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Variability of *Peronospora sparsa* (syn. *P. rubi*) in Finland as measured by amplified fragment length polymorphism

Hannele Lindqvist-Kreuze[1], Hilkka Koponen[2] and Jari P.T. Valkonen[1,2,*]
[1]*Department of Plant Biology, Genetic Center, Swedish University of Agricultural Sciences (SLU), PO Box 7080, S-750 07 Uppsala, Sweden, and Institute of Biotechnology, University of Helsinki, Finland;*
[2]*Department of Applied Biology, PO Box 27, FIN-00014 University of Helsinki, Finland;*
[*]*Author for correspondence (Fax: +4618673392; E-mail: jari.valkonen@vbiol.slu.se)*

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Abstract

Downy mildew caused by *Peronospora sparsa* (syn. *P. rubi*) is a serious threat to commercial cultivation of arctic bramble (*Rubus arcticus* subsp. *arcticus*) in Finland. *P. sparsa* is distributed throughout the country in cultivated and wild arctic bramble and in cloudberry (*R. chamaemorus*). A total of 36 isolates of *P. sparsa* collected from these hosts was analysed for amplified fragment length polymorphism (AFLP). Of the 226 markers scored, 223 were polymorphic and all isolates of *P. sparsa* had unique AFLP fingerprints, which indicated high levels of genetic variability. An UPGMA clustering analysis of the isolates did not reveal any genetically distinguishable strains. The isolates were grouped neither according to the geographic origin nor the host from which they were isolated. Isolates of *P. sparsa* obtained from wild arctic bramble and one from cloudberry readily infected the leaves of the cultivated arctic bramble (cultivar 'Pima'). Also, *P. sparsa* isolated from cultivated arctic bramble infected the leaves of wild arctic bramble. These data suggest that *P. sparsa* may be disseminated from wild arctic bramble and cloudberry to cultivated arctic bramble in the field.

Introduction

Downy mildew caused by *Peronospora sparsa* Berkeley (syn. *P. rubi* Rabenshorst) (Oomycetes; order Peronosporales; family Peronosporaceae) (Hall and Shaw, 1982; Francis and Waterhouse, 1988; Hall, 1989; Breese et al., 1994) has become a severe hindrance to cultivation of arctic bramble (*Rubus arcticus* L. subsp. *arcticus*) in Finland (Koponen and Valkonen, 1996; Parikka, 1996; Lindqvist et al., 1998; Kokko et al., 1999; Koponen et al., 2000). Arctic bramble is native to subarctic Eurasia where it produces berries most abundantly between 62 and 66 degrees latitude (Hultèn, 1971). After 40 years of experimentation on native strains of *R. arcticus*, two strains (cv. Mespi and cv. Mesma) were selected for cultivation in Finland at the beginning of the 1970s (Ryynänen, 1973). Later, a new cultivar (Pima) with improved yield characteristics was obtained from a cross between Mesma and Mespi (Ryynänen and Dalman, 1983). Arctic bramble is self-incompatible and two genotypes must be grown together for berry production (Ryynänen, 1973). Cultivars Mespi and Pima correspond to over 90% of the cultivated area. In the middle of the 1990s, ca. 30 ha were in commercial production by several farmers. Prospects for expanded production of the highly valued, aromatic berries were good. However, during 1994–1996, large yield losses were reported due to a 'dryberry disease' throughout the whole cultivation area, and the main causal agent was found to be *P. sparsa* (Koponen and Valkonen, 1996; Lindqvist et al., 1998; Koponen et al., 2000). The conclusion that *P. sparsa* was the causal agent was further supported when significant yield increases were obtained in field trials using oomycete-specific fungicides including tolylfluanid (Euparen) (Parikka,

1996; Koponen et al., 1998) and metalaxyl (Ridomil) (Parikka, 1996; Kokko et al., 1999). Cultivars Pima and Mespi are both susceptible to *P. sparsa*. There are new cultivars available (Pirinen et al., 1998), but they are not commonly in use and their resistance, if any, has not been reported. Hybrids produced by crossing the Canadian subspecies *R. arcticus* subsp. *stellatus* with the Scandinavian *R. arcticus* subsp. *arcticus* (Larsson, 1968) seem, however, to have some resistance to *P. sparsa* (Koponen et al., 2000).

Research to improve control of *P. sparsa* in arctic bramble was initiated a few years ago. To date, a polymerase chain reaction (PCR)-based method has been developed for detection of *P. sparsa* in planting materials (Lindqvist et al., 1998). The dryberry disease in arctic bramble can be suppressed during the beginning of the growing season, but not prevented, using tolylfluanid or metalaxyl (Parikka, 1996; Koponen et al., 1998; Kokko et al., 1999). One disadvantage of this approach may be the development of resistance to these chemicals in *P. sparsa*. Treatment with commercially available preparations, including Aliette 80WG or Bion, which induce general host resistance to pathogens increased the yields up to 3-fold in growing seasons inducive to the dryberry disease (Parikka, 1996; Koponen et al., 1998).

While the distribution of *P. sparsa* in Finland has been studied (Koponen et al., 2000), the genetic variability of *P. sparsa* is unknown. The three main forces introducing genotypic variation into a pathogen population are mutation, recombination and migration (Burdon and Silk, 1997). Although mutations contribute significantly to the evolution of pathogenicity (Goodwin et al., 1995b), the overall level of genotypic variation increases significantly in the presence of sexual reproduction (Sujkowski et al., 1994; Goodwin et al., 1995a; Brurberg et al., 1999). Long-distance migration, most likely associated with infected plants transported by humans (Fry et al., 1992), has contributed to increased variation and establishment of new virulent and more aggressive strains of the potato late blight pathogen *Phytophthora infestans* in Europe (Sujkowski et al., 1994). However, since *Peronospora sparsa* is endemic in Finland (Koponen et al., 2000), populations may be in equilibrium, which would hamper distinguishing human-aided from natural migration. Knowledge on diversity of the pathogen population is important for identifying effective resistance and breeding for resistant varieties. Inadequate screening of resistance sources with genetically different strains of the pathogen could make resistance prone to break-down in the field where other more virulent variants of the pathogen might be present.

Host species specificity of isolates of *P. sparsa* has not been studied in Finland. Knowledge on host specificity of a pathogen has important implications, such as understanding the role of alternate hosts in the epidemiology of the disease or the potential for host range expansion. In Finland, *P. sparsa* is widely distributed in wild arctic bramble and cloudberry (*R. chamaemorus* L.) populations and, apparently, is not a recently introduced pathogen in these species since herbarium samples collected over the last century were found to be infected (Koponen et al., 2000). *P. sparsa* infects raspberry, boysenberry, Tummelberry and other *Rubus* (Breese et al., 1994; Francis and Waterhouse, 1988; McKeown, 1988; Hall, 1989; Koponen et al., 2000) and *Rosa* species (Francis and Waterhouse, 1988; Breese et al., 1994) in other parts of Europe and in New Zealand. So far, host species specificity has been tested only with *P. sparsa* isolates originating from roses and Tummelberry: isolates from these hosts could cross-infect the two hosts (Breese et al., 1994). Since wild arctic bramble and cloudberry occur all over Finland and often in close proximity to the fields of cultivated arctic bramble, the wild plants might serve as an important source of inoculum. Therefore, exchangeability of isolates of *P. sparsa* between cloudberry and wild and cultivated arctic bramble needs to be tested. On the other hand, if *P. sparsa* is specialised on certain hosts, populations genetically isolated by host specificity would be expected to be evolving independently and this could be detected as genetic differences in a cluster analysis.

For measuring the variability of *P. sparsa* in Finland we chose the amplified fragment length polymorphism (AFLP) technique (Vos et al., 1995). AFLPs are highly reproducible, relatively fast to generate and have been used to study genetic variation of fungi (O'Neill et al., 1997; Rosendahl and Taylor, 1997; Gonzáles et al., 1998; DeScenzo et al., 1999; Peever et al., 1999; Tredway et al., 1999). Importantly, since the DNA restriction fragments are amplified by polymerase chain reaction (PCR) in AFLP, only a little genomic DNA is needed for production of hundreds of AFLP markers. This is essential with a biotrophic organism such as *P. sparsa* that sporulates weakly, thus limiting the amount of material available for DNA extraction.

The aim of this study was to measure the genetic variability of *P. sparsa* in Finland for evidence of substructuring by host or geographic distance.

Materials and methods

Isolate collection and cross-infection studies

Isolates of *P. sparsa* were obtained from nine locations in Finland (Figure 1, Table 1). Leaf samples were collected from plants showing foliar symptoms resembling those caused by *Peronospora* (McKeown, 1988; Lindqvist et al., 1998). Most of the isolates from wild and cultivated *R. arcticus* were obtained from samples collected at the end of June 1998, and the isolate from *R. chamaemorus* was from samples collected at the end of September 1998. Four isolates (Lumi1, Lumi2, Lumi3, Ruuk2) (Table 1) were from samples collected in July 1999. The sampled leaves were placed on water agar in Petri dishes within 12 h of sampling and incubated under dim light at 17 °C for 21 days. Fungal growth was checked under a preparation microscope. Pure cultures were produced by picking spores of *P. sparsa* and placing them in a droplet of water on the abaxial side of healthy leaves of *in vitro*-propagated *R. arcticus* (cv. Pima) or *R. chamaemorus* (protocols for culture media and conditions kindly provided by Laukaa Healthy Plant and Research Station, Laukaa, Finland). Some healthy plants were grown in a growth chamber and the leaves of these plants were sterilised by keeping both sides of the leaf in 0.1% sodium hypochlorite solution for 1 min and rinsing them with sterile distilled water before inoculation with *P. sparsa*.

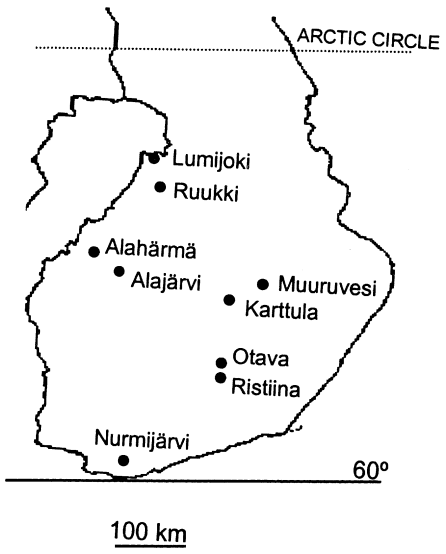

Figure 1. The sampling sites in Finland from which the *P. sparsa* isolates used in this study were obtained.

The inoculated leaves were incubated on moist filter papers in Petri dishes closed with a lid under dim light at 17 °C until the sporangia covered most of the leaf surface (7–8 days). They were also studied under a preparation microscope. Sporangia growing on an inoculated leaf were considered to represent a single isolate. From pure cultures, the sporangia were dislodged in sterile distilled water and stored at −70 °C.

DNA extraction

The method for DNA extraction was kindly provided by Dr. Annemarie Fejer-Justessen (Danish Institute of Agricultural Sciences, Research Center Flakkebjerg, Denmark). Two DNA extractions were made of each

Table 1. Isolates of *P. sparsa* used in the analysis for amplified fragment length polymorphism (for locations, see also Figure 1)

Isolate	Location	Host
Alah1	Alahärmä	Wild *Rubus arcticus*
Alah2	Alahärmä	Cultivated *R. arcticus*
Alaj1	Alajärvi	Cultivated *R. arcticus*
Alaj2	Alajärvi	Cultivated *R. arcticus*
Alaj3	Alajärvi	Cultivated *R. arcticus*
Alaj4	Alajärvi	Cultivated *R. arcticus*
Kart1	Karttula	Cultivated *R. arcticus*
Kart2	Karttula	Cultivated *R. arcticus*
Kart3	Karttula	Cultivated *R. arcticus*
Kart4	Karttula	Cultivated *R. arcticus*
Kart5	Karttula	Cultivated *R. arcticus*
Kart6	Karttula	Cultivated *R. arcticus*
Kart7	Karttula	Cultivated *R. arcticus*
Muur1	Muuruvesi	Cultivated *R. arcticus*
Muur2	Muuruvesi	Cultivated *R. arcticus*
Muur3	Muuruvesi	Cultivated *R. arcticus*
Muur4	Muuruvesi	Cultivated *R. arcticus*
Muur5	Muuruvesi	Cultivated *R. arcticus*
Muur6	Muuruvesi	Cultivated *R. arcticus*
Muur7	Muuruvesi	Cultivated *R. arcticus*
Muur8	Muuruvesi	Cultivated *R. arcticus*
Muur9	Muuruvesi	Cultivated *R. arcticus*
Rist1	Ristiina	Cultivated *R. arcticus*
Rist2	Ristiina	Cultivated *R. arcticus*
Rist3	Ristiina	Cultivated *R. arcticus*
Rist4	Ristiina	Cultivated *R. arcticus*
Rist5	Ristiina	Cultivated *R. arcticus*
Ruuk1	Ruukki	Cultivated *R. arcticus*
Ruuk2	Ruukki	Cultivated *R. arcticus*
Lumi1	Lumijoki	Wild *R. arcticus*
Lumi2	Lumijoki	Wild *R. arcticus*
Lumi3	Lumijoki	Wild *R. arcticus*
Otav1	Otava	Wild *R. arcticus*
Otav2	Otava	Wild *R. arcticus*
Otav3	Otava	Wild *R. arcticus*
Nurm1	Nurmijärvi	*R. chamaemorus*

isolate. Spores stored in water in a microcentrifuge tube at −70 °C were collected by centrifuging at 6100*g* for 10 min, removing the supernatant with a pipette, and freezing the spore pellet in liquid nitrogen. The pellet was ground in liquid nitrogen with a pestle and a mortar, using washed sand as an abrasive, and the powder was transferred into a clean microcentrifuge tube. An aliquot (500 µl) of extraction buffer (25 g D-sorbitol, 10 g sarkosyl, 8 g *N*-acetyl-*N*,*N*,*N*-trimethyl ammonium bromide, 47 g NaCl, 8 g ethylenediamine tetra-acetic acid disodium salt (EDTA) and 10 g polyvinyl pyrrolidone per litre; 0.1 M Tris; pH 8.0) and 5 µl Proteinase K (10 mg/ml) were added to the powder, mixed and incubated at 65 °C for 1.5 h. Chloroform (500 µl) was added and after mixing, the mixture was centrifuged at 13,700*g* for 20 min. The upper phase was treated with 10 µl of RNase A (10 mg/ml) for 1 h at 37 °C. Chloroform extraction was repeated. DNA was precipitated for 5 min at room temperature with 1 volume of ice cold isopropanol. The pellet was washed with 70% ethanol, air dried and dissolved in 20 µl of TE buffer (10 mM Tris–HCl pH 8.0, 1 mM EDTA).

AFLP procedure

AFLP analysis was done primarly according to the AFLP Plant Mapping Kit Protocol (PE-Applied Biosystems, Foster City, CA) with modifications as follows. For the DNA digestion and adapter ligation step, 5U *Tru*9I and 5U T4 DNA Ligase were used, the adapter pair concentrations were 50 pmol for the *Mse*I adapter pair and 5 pmol for the *Eco*RI adapter pair, and the total reaction volume was 50 µl. Primers and adapters (Table 2) were synthesized at Life Technologies (Täby, Sweden) or at PE-Applied Biosystems (Warrington, UK). All the enzymes for DNA digestion and adapter ligation were obtained from Roche Diagnostics (Mannheim, Germany). The preselective and selective PCR amplification steps were run in 20 µl total volume containing 0.2 mM dNTPs (MBI Fermentas, Vilnius, Lithuania), 2 mM $MgCl_2$ and 0.4 U of Taq DNA Polymerase (MBI Fermentas). Pre-selective amplification used 30 ng of both of the non-labelled primers (E01 and M02) each containing one selective nucleotide. Selective amplification used 0.05 µM of fluorescently labelled *Eco*RI-primer (E14) with two selective nucleotides and 0.5 µM of non-labelled *Mse*I primer (M47 or M49) with three selective nucleotides. The running conditions for pre-selective amplification consisted of a denaturation step at 94 °C for 30 s, an annealing step at 60 °C for 1 min, and an elongation step at 72 °C for 1 min. All three steps were repeated 35 times and amplification products were verified on agarose gels before selective amplification. PCR conditions for selective amplification were as described by Vos et al. (1995), except for an additional elongation step of 5 min at 72 °C. Samples were analysed on an ABI PRISM XL377 DNA sequencer (PE Applied Biosystems, Foster City, CA). Denatured samples (1 or 1.6 µl) were loaded onto 48- or 64-well denaturating acrylamide gels. The gels were run at 3000 V for 3 h with 1x TBE buffer (Tris 108 g, Boric acid 55 g in 1 l of H_2O and 20 mM EDTA).

Table 2. Nucleotide sequences of the adapters and primers used for the amplified fragment length polymorphim analysis in this study. The selective nucleotides at the 3′ end are indicated in bold

Name	Sequence
*Eco*RI adapter 1	5′-CTCGTAGACTGCGTACC-3′
*Eco*RI adapter 2	5′-AATTGGTACGCAGTC-3′
*Eco*RI primer 1 (E01)	5′-AGACTGCGTACCAATTC**A**-3′
*Eco*RI primer 2 (E14)	5′-GACTGCGTACCAATTC**AT**-3′
*Mse*I adapter 1	5′-GACGATGAGTCCTGAG-3′
*Mse*I adapter 2	5′-TACTCAGGACTCAT-3′
*Mse*I primer 1 (M02)	5′-GACGATGAGTCCTGAGTAA**C**-3′
*Mse*I primer 2 (M47)	5′-GATGAGTCCTGAGTAA**CAA**-3′
*Mse*I primer 3 (M49)	5′-GATGAGTCCTGAGTAA**CAC**-3′

Analysis of AFLP data

Data were acquired using GeneScan version 2.1.1 (PE Applied Biosystems) where lanes were tracked manually. The analysis parameters for sizing the fragments were set as follows: Analysis range: all scans; Data Processing: Multicomponent and Baseline; Smooth Options: light; Peak Detection: Dye Amplitude Threshold 50; Minimum Peak Half Width 3 Pts; Size Call Range: All Sizes; Size Calling Method: Local Southern; Split Peak Correction: None. After initial analysis samples were imported into GenoTyper version 2.0 (PE Applied Biosystems). Categories were defined by labelling the 10 highest peaks corresponding to fragment sizes of 75–300 bp in each sample. The labels in these categories were filtered to remove shoulder peaks. The remaining labelled peaks were used to create categories with a tolerance of ±0.5 bp. The corresponding peaks in all samples were labelled. Overlapping categories were either removed or adjusted to be non-overlapping. The total number of reliable categories (loci) was 226, of which 113 were obtained with the primer pair E14M49 and 113 were

obtained with the primer pair E14M47. Data were tabulated with 1 corresponding to an existing fragment and 0 to the absence of that fragment at each locus using GenoTyper.

Genetic distances were measured between all pairs of isolates using two methods. First, Jaccard's genetic distance as implemented in the Rapdistance package (Version 1.04; J Armstrong, A Gibbs, R Peakall and G Weiller, Australian National University, Canberra) was used to generate a distance matrix for UPGMA clustering analysis and for correlation analysis. Pearson's product moment correlation coefficient (r) for the geographic distance and Jaccard's genetic distance were calculated between each pair of isolates using a standard spread-sheet program. Second, Nei and Li's restriction fragment distance as implemented in the PHYLIP package (version 3.6alpha; J Felsenstein, Department of Genetics, University of Washington, Seattle) was used to generate a distance matrix for another UPGMA clustering analysis to test the statistical significance of the phenogram branching by bootstrapping the dataset for 100 times. The phenograms based on the resulting distances were constructed using the UPGMA method as implemented in the program NEIGHBOR from the PHYLIP package.

Results and discussion

Genetic variability of *P. sparsa* has not been studied, except for an analysis of the internal transcribed spacer sequences (ITS) of the rRNA genes (Lindqvist et al., 1998; Kokko et al., 1999) in a limited number of isolates which were not available for this study. Therefore, 36 new isolates of *P. sparsa* were collected from different parts of Finland and examined for variability using AFLP.

The two primer combinations used yielded 226 AFLP markers within the size range of 76–300 bp that could be scored unambiguously. On average, 52 markers (s.d. 10.4) were obtained per isolate. All samples (two DNA extractions of each) were run on at least two gels and the data combined for analysis with Genotyper. Repeated analyses using the same pre-amplification reaction provided identical AFLP profiles. Also, the results were highly reproducible using different DNA extractions of the same genotype, but some differences were observed. On average, the proportion of bands that were present in only one of the two DNA extracts was 8.5% (s.d. 4.2). Most of the previous studies on fungi have not compared the AFLP data between different DNA samples extracted from the same specimen. Alternatively, the results from such comparisons have been referred to as 'highly reproducible' with data not shown. Thus, it was difficult to make comparisons on reproducibility between the different AFLP studies on fungi.

Hansen et al. (1999) studied the genetic variability amongst plants of *Beta* species and found three types of reproducibility errors in the AFLP: digitisation mistakes, differences in gel resolution and the presence of certain marker bands in a specific sample in one experiment but not in another. The two first mentioned sources of error can be very effectively removed in the automated analyses using fluorescent labels, such as our study. The accurate sizing and digitisation of each fragment is assured by loading the size standard in every lane together with the sample and analysing them by GeneScan software. The fragments not clearly differentiated from one another are detected as overlapping fragments by the GenoTyper software and can be removed automatically. In contrast, the third type of error, i.e., poor reproducibility of certain AFLP markers, is more difficult to control. Hansen et al. (1999) found that 0.5% of the AFLP fragments were of the erratic type in DNA samples extracted from sugarbeet. *P. sparsa* is a biotrophic pathogen, and the amounts of DNA obtained from axenic cultures established on sterile leaves in the laboratory are limited. Therefore, the higher proportion of poorly reproducible AFLP bands in our study as compared to Hansen et al. (1999) may be due to quality differences between the DNA extracts.

To obtain a single value for Jaccard's genetic distance between pairs of isolates, the non-reproducible markers between the two DNA extractions were removed. Jaccard's distances ranged from 0.53 to 0.88. The fingerprinting patterns were unique for each of the 36 *P. sparsa* isolates tested and all but three markers were polymorphic. The geographic distance between the sampling sites of isolates ranged from a few meters to 495 km. As shown in Figure 2, the scattering of datapoints indicates no significant correlation between the genetic distance and the geographic distance of origin (Pearson's correlation coefficient $r = 0.26$).

Due to the slight differences in the fingerprints between two DNA extractions of the same isolate of *P. sparsa*, the data corresponding to each DNA isolation were subjected to cluster analysis as a separate entity (Figure 3). Jaccard's as well as Nei and Li's similarity coefficients produced a similar UPGMA phenogram. However, no branch of the phenogram was significantly supported by a bootstrap analysis

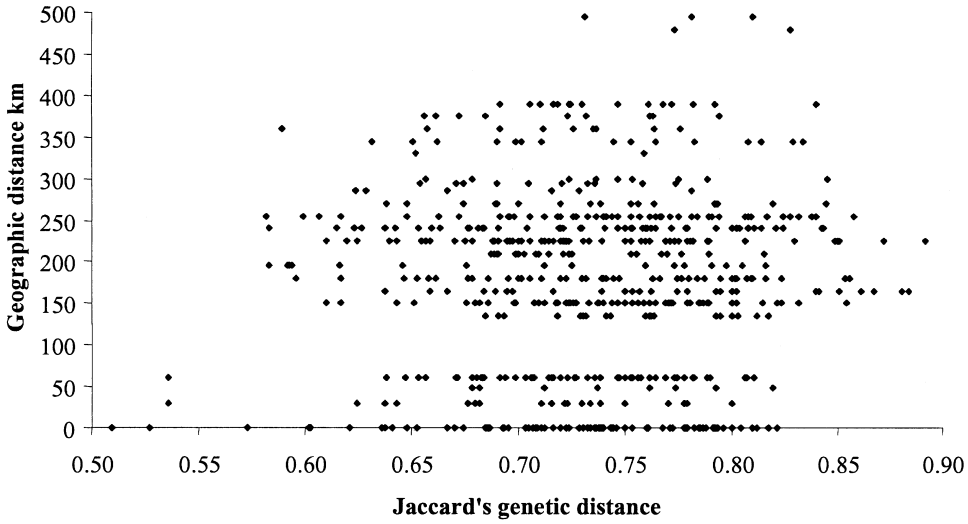

Figure 2. Plot of pairwise comparisons of Jaccard's genetic distance and geographic distance among 36 isolates of *P. sparsa* from Finland.

(bootstrap values < 60), except the two DNA extractions of the same genotype which always grouped together (bootstrap values of 100). Similar results were obtained when non-reproducible bands were removed prior to analysis (data not shown). Thus, the AFLP analysis revealed no systematic genetic substructuring of *P. sparsa* isolates by either the geographical origin or the host (wild or cultivated arctic bramble) from which the isolates were obtained. Only a single isolate of *P. sparsa* from cloudberry was available for study. AFLP analysis did not reveal significant differences between this isolate and the isolates from arctic bramble. However, a single isolate is obviously not sufficient for this comparison and further studies are needed to reveal whether or not genetically distinguishable strains of *P. sparsa* occur on cloudberry.

The lack of detectable geographic differentiation of *P. sparsa* in cultivated arctic bramble may be because *P. sparsa* is endemic and has existed for a long time in Finland (Koponen et al., 2000), and populations may have reached an equilibrium. Examination of three isolates of *P. sparsa* collected from wild arctic bramble at two distant geographic locations each (500 km apart) (Table 1, Figure 1), neither of which was at a close proximity to cultivations of arctic bramble, served to confirm the lack of genetic differentiation between the two distant groups of isolates. Therefore, dissemination of *P. sparsa* in infected planting material, which is known to have occurred between different parts of Finland in several years in the 1990s, has probably not significantly changed the genetic structure of local populations of *P. sparsa*. However, infected planting materials may have enhanced the dispersal of *P. sparsa* in cultivations and contributed to the development of an epidemic. Currently, more accurate indexing methods are available for healthy plant production (Lindqvist et al., 1998).

Despite the lack of substructuring, considerable genetic variability was observed in *P. sparsa*. For example, the variability observed amongst five isolates (Rist1–Rist5) collected from one small garden plot in Ristiina was as extensive as that found amongst the rest of the isolates (Figures 2 and 3). The extent of the genetic diversity and the fact that each isolate had a unique AFLP fingerprint was not suggestive of asexually reproducing clonal lineages seen, for example, in *Phytophthora infestans* (Koh et al., 1994; Goodwin et al. 1995b). Sexual oospores of *P. sparsa* have been found on Tummelberry (*Rubus* hybrid; blackberry × red raspberry) in the United Kingdom (Williamson et al., 1995) and on several wild and cultivated *Rubus* species in New Zealand (Hall and Shaw, 1987), but their occurrence on arctic bramble requires further study. Our data are suggestive of sexual reproduction of *P. sparsa* in Finland.

The genetic distances between pairs of isolates of *P. sparsa* ranged from 0.25 to 0.40 according to UPGMA. Genetic distances within a similar range have been observed among isolates of *Peronospora parasitica* using AFLP analysis (Rehmany et al., 2000). Distances on Jaccard's scale ranged from 0.35 to

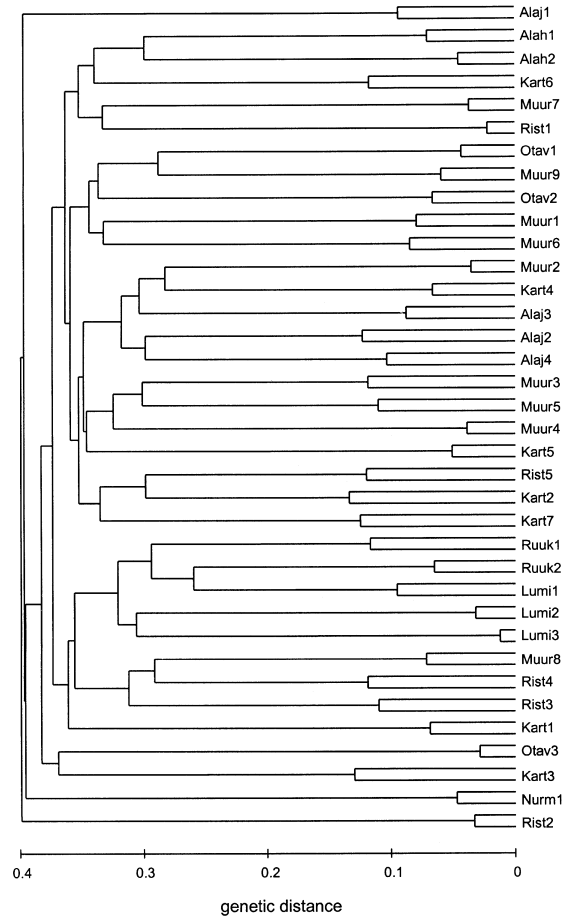

Figure 3. Unweighted pair-group method dendrogram based on the arithmetic average analysis of Jaccard's genetic distance between 36 isolates of *P. sparsa* from Finland. The analysis was based on two DNA extractions for each isolate. Bootstrap values for all nodes were <60 except for the different DNA extractions of each isolate which were always 100.

0.70 among isolates of *P. parasitica* originating from *Brassica oleracea* and from 0.10 to 0.40 among isolates originating from *Arabidopsis thaliana*. However, the distance between the two groups of isolates was 0.90 and, consequently, the isolates were grouped to two phenogram clusters according to the host. Such substructuring of isolates, e.g., based on origin in wild or cultivated arctic bramble, was not observed with *P. sparsa* in this study.

There were no apparent differences in the growth rate or sporulation among the isolates. Hence, all isolates of *P. sparsa* readily infected cultivated arctic bramble, irrespective of the host of origin (cloudberry or wild arctic bramble). These data are consistent with the AFLP analysis indicating no systematic genetic differentiation. Such lack of genetic differentiation is perhaps not surprising since the cultivars of arctic bramble are, in fact, genotypes collected relatively recently from the wild (Ryynänen, 1973; Ryynänen and Dalman, 1983) which, in turn, would likely be reflected in a lack of differentiation in *P. sparsa* populations infecting them.

The data of this study facilitate evaluation of strategies for better control of downy mildew in arctic bramble. Establishment of cultivations using healthy planting materials does not assure escape from losses caused by *P. sparsa* because the sporangia produced on infected plants of arctic bramble and cloudberry in the wild vegetation at the proximity of cultivations may cause re-infestation. The asexual sporangia of the downy mildew pathogens are usually short-lived and sensitive to environmental extremes and cannot be disseminated over long distances by wind (Michelmore et al., 1988). For example, only relatively short distance migrations of *P. infestans* can occur by wind (Fry et al., 1992). However, we have found wild arctic bramble infected with *P. sparsa* in close proximity (50–200 m) to many cultivations. It seems unlikely that such short distances would prevent dissemination of *P. sparsa* between wild and cultivated arctic bramble. Therefore, cultivars resistant to downy mildew are needed. The high genetic variability within populations of *P. sparsa* implies that breeding lines of arctic bramble should be challenged with several isolates of *P. sparsa* for selection of durable resistance. The use of fungicides as a control strategy should be considered cautiously because the high genetic variability of *P. sparsa* may provide the means for a rapid selection of fungicide-intolerant strains.

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