditions. As a result oospores may be proportionally more important as a source of inoculum resulting in a population more influenced by sexual recombination. Frost in the soil might also synchronize the germination of oospores with the planting of the potato crop (Widmark *et al.* 2007) and in this way further increases the importance of oospores as inoculum of *P. infestans*. Oospores have indeed been detected in numerous studies in the Nordic countries and are considered important inoculum sources (Andersson *et al.* 2009).

Other mechanisms, such as mutation and mitotic recombination, may also contribute to genetic variation in the *P. infestans* populations. Asexual progenies of *P. infestans* have previously been shown to differ from their parents in several characteristics such as aggressiveness, growth rate, colony morphology and virulence (Caten & Jinks 1968; Abu-El Samen *et al.* 2003). Mitotic gene conversion was observed to occur at remarkably high frequencies in *Phytophthora sojae* (Chamnanpunt *et al.* 2001) documenting potential for rapid generation of variation. In general populations with large effective sizes, such as populations of *P. infestans*, tend to have higher gene diversity, as more alleles can emerge through mutation and fewer alleles will be lost due to random genetic drift (Hartl & Clark 1997). However, there is no known reason that these mechanisms should contribute to more diversity in *P. infestans* in the Nordic region than elsewhere.

This study, conducted with isolates of *P. infestans* taken from different fields early in the late blight epidemic indicates that the pathogen population is extremely diverse, with 169 unique multilocus genotypes detected among 191 isolates. While allele frequencies are similar in the different Nordic countries, only a few genotypes occurred more than once. This study, together with others on the equal numbers of both mating types, presence of oospores in field material, inoculum sources in the soil, and variability between and within infection foci, indicate that sexual reproduction is a major determinant of the population structure of *P. infestans* in the Nordic countries.

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