

Genetic Variation among European *Lophodermium piceae* Populations - Preliminary Results

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Abstract – *Lophodermium piceae* is a common needle endophyte of Norway spruce (*Picea abies*). The aim of the present study was to examine the degree of differentiation within and among European populations separated by various distances and geographical obstacles. For this purpose, populations (including > 10 isolates/subpopulation) were collected along a north-south transect stretching from the northern timberline in Finnish Lapland to the southern border of the distribution area of Norway spruce in northern Italy. Differentiation between *L. piceae* populations was determined from DNA sequences of three genetic markers. One of the markers was the internal transcribed spacer (ITS) of the ribosomal DNA and the other two (LP1 and LP2) were based on sequence characterized amplified regions (SCAR) designed for *L. piceae*. Preliminary results including sequences of Finnish, Swiss and Italian isolates show low differentiation among populations. According to analysis of molecular variance the among population variation was 1%, 5% and 0% in ITS, LP1 and LP2 markers, respectively.

Norway spruce / *Picea abies* / endophyte / genetic differentiation / gene flow

Kivonat – Genetikai variáció a *Lophodermium piceae* európai populációi között - előzetes eredmények. A *Lophodermium piceae* a lucfenyő (*Picea abies*) tűiben élő gyakori endofita gomba. E tanulmány célkitűzése megvizsgálni a differenciálódás fokát a különböző távolságok és földrajzi akadályok által elválasztott európai populációk között, és azokon belül. Ennek érdekében populációkat (> 10 izolátum/alpopuláció) gyűjtöttünk egy észak-déli metszéspont mentén, a finnországi lapp erdőhatártól Észak-Olaszországig, a lucfenyő elterjedési területének déli határáig. A *L. piceae* populációk közötti differenciálódást genetikai markerek DNS szekvenciája alapján határoztuk meg. Egyik marker a riboszomális DNS ITS szakasza, a másik kettő pedig (LP1 és LP2) a *L. piceae*-ra jellemző SCAR szekvenciák voltak. A finnországi, svájci és olaszországi izolátumok szekvenciáit tartalmazó előzetes eredmények alacsony differenciálódást mutatnak a populációk között. A molekuláris variancia-analízis szerint a populációk közötti variáció 1%, 5% és 0% volt az ITS, LP1 és LP2 markerek tekintetében.

lucfenyő / *Picea abies* / endofita / genetikai differenciálódás / génáramlás

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1 INTRODUCTION

This study is part of a project studying the differentiation and gene flow between distant populations of several fungal species occurring on Norway spruce and Scots pine (*Pinus sylvestris*). Knowledge on natural long-distance dispersal (>1000 km) of forest pathogenic fungi, i.e. by wind or animal vectors (insects, birds), is presently scant. Long-distance dispersal of pathogens attributable to human activities, e.g. by the import of seedlings or wood, has been disastrous in many instances, e.g. chestnut blight or Dutch elm disease. Sporal spread by wind over long distances is undisputable in the case of some pathogenic fungi of agricultural plants (Gregory, 1973; Schmale *et al.* 2006), but the dissemination potential of most forest pathogenic fungi and the significance of geographical barriers (e.g. areas without host trees, mountains or the sea) for dispersal is poorly known. Consequently, we do not know how rapidly these pathogens may invade new locations which have turned climatically suitable to them. Neither do we know what kind of barriers prevent long-distance translocation of fungi nor how different fungi vary in their ability to spread over long distances. Comparisons of genetic variation among and within populations from distant sites may help to reduce this lack of knowledge.

Lophodermium piceae is an extremely common endophyte in Norway spruce needles all over Europe, and the population size is relatively stable. It can generally be found in the majority of older (> 3 yr) needles of single trees and it may be one of the most numerous fungi in spruce forests. Locally, *L. piceae* is a highly diverse fungus and it is difficult to find identical (characterized by DNA markers) isolates even within a single needle. The fungus is transmitted by aerial spread of ascospores, which are formed on dead needles still attached to twigs in the tree crown or on fallen senescent needles. It is not known how the ascospores are dispersed over long distances. Remote populations are expected to differ distinctly genetically due to isolation if long-distance dispersal of ascospores is negligible. In this research, genetic differentiation and gene flow among several populations of *L. piceae* from Finland, Italy and Switzerland are studied using DNA sequence comparisons at three loci.

2 MATERIAL AND METHODS

One part of the isolates used in this study is identical to that described in Müller *et al.* (in press). The majority of the isolates were obtained from three to four year old healthy looking needles detached from mature Norway spruce trees. Each sampling site consisted of a mature forest with a size in the range of 2-100 ha. Two of the sampling sites included in this study represent extreme environments for Norway spruce. The permafrost site in Switzerland (Jura Mountains, Creux du Van, Neuchâtel) is a *Tofieldio-Piceetum* association on a cold scree slope at ca. 1200 m altitude. The presence of ice in the ground or between the blocks is often visible during early summer, and a cold wind is easily perceptible coming out of the block field (Pancza 1989). The mean annual ground temperature ranges between 0.5°C and 2.0°C with a maximum of 5.5°C (Delaloye *et al.* 2003). The Norway spruce trees at this site exhibit dwarfed growth. Trees older than 50 years, as determined by tree ring analysis, are only ca. 20 cm high. The North Finnish site at Ylläs is close to the timberline of Norway spruce in Finland. Sampling sites in Italy, Central Finland and North Switzerland are situated 20-100 km from each other, and those in South Finland maximally 280 km from each other. Surface sterilization of the needles and fungal isolation was carried out as described by Müller *et al.* (2001). Each isolate was obtained from a different tree individual within a sampling site. The number of isolates and origin are listed in *Table 1*.

DNA isolations, PCR, sequencing and design of the SCAR-markers LP1 and LP2 are described by Müller et al. (in press). Analysis of molecular variance (AMOVA) was calculated with Arlequin ver. 3.0 (Excoffier et al. 2005).

Table 1. Origin and number of isolates used for sequence analysis of three DNA-markers: ITS, LP1 and LP2.

Origin of isolates	Number of sampling sites	Number of isolates		
		ITS	LP1	LP2
Finland, North (at timberline)	1	13	12	12
Finland, Central	2	11	23	23
Finland, South	4	41	46	45
Switzerland, North	2	22	-	-
Switzerland, West (permafrost site)	1	13	11	11
Italy, North	2	25	23	23
Total number of isolates		125	115	114

3 RESULTS AND DISCUSSION

All three markers showed to be highly variable. Between 34 and 55 alleles could be detected among the examined isolates (Table 2). The majority of the alleles occurred in only one isolate each. The most common ITS, LP1 and LP2 allele occurred in 35, 23 and 25% of the isolates, respectively. Variation of allele frequencies was much greater within than among populations. Greatest among-population variation was found in LP1-sequences, amounting to 5% of the total variation (Table 2). All or almost all of the variation between LP2 and ITS allele frequencies was found within populations.

Table 2. Distribution (%) of the total variance among and within populations (AMOVA) and number of alleles found from each of the investigated markers

Source of variation	d.f.	Marker		
		ITS	LP1	LP2
Among populations	4-5	1	5	0
Within populations	109-119	99	95	100
Number of alleles		34	55	44

These results suggest significant gene flow among remote *L. piceae* populations in Europe. Presence of a diverse *L. piceae* population in the extreme climatic conditions at timberline in North Finland and the permafrost site in Switzerland is noteworthy. The mode of life of *L. piceae* includes periods dwelling at low activity as “dormant” microthalli within apparently healthy needles (Suske - Acker 1987, Suske - Acker, 1989, Müller et al. 2001). *L. piceae* is protected against harsh weather conditions during this dormant phase. *L. piceae* thalli resume growth in senescing needles and sporulate in spring that follows death of the needles. Weather conditions must probably be favourable only for a comparatively short period of time for sporulation and infection. Thus, as long as favourable conditions are sometimes met and if *L. piceae* ascospores get dispersed over long distances, isolation of *L. piceae* populations is prevented. It remains to be investigated in future studies, whether subpopulations of *L. piceae* show any phenotypic adaptation to extreme climatic conditions compared to subpopulations in favourable climatic conditions.

Acknowledgements - We thank Rauni Björkman and Minna Sinkkonen for technical help and Dr. Nicola LaPorta for providing needle samples from Italy for fungal isolations.

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