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[1]*Department of Silviculture and* [2]*Department of Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umeå, Sweden*

# RAPD variation in *Gremmeniella abietina* attacking *Pinus sylvestris* and *Pinus contorta* in northern Sweden

By P. HANSSON[1], X-R. WANG[2], A. E. SZMIDT[2] and M. KARLMAN[1]

## Summary

Genomic DNA from 81 isolates of *Gremmeniella abietina* collected from eleven plantations each of *Pinus sylvestris* and *Pinus contorta* in northern Sweden was studied using RAPD markers. The DNA variation between and within populations and the race and type distribution of *G. abietina* populations, causing symptoms similar to those of the North American race, were studied. The degree of genetic similarity was greater among *G. abietina* isolates from the same geographical areas than among isolates from different geographical areas, regardless of whether they were isolated from *P. sylvestris* or *P. contorta*. RAPD variation was greatest in the central parts of northern Sweden, suggesting that sexual reproduction has been somewhat more important there than further north or south. Only the RAPD fragments characteristic of the EU race of *G. abietina* were found in the material tested. The RAPD pattern described as characteristic of the northern type within the EU race was identified in 62% of the isolates. Divergence from the expected profile was due to differences in occurrence of fragments OPA12-1400 and 12-1500. This indicates that this part of the RAPD profile cannot be treated as diagnostic for the northern type. A conclusion of practical importance is that there is a considerable risk of *G. abietina* spreading from infected *P. contorta* plantations to adjacent areas with indigenous *P. sylvestris* regeneration, and vice versa, owing to the indicated lack of host-specificity of the pathogen. It is possible, however, that host-specific strains exist, but do not differ in their RAPD profiles.

## 1 Introduction

*Gremmeniella abietina* (Lagerb.) Morelet (*Scleroderris* canker) is responsible for substantial mortality in coniferous plantations in Europe (DONAUBAUER 1972; STEPHAN 1979, 1990), North America (DORWORTH 1972; SKILLING 1977) and Asia (YOKOTA et al. 1974). In Scandinavia, the introduced *Pinus contorta* (Dougl.) ex Loud. is highly susceptible (KARLMAN 1990; DONAUBAUER et al. 1992; KAITERA and JALKANEN 1992; KARLMAN et al. 1994).

On the basis of serological and morphological characteristics, *G. abietina* has been divided into three races: North-American (NA), European (EU) and Asian (DORWORTH and KRYWIENCZYK 1975). The NA race typically causes stem cankers and shoot disease in young pine plantations. Usually, only trees less than 2 m high are killed by this race, which produces both pycnidia and apothecia (SKILLING 1972, 1977). In addition to infecting young pines, the EU race also affects the upper crown of older trees. In the central and western parts of Europe, it produces pycnidia but very few apothecia (GIBBS 1984; HELLGREN and BARKLUND 1992; ROLL-HANSEN 1993). In northern Sweden, where large areas of the introduced *P. contorta* have been infected by *G. abietina*, the typical symptoms are similar to those of the NA race (KARLMAN et al. 1994). Since the mid-1970s the EU race has been present in the eastern parts of Canada and the north-eastern parts of the USA (SETLIFF et al. 1975; DORWORTH et al. 1977). *G. abietina* (NA race) occurs only sporadically within the natural range of *P. contorta* in western North America (HIRATSUKA and FUNK 1976).

Present knowledge of the genetic structure of the European populations of *G. abietina* is largely based on morphological studies, i.e. on conidial length and septation (MORELET 1980; PETRINI et al. 1990). Two different morphological types of *G. abietina* have been found in

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Finland (Uotila 1983), as well as in Sweden (Hellgren 1995). These observations indicate that the European populations of this fungus are genetically differentiated.

The occurrence of the EU and NA races within *G. abietina* was confirmed by studies of variation in total protein patterns (Benhamou et al. 1984; Petrini et al. 1989, 1990) and pectic enzym patterns (Lecours et al. 1994). Further genetic evidence was recently demonstrated by analysis of the DNA variation (Hamelin et al. 1993; Bernier et al. 1994). The EU race of *G. abietina* was divided into three geographically separated types based on an analysis of RAPD profiles (Hamelin et al. 1995). A similar study suggests that two different 'ecotypes' of *G. abietina* exist in Scandinavia (Hellgren and Högberg 1995).

It is possible that this pathogen has a number of distinct strains that may differ in their impact on hosts, as seen for *Armillaria mellea* (Vahl:Fr.) Kumm., which is composed of a number of reproductively isolated strains (Korhonen 1978). Genetic methods of taxonomic classification are based on the premise that genetic identity among individuals, at least for a certain group of genes, will be high within biological species sharing the same gene pool, whereas significant genetic divergence will be found among biological species (Ennos 1991). A wide array of genetic markers is now available for measuring degrees of genetic relationship among groups. Of these, the random amplified DNA-polymorphism (RAPD) markers appear to be the most feasible for genetic analysis of populations of haploid parasitic fungi. Indeed, knowledge about the genetics of this group of organisms has been recently accumulating through the use of RAPD analysis (Hubbes 1993; Ouellet and Seifert 1993; Andebrhan and Furtek 1994). Since inheritance studies show that RAPDs behave like dominant Mendelian characters, it should be possible to use them as genetic markers (Lu et al. 1995). Inheritance tests on fungi are difficult because of the dual mode of reproduction. One can assume, however, that RAPD genetics in fungi are similar to that in other organisms.

The aim of this study was to characterize the genetic variation between and within geographically separated populations of *G. abietina* in northern Sweden, attacking *P. contorta* and *P. sylvestris* (L.), and to determine the race and type identity and distribution of *G. abietina* populations causing symptoms similar to those caused by the North American race.

## 2 Materials and methods

### 2.1 Fungal material

The fungal material was collected from eleven areas in northern Sweden (Fig. 1). Within each area, one plantation of *P. contorta* 1–3 m high, and at least moderately affected by *G. abietina*, was selected for material collection. Material was also collected from adjacently planted or naturally regenerated *P. sylvestris* trees that were 1–2 m high, (no more than 300 m from the most distant selected *P. contorta* tree). In each sampled stand, four well-separated (15–200 m) pairs of affected trees of each species were selected. The distance between individuals in the pairs was 1–5 m. From each tree 2–4 twigs with apothecia, or in some cases, pycnidia of *G. abietina* were collected.

On twigs with apothecia, the fungus was first isolated from a limited number of asci in the hymenium. When, after approximately 3 weeks, this multiple ascospore mycelium produced pycnidia *in vitro*, a few conidia from the same pycnidia were used to re-isolate the fungus (multiconidial sample). On twigs with pycnidia, the procedure started with the multiconidial isolation from *in vivo* pycnidia. Microscopic studies of conidia verified the species identity. From the southernmost area, four single-spore isolates, two from conidia and two from ascospores, were added.

Small (5 × 5 mm) pieces of 2–3-week-old mycelium were transferred to 1.5-ml Eppendorf tubes with autoclaved liquid medium and allowed to grow for about 1 week at 17°C under continuous light. The growing medium consisted, per litre, of 200 ml Granini® vegetable juice (Deutsche Granini, Bielefeld, Germany), 10 g glucose, 20 g Difco agar and 800 ml ultrapure Milli-Q® Plus water (18.2 MΩ/cm).

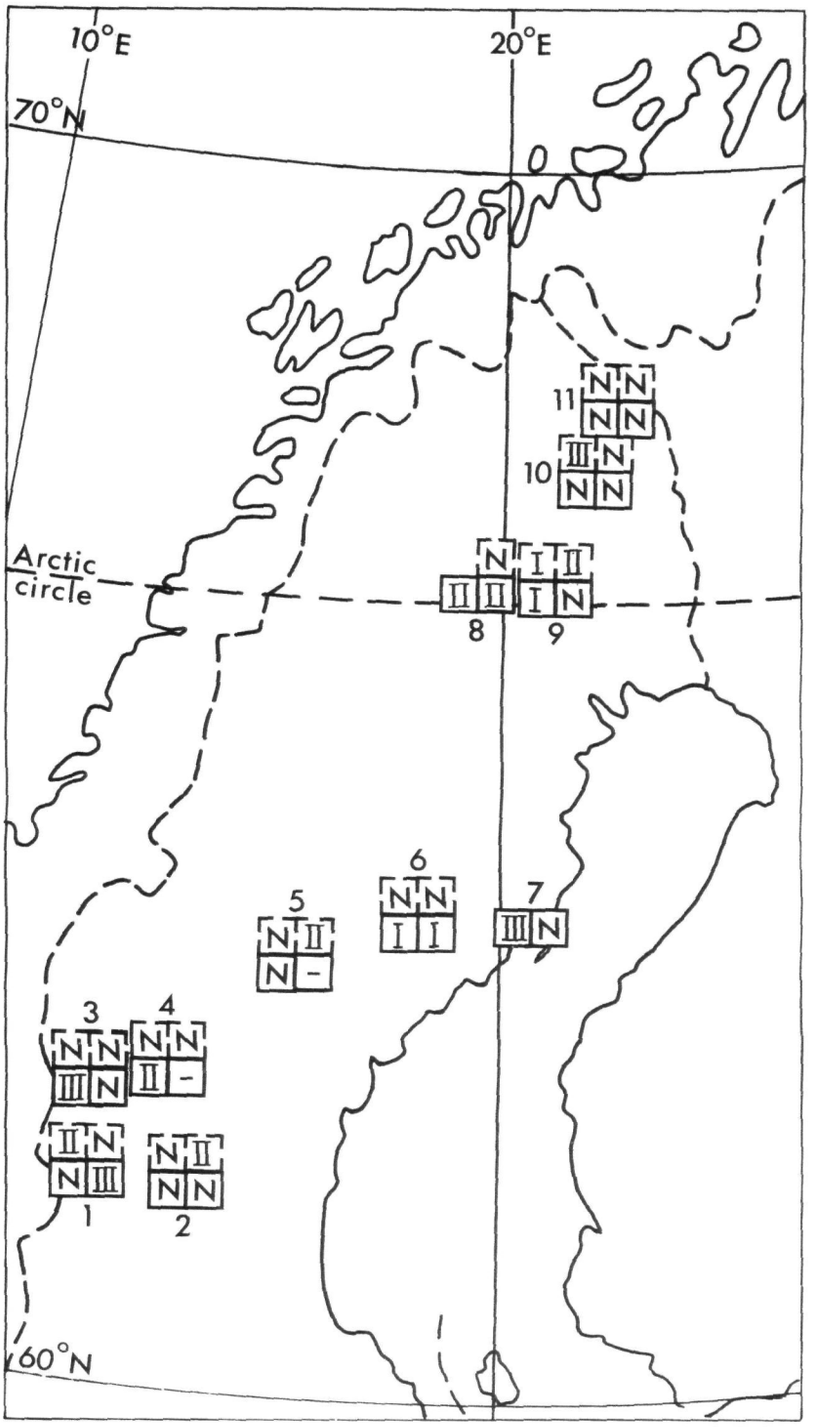


*Fig. 1.* Origin of the *G. abietina* isolates used. The numbering is the same as in Table 1. The distribution of the four different amplification profiles of the RAPD fragments OPA08-600, OPA12-1400 and OPA12-1500 in the race/type test is shown. The broken lines represent isolates from *P. sylvestris* and the solid lines isolates from *P. contorta.* N = northern type (profile 1,1,1); I = profile 1,1,0; II = 1,0,1; III = 1,0,0; – = missing value)

A total of 81 different isolates were investigated. In the study aimed at characterizing the overall genetic variation, 52 isolates collected from southern, central and northern areas were investigated (nos. 1, 5 and 10 in Fig. 1 and Table 1). In the race/type study, a total of 41 isolates were selected. Selections were made from both pine species with the aim of maximizing the distance between isolates from the 11 areas (Fig. 1; Table 1).

## 2.2 Extraction of DNA

The DNA was extracted using a modified sodium disulphate (SDS) protocol (Cenis 1992). Eppendorf tubes with crushed mycelium and extraction buffer (200 mm Tris-HCl pH 8.5,

*Table 1.* **Origin and numbers of *G. abietina* isolates from each area and host species**

| Area[1] | Latitude (°N) | Longitude (°E) | Elevation (m.a.s.l.) | Host species[2] | No. tree-pairs[3] | No.trees[3] | No. isolates*** |
|---|---|---|---|---|---|---|---|
| 1 | 61°40′ | 12°30′ | 620 | *Ps* | 2 (4) | 2 (7) | 2 (9) |
| | | | | *Pc* | 2 (4) | 2 (7) | 2 (8) |
| 2 | 61°40′ | 14°40′ | 580 | *Ps* | 2 | 2 | 2 |
| | | | | *Pc* | 2 | 2 | 2 |
| 3 | 62°35′ | 13°10′ | 680 | *Ps* | 2 | 2 | 2 |
| | | | | *Pc* | 2 | 2 | 2 |
| 4 | 63°00′ | 14°10′ | 610 | *Ps* | 2 | 2 | 2 |
| | | | | *Pc* | 2 | 2 | 2 |
| 5 | 63°20′ | 16°20′ | 460 | *Ps* | 2 (4) | 2 (6) | 2 (7) |
| | | | | *Pc* | 2 (4) | 2 (6) | 2 (7) |
| 6 | 64°00′ | 18°45′ | 420 | *Ps* | 2 | 2 | 2 |
| | | | | *Pc* | 2 | 2 | 2 |
| 7 | 63°50′ | 20°30′ | 10 | *Pc* | 2 | 2 | 2 |
| 8 | 66°45′ | 19°45′ | 500 | *Ps* | 1 | 1 | 1 |
| | | | | *Pc* | 2 | 2 | 2 |
| 9 | 66°45′ | 21°20′ | 370 | *Ps* | 2 | 2 | 2 |
| | | | | *Pc* | 2 | 2 | 2 |
| 10 | 67°45′ | 22°20′ | 300 | *Ps* | 2 (4) | 2 (8) | 2 (11) |
| | | | | *Pc* | 2 (4) | 2 (8) | 2 (10) |
| 11 | 68°20′ | 23°05′ | 340 | *Ps* | 2 | 2 | 2 |
| | | | | *Pc* | 2 | 2 | 2 |
| Total | | | | *Ps* | 19 (12) | 19 (21) | 19 (27) |
| | | | | *Pc* | 22 (12) | 22 (21) | 22 (25) |
| | | | | *Ps+Pc* | 41 (24) | 41 (42) | 41 (52) |

[1]Correspond to the numbers in Fig. 1
[2]*Ps* = *Pinus sylvestris*; *Pc* = *P. contorta*
[3]Values within parentheses represent the number used in the study of overall genetic variation

250 mM NaCl, 25 mM EDTA, 0.5% SDS) were placed in a water bath (60°C) for 30 min before adding 3 M sodium acetate, pH 5.2. DNA pellets were dissolved in TE buffer (Tris 10 mM, 1 mM EDTA, pH 8.0).

## 2.3 PCR amplification

The PCR amplifications were performed in volumes of 25 μl containing 5–15 ng template DNA, 150 μM of each dNTP (Pharmacia, Uppsala, Sweden), 0.2 μM primer and 0.75 U *Taq* DNA polymerase (Promega, Madison, WI, USA). The amplifications were performed in a DNA thermal cycler PTC 100 (MJ Research Inc. Watertown, MA, USA), programmed as described by Hamelin et al. (1995); namely, a denaturation step at 94°C for 3 min, was followed by 40 cycles at 94°C for 1 min, 35°C for 1.5 min and 72°C for 2 min. The final step was 5 min at 72°C. Amplification products were separated by electrophoresis on 1.4% agarose gels (1 × TAE buffer), containing ethidium bromide and visualized on a UV transilluminator. As a reference, BRL's 1-kb ladder was used. The gels were photographed with a Polaroid camera and the amplification patterns were examined. Fragment sizes were determined by measuring images on the photographs using a vernier caliper and comparing the values with those of the 1-kb ladder.

Thirteen arbitrary primers in Kit-A (OPA01–05, 07–08 and 10–15; Operon Technologies, Alameda, CA, USA), were screened on 10 representative isolates (from all three locations and two species) to find primers showing extensive RAPD variation between isolates and reproducible, strong amplification products.

To verify that the multiconidial isolates correspond to individuals, two tests were performed with the chosen primers on four different multiconidial isolates. First, ten small (5 × 5 mm) pieces systematically distributed over the mycelium plate of each of the four multiconidial isolates were compared with each other in terms of their RAPD patterns. Second, 3–5 single conidial and 1–2 multiconidial isolates from the same *in vivo* pycnidia were compared.

To determine the race and type identity of the material, the RAPD primers (OPA03, 08 and 12) and thermal-cycler programme described in HAMELIN et al. (1995) were used.

### 2.4 Scoring the data

In the study of overall genetic variation, only amplification fragments that were readable and reproducible were used. In the race/type study, the amplifications for all samples were repeated for primers OPA08 and 12. Primer OPA03 was used only once per sample since it always showed complete profiles. A fragment was accepted if the sample showed a readable and complete profile or if profiles, from at least two gels with indistinct profiles, showed the same pattern in the reading region.

### 2.5 Analysis

In the study concerning overall genetic variation, the frequency of polymorphic fragments was screened for different populations consisting of the whole material, the two host species and the three locations. A similarity index was calculated as $Sim_{xy} = N_{xy}/(N_x + N_y - N_{xy})$ where $N_x$ is the number of fragments present in population $x$, $N_y$ the number of fragments present in population $y$, and $N_{xy}$ the number of fragments common to $x$, and $y$ as described by MOREAU et al. (1994). Furthermore, the number of different RAPD profiles (haplotypes) was determined for each population.

Treatment of data from the race/type study was restricted to comparisons between the recorded amplification profiles and the fragments characteristic of the race and the three types within the European race (HAMELIN et al. 1995).

## 3 Results

### 3.1 Overall genetic variation

Two primers (OPA02 and 13) selected after the screening test gave a total of 23 readable and reproducible fragments. These RAPD fragments did not vary among samples from the same multiconidial isolate or between single and multiple conidia isolates from the same *in vivo* pycnidia.

Ten out of the 23 scored fragments were common to all populations (Table 2; Fig. 2), while five were absent or rare. Four (OPA02–700, OPA02–950, OPA13–650, and OPA13–900) of the remaining eight fragments were absent in the northern area, rare in the southern area, but common in the central area. In one case, the central area lacked a fragment (OPA13–1320) that was common in the northern and southern areas. None of the fragments showed pronounced differences in frequency between host species.

The matrix of similarity indices (Table 3) illustrates that the degree of similarity was greater between different host species than between geographical areas. The similarity index between isolates originating from different host species over all three geographical areas was 0.783. Table 4 shows that the proportion of polymorphic fragments was highest in the central area and lowest in the northern area.

A total of 26 different haplotypes were found in the material from northern Sweden (Table 5). There were more haplotypes in *P. sylvestris* than in *P. contorta*, and the relative number of haplotypes was greatest in the central area.

*Table 2.* **Frequencies of RAPD fragments amplified by primers OPA02 and OPA13 in the investigated populations of *G. abietina***

| Population | n | OPA02 -520 | OPA02 -700 | OPA02 -900 | OPA02 -920 | OPA02 -950 | OPA02 -990 | OPA02 -1100 |
|---|---|---|---|---|---|---|---|---|
| N. Sweden | 52 | 1.000 | 0.089 | 1.000 | 0.964 | 0.036 | 0.018 | 1.000 |
| *P. sylvestris* | 27 | 1.000 | 0.097 | 1.000 | 0.968 | 0.032 | 0.000 | 1.000 |
| *P. contorta* | 25 | 1.000 | 0.080 | 1.000 | 0.960 | 0.040 | 0.040 | 1.000 |
| Northern area | 21 | 1.000 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 1.000 |
| Central area | 14 | 1.000 | 0.143 | 1.000 | 0.857 | 0.143 | 0.071 | 1.000 |
| Southern area | 17 | 1.000 | 0.143 | 1.000 | 1.000 | 0.000 | 0.000 | 1.000 |

| Population | n | OPA13 −650 | OPA13 -700 | OPA13 -750 | OPA13 -790 | OPA13 -850 | OPA13 -900 | OPA13 -1100 | OPA13 -1150 |
|---|---|---|---|---|---|---|---|---|---|
| N. Sweden | 52 | 0.143 | 1.000 | 0.946 | 0.036 | 1.000 | 0.071 | 0.250 | 0.018 |
| *P. sylvestris* | 27 | 0.194 | 1.000 | 0.968 | 0.000 | 1.000 | 0.065 | 0.290 | 0.032 |
| *P. contorta* | 25 | 0.080 | 1.000 | 0.920 | 0.080 | 1.000 | 0.080 | 0.200 | 0.000 |
| Northern area | 21 | 0.000 | 1.000 | 0.952 | 0.048 | 1.000 | 0.000 | 0.238 | 0.000 |
| Central area | 14 | 0.286 | 1.000 | 0.929 | 0.071 | 1.000 | 0.214 | 0.214 | 0.071 |
| Southern area | 17 | 0.190 | 1.000 | 0.952 | 0.000 | 1.000 | 0.048 | 0.286 | 0.000 |

| Population | n | OPA13 −1250 | OPA13 -1300 | OPA13 -1320 | OPA13 -1350 | OPA13 -1400 | OPA13 -1580 | OPA13 -1700 | OPA13 -1950 |
|---|---|---|---|---|---|---|---|---|---|
| N. Sweden | 52 | 0.571 | 0.339 | 0.232 | 1.000 | 0.018 | 1.000 | 0.018 | 0.929 |
| *P. sylvestris* | 27 | 0.581 | 0.290 | 0.258 | 1.000 | 0.000 | 1.000 | 0.000 | 0.903 |
| *P. contorta* | 25 | 0.560 | 0.400 | 0.200 | 1.000 | 0.040 | 1.000 | 0.040 | 0.960 |
| Northern area | 21 | 0.714 | 0.333 | 0.429 | 1.000 | 0.000 | 1.000 | 0.000 | 0.905 |
| Central area | 14 | 0.357 | 0.643 | 0.000 | 1.000 | 0.000 | 1.000 | 0.071 | 0.857 |
| Southern area | 17 | 0.571 | 0.143 | 0.190 | 1.000 | 0.048 | 1.000 | 0.000 | 1.000 |

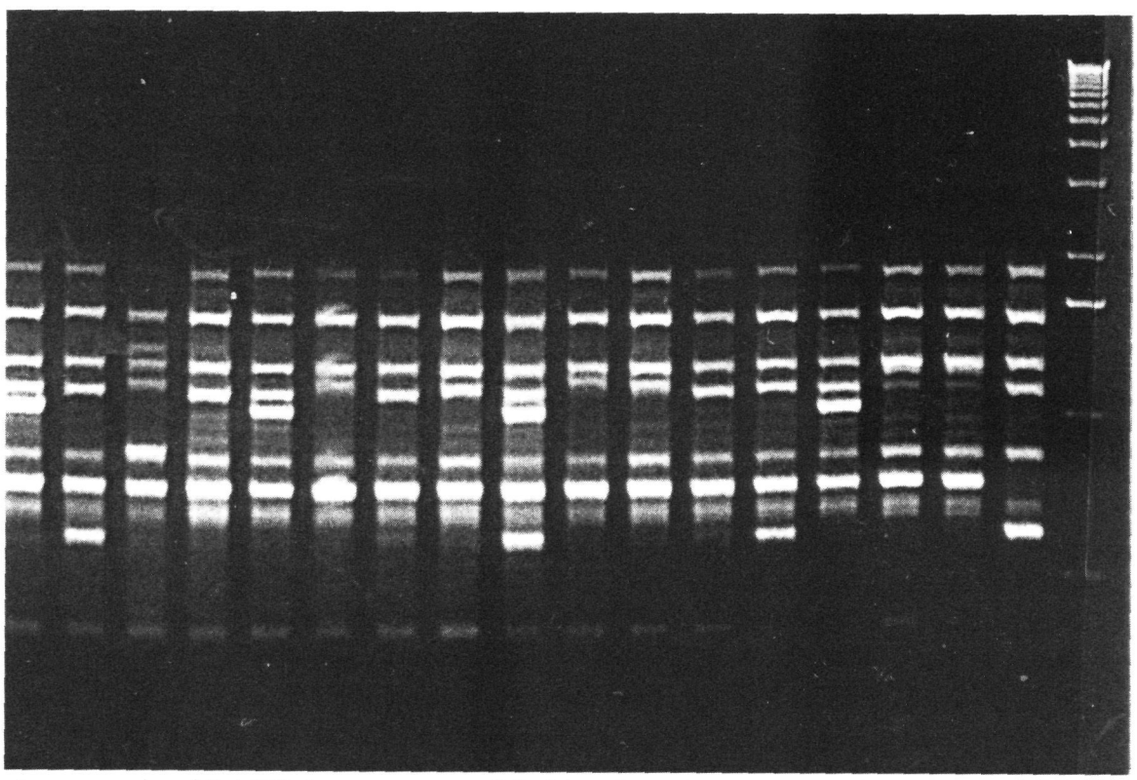

*Fig. 2.* An example of RAPD fragments amplified by the primer OPA13 in isolates of *Gremmeniella abietina* from the southern area. Lanes 1–8: isolates from *Pinus contorta*; lanes 9–17: isolates from *Pinus sylvestris*; lane 18: 1-kb ladder

### 3.2 Race and type distribution

All 41 samples used in this study possessed the fragment OPA03-750 and lacked OPA03-1700, which, according to HAMELIN et al. (1993), is typical for the EU race of *G. abietina*. Out of 39 samples giving satisfactory profiles, only 24 (62%) showed the pattern typical of the northern type (HAMELIN et al. 1995). The remaining samples showed three additional amplification profiles that could be distinguished based on fragments OPA12-1400 and OPA12-1500. The geographical distributions of these four profiles are shown in Figure 1. Fragment OPA08-600 was found in all samples. Neither the alpine nor the central European type (HAMELIN et al. 1995) was found in the sample. When the results from fragments OPA08-800, OPA08-1800 and OPA08-1900 were added, 16 different profiles could be seen.

The primers used to distinguish the race and type (OPA03, 08 and 12) did not reveal any differences in profiles between isolates originating from single-conidia and those originating from multiple-conidia isolates.

## 4 Discussion

### 4.1 Overall genetic variation

As discussed by ENNOS (1991), a typical shortcoming of many studies addressing genetic variation in fungi is small sample size. Furthermore, in many studies, it is difficult to determine whether sampled 'isolates' represent 'individuals' derived from a single spore or mixtures of individuals derived from different spores. This problem is particularly important in population genetics where analyses require samples consisting of known and comparable numbers of accessions from individual populations. To minimize the afore-mentioned problem, we collected fruiting bodies of *G. abietina* from several widely separated trees in each of several plantations that were geographically widely distributed over northern Sweden. To make the isolates as comparable as possible, only isolates derived from multiple conidia from a single pycnidium *in vitro* or, to lesser extent, *in vivo* were used. In view of the uniform results of the test with multiple-/single-conidial isolates, it can be assumed that the isolates, although of multiconidial origin, can be treated as separate individuals.

The considerable genetic variation in *G. abietina* found in northern Sweden suggests that the sexual reproduction system of this fungus is responsible for a substantial part of the spread. This is in accordance with the rich production of apothecia recorded in northern

*Table 3.* **Indices of similarity between *G. abietina* populations from different geographical areas and host species in northern Sweden. The bold italic numbers express the degree of similarity between geographical areas regardless of host species. *Ps* stands for the host *Pinus sylvestris* and *Pc* for *P. contorta***

| | | Northern area | | | Central area | | | Southern area | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Ps* | *Pc* | *Ps+Pc* | *Ps* | *Pc* | *Ps+Pc* | *Ps* | *Pc* | *Ps+Pc* |
| Northern area | Ps | – | | | | | | | | |
| | Pc | 0.933 | – | | | | | | | |
| | Ps+Pc | | | – | | | | | | |
| Central area | Ps | 0.684 | 0.650 | | – | | | | | |
| | Pc | 0.619 | 0.667 | | 0.810 | – | | | | |
| | Ps+Pc | | | ***0.609*** | | | – | | | |
| Southern area | Ps | 0.875 | 0.824 | | 0.790 | 0.714 | | – | | |
| | Pc | 0.778 | 0.737 | | 0.800 | 0.727 | | 0.889 | – | |
| | Ps+Pc | | | ***0.700*** | | | ***0.696*** | | | – |

*Table 4.* **Proportions of 23 polymorphic RAPD fragments (P) in the tested populations of *G. abietina* from northern Sweden**

| Population | Number of isolates | P[1] |
|---|---|---|
| 1. Northern *Ps* | 11 | 21.7 |
| 2. Northern *Pc* | 10 | 26.1 |
| 3. Central *Ps* | 7 | 43.5 |
| 4. Central *Pc* | 7 | 56.5 |
| 5. Southern *Ps* | 9 | 30.4 |
| 6. Southern *Pc* | 8 | 34.8 |

[1]A fragment is considered polymorphic if its frequency does not exceed 0.95

*Table 5.* **Absolute and relative numbers of different haplotypes in *G. abietina* in different populations**

| Population | Number of isolates | Different haplotypes | |
|---|---|---|---|
| | | Number | Proportion |
| Northern Sweden | 52 | 26 | 50 |
| *Pinus sylvestris* | 27 | 18 | 67 |
| *Pinus contorta* | 25 | 14 | 56 |
| Northern area | 21 | 10 | 48 |
| Central area | 14 | 10 | 71 |
| Southern area | 17 | 11 | 52 |

Sweden (KARLMAN et al. 1994) and the suggestion that apothecia formation is promoted by a harsh climate (ROLL-HANSEN 1982). The fact that the variation was greater between than within geographical locations confirms that the vegetative reproduction system is more important in short-distance spread and the sexual system in long-distance spread (SKILLING 1972).

One of the most important findings was the lack of obvious differences in the genetic composition between isolates attacking *P. sylvestris* and those attacking *P. contorta*. *G. abietina* isolates from *P. sylvestris* in southern Sweden and from three *P. contorta* plantations in one restricted area in northern Sweden differed with respect to conidial measurements and septation, but not with regard to host colonization (HELLGREN 1995). Analogous findings, indicating a lack of host-specificity, were revealed by a recent analysis of RAPD variation of essentially the same material (HELLGREN and HÖGBERG 1995), Nor did HAMELIN et al. (1995) find any evidence of host-specificity in a survey of European isolates. The fungus has probably not developed host-specific populations with regard to these species. It is possible that host-specific strains exist, but do not differ in their RAPD profiles.

### 4.2 Race and type distribution

HAMELIN et al. (1993) described the EU race as being characterized by the presence of fragment OPA03-750 and the absence of OPA03-1600. The results from primer OPA03 in the study presented here indicate that the EU race of *G. abietina* is the only one present in the material. These findings, together with results from the survey of European isolates (HAMELIN et al. 1995), suggest that the NA race is rare or absent in northern Sweden.

HAMELIN et al. (1995) distinguished three different geographical types within the EU race of *G. abietina* in Europe. The alpine type was characterized by the presence of the RAPD

fragments OPA08-1800 and OPA12-1400 and the absence of OPA08-600, OPA12-1350 and OPA12-1500. The central European type was characterized by the presence of the fragments OPA08-800, OPA08-1900, OPA12-1350 and OPA12-1400 and the absence of OPA08-600 and OPA12-1500. The northern type was characterized by the presence of OPA08-600, OPA12-1400 and OPA12-1500. The results presented here indicate that only the northern type exists in the investigated material from northern Sweden. According to HAMELIN et al. (1995), the northern type occurs north of latitude 63°N in Sweden and produces symptoms similar to those of the NA race. The alpine type is found at elevations above 2000 m in the Alps. The intermediate central European type, which occurs from the Apennines in Italy northwards up to central Sweden, affects the upper part of the crown of 30–40-year-old trees, and seldom produces apothecia. Although some samples were collected south of latitude 63°N, it is probable that they all represent the typical northern type, since the isolates have been collected in plantations and naturally regenerated stands (*P. sylvestris*) showing the 'small tree type' (HELLGREN and HOGBERG 1995) or 'type B' (UOTILA 1983) symptoms. The results indicate, however, that there is a considerable variation in the occurrence of fragments OPA12-1400 and OPA12-1500, which have been described as being diagnostic for the northern type by HAMELIN et al. (1995). Of the tested isolates, no fewer than 38% showed divergent profiles. Isolates with divergent profiles showed a wide geographic distribution and were obtained from both hosts (Fig. 1). This contradiction might be explained by the differences in the size and distribution of the samples collected between studies. In this study, the increase in examined isolates and completely distributed sampling areas expose a genetic variation that might have been hard to detect within the limited number of isolates, originating from only a few *P. contorta* plantations in northern Sweden, used by HAMELIN et al. (1995). HELLGREN and HÖGBERG (1995) reported that different isolates classified as belonging to the 'small tree type', all showed different amplification profiles. That study was, however, based on *P. contorta* isolates with unclear sampling documentation from one restricted area in northern Sweden (approximate latitude: 65°N).

In conclusion, the risk of spread from infected *P. contorta* plantations to adjacent areas with indigenous *P. sylvestris* regeneration, and vice versa, should be considered high in view of the indicated lack of host-specificity of *G. abietina*.

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## Résumé

*Variation RAPD chez Gremmeniella abietina parasite de Pinus sylvestris et P. contorta dans le nord de la Suède*

L'ADN génomique de 81 isolats de *G. abietina* récoltés dans 11 plantations de *P. sylvestris* et de *P. contorta* dans le nord de la Suède, a été étudié par marqueurs RAPD. La variation de l'ADN inter- et intra-populations a été etudié, ainsi que la race et le type de distribution des populations de *G. abietina*, responsable de symptômes semblables à ceux provoqués par le race nord-américaine. Le degré de similarité génétique était plus grand parmi les isolats issus des mêmes zones géographiques que parmi ceux d'origines différentes, qu'ils aient été isolés de *P. sylvestris* ou de *P. contorta*. La variation RAPD était plus grande dans les zones centrales du nord de la Suède, ce qui suggère que la reprodution sexuée y a été un peu plus importante que plus au nord ou plus au sud. Seuls les fragements RAPD

caractéristiques de la race EU de *G. abietina* ont été trouvés dans le matériel testé. Le profil RAPD décrit comme caractéristique du type nordique de la race EU a été identifié chez 62% des isolats. Les divergences par rapport au profil attendu étaient dues aux différences d'apparition des fragements OPA12-1400 et 12-1500. Ceci indique que cette partie du profil RAPD ne serait pas utilisable pour diagnostiquer le type nordique. Une conclusion d'importance pratique est qu'il y a un risque considérable de développment de *G. abietina* à partir des plantations infectées de *P. contorta* adjacentes aux zones indigènes de régénération du pin sylvestre et vice-versa, du fait de l'absence de spécificité d'hôte. Il est cependant possible que des souches hôte-spécifiques existent sans qu'elles différent par leurs profils RAPD.

## Zusammenfassung

*Variabilität von RAPDs bei Gremmeniella abietina von Pinus sylvestris und Pinus contorta in Nordschweden*

Genomische DNA von 81 *Gremmeniella abietina*-Isolaten aus je 11 Beständen von *Pinus sylvestris* und *P. contorta* im Norden von Schweden wurde auf RAPD-Marker untersucht. Dabei wurde versucht, die Variabilität zwischen und innerhalb von Populationen von *G. abietina*-Isolaten aus Wirten mit für die nordamerikanische Rasse typischen Symptomen zu erfassen. Unabhängig von der Wirstbaumart waren *G. abietina*-Isolate aus der gleichen geographischen Region untereinander genetisch ähnlicher als solche aus entfernten Regionen. Die Variabilität von RAPDs war im zentralen Teil von Nordschweden am größten, was vermuten läßt, daß die sexuelle Vermehrung dort von größerer Bedeutung ist als nördlicher oder südlich davon. Es wurden nur für die europäische (EU) Rasse von *G. abietina* typische RAPD-Fragmente gefunden. Aufgrund der RAPD-Analyse konnten 62% der Isolate der nördlichen EU Rasse zugeordnet werden. Allerdings wurden Abweichungen vom erwarteten Profil hinsichtlich des Vorhandenseins der Fragmente OPA12-1400 und 12-1500 festgestellt. Das bedeutet, daß diese Fragmente keinen diagnostischen Wert haben und zur Identifikation der nördlichen EU Rasse nicht dienen können. Von praktischer Bedeutung ist, daß sowohl ein großes Risiko der Ausbreitung von *G. abietina* von *P. contorta* Beständen auf solche mit einheimischer *P. sylvestris* besteht als auch umgekehrt, da *G. abietina* aufgrund der gefundenen RAPDs keine Wirtsspezifität aufweist. Allerdings kann die Existenz von Wirtsspezifität nicht völlig ausgeschlossen werden, da die gefundenen RAPDs nur einen kleinen Ausschnitt aus dem Genom darstellen.

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*Authors' addresses:* P. HANSSON and M. KARLMAN Department of Silviculture, Swedish University of Agricultural Sciences, Faculty of Forestry, S-901 83 Umeå, Sweden; A. E. SZMIDT and X-R. WANG Department of Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Faculty of Forestry, S-901 83 Umeå, Sweden

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