Population structure of *Serpula lacrymans* in Europe with an outlook to the French population

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**Abstract:** In this study the genetic variation and population structure in a French population of the dry rot fungus *S. lacrymans* was investigated using 14 microsatellites markers and compared to the rest of Europe. In that comparison the French population possessed the same allelic diversity as rest of Europe. A weak geographic structuring of the genetic variation was observed across Europe, where the French isolates to some extent separated from the rest of Europe, indicating that weak barriers to gene flow exists. Eighty percent of the isolates had unique multilocus microsatellite genotypes, which corresponds to high recombination and dispersal by sexual spores. Deviations from Hardy-Weinberg expectations were observed in multiple loci. In most loci there was an excess of heterozygotes, which could be due to either non-random mating, presence of more than two nuclei in the secondary mycelia or another unrecognized process. A total of six vegetative compatibility (VC) groups were present in Europe, out of which four were sampled in France. One VC group was over-represented in France while two others were underrepresented, as compared to the rest of Europe.

**Key words:** dry rot fungi, heterozygosity excess, population genetics, Serpulaceae, vegetative compatibility

**INTRODUCTION**

In temperate regions the dry rot fungus *Serpula lacrymans* is probably the most severe biodeterioration agent in wooden buildings (Jennings and Bravery 1991). It is highly prevalent in buildings in mid- and northern Europe, causing billions of Euros in damage every year due to its brown rot activity (Rayner and Boddy 1988, Schmidt 2006). Systematically *S. lacrymans* belongs to Serpulaceae, Boletales, Agaricomycotina.

Although not thoroughly investigated, it is believed that *S. lacrymans*, like most other basidiomycetes, has a short-lived monokaryotic stage before dikaryotization, resulting in a long-lived dikaryotic mycelia, which is the predominant form occurring in buildings. *Serpula lacrymans* propagates sexually by producing large resupinate pancake-like fruit bodies harboring billions of tetra-spore basidia. The monokaryotic primary mycelium (n) produces arthrospores—disarticulated propagules (Falck 1912, Schmidt and Moreth 1991). Moreover, it has been observed that the dikaryotic hyphae (n+n) can break off and disperse as arthrospores as well (Maurice et al. 2011b). More locally within buildings, *S. lacrymans* also may spread by vegetative mycelia, and strands can cover several square meters on floors, ceilings and walls (Savory 1964, Huckfeldt and Schmidt 2006).

*Serpula lacrymans* includes two genetically well separated lineages, var. *lacrymans* and var. *shastensis*, that probably represent separate species (Kauserud et al. 2007). While var. *shastensis* resides in natural habitats in Pacific North America, the aggressive form var. *lacrymans* has spread worldwide from its natural range in northeastern Asia. The European population is genetically homogeneous because it probably has expanded into buildings in northern and mid-Europe from a few founder events (Kauserud et al. 2004, 2007). In previous studies on genetic diversity of *S. lacrymans* in Europe, little genetic variation and geographic structuring has been detected among samples found mainly in northern Europe (Palfreyman et al. 2003; White et al. 2001; Kauserud et al. 2004, 2007; Engh et al. 2010). Moreover, only six
vegetative compatibility groups (VC) have been observed in European populations (Kaiserud 2004, Engh et al. 2010). This is a very low number of VC groups compared to natural populations of other Basidiomycetes because VC groups correspond to genetic individual in most naturally occurring isolates (Malik and Vilgalys 1999).

It has been hypothesized that the limited number of VC groups is due to a limited number of vic alleles being introduced to Europe. This means that different genets often will share the same vic alleles, making them unable to recognize self from nonself (Kaiserud et al. 2007).

Little emphasis has been put on understanding the diversity within the southern range of S. lacrymans in Europe, including France. The dry rot fungus is common in the northern and western part of France (Rioul and Bourreau 2002, Maurice et al. 2011a). Recently many wooden buildings in the Alps also have been infected. Infection rates in Mediterranean France are low, likely due to higher temperatures.

In this study the genetic variation and population structure of a French population of S. lacrymans was investigated, using microsatellites markers and multilocus sequencing and compared to the rest of Europe. Because several isolates were available from the same building in France, we also were able to investigate if multiple genets might co-occur in one building. Further, we wanted to reveal whether unique VC groups might occur in the French population.

MATERIALS AND METHODS

Sample.—A total of 76 European isolates of S. lacrymans were studied, among which 17 isolates were sampled from French buildings and 59 were collected from other European countries: Belgium (four), England/Scotland (six), Germany (19), Finland (seven), Norway (16), Poland (one), and six isolates with unknown European origins (Supplementary Table I). The French isolates were obtained from fruiting bodies or mycelium and isolated on an agar-based semiselective media for basidiomycetes, as described by Maurice et al. (2011a).

Culturing.—In vitro culturing of strains of S. lacrymans was performed to test for vegetative compatibility. All strains were grown on malt extract peptone agar (MEA) and incubated at 20°C. The strains were microscopically checked for clamp connections, and dikaryotic strains were confronted pairwise to reveal vegetative incompatibility groups (VC).

Molecular analyses.—Total DNA was extracted from 76 isolates (fruiting bodies and pure cultures) of S. lacrymans with the 2-CTAB (Cetyl trimethylammonium bromide) miniprep method (Murray and Thompson 1980). The DNA concentrations were measured with a spectrophotometer (NanoDrop ND-1000, Thermo Fisher Scientific, Wilmington, Delaware) and diluted to obtain a concentration of approximately 30 ng μL⁻¹. For 17 French and six other European isolates, the ITS nrDNA region and part of the beta tubulin (tub) gene were amplified with the primer pairs ITS1/ITS4 (White et al. 1990) and B36F/B12 (Thone and Royse 1999) respectively, as specified in Kaiserud et al. (2007).

Microsatellite analysis.—Fourteen microsatellite markers developed by Högb erg et al. (2006) and used by Kaiserud et al. (2007) and Engh et al. (2010) to differentiate between European and Japanese isolates of S. lacrymans were used to genotype European isolates. The microsatellite markers were run on an ABI 3730 DNA analyzer (Life Technologies, Foster City, California). The microsatellite markers were manually scored with GeneMapper 4.0 (Applied Biosystems). The dataset was converted into Arlequin 3.1 (Excoffier et al. 2006), STRUCTURE 2.3.3 (Pritchard et al. 2000) input formats using convert 1.31 (Gläubitz 2004).

Population genetic analyses.—Bayesian population structure analyses were conducted using the program STRUCTURE 2.3.3, a model-based clustering method for multilocus genotype data, to infer population structure and assign individuals to populations (Falush et al. 2003, Hubisz et al. 2009). A number of potential populations (K), 1–10, were tested, with simulations that were run 10⁶ Markov chain Monte Carlo (MCMC) iterations following a burn-in of 10⁵ iterations. STRUCTURE was set up to use an admixture ancestry model with correlated allele frequencies. Ten replicate analyses for each K, 1–10, were conducted. No structure was found for any K using STRUCTURE. The dataset were then analyzed in TESS 2.3, a Bayesian clustering approach that incorporates spatial information as prior (Durand et al. 2009). K = 2–K = 10 were analyzed with 10 replicates for each K. A boxplot was made of the 90% highest deviance information criterion (DIC) values of the 10 runs for each K. The decision of the Kmax was based on the significantly lowest DIC. The TESS admixture output for Kmax was further analyzed in R using the tessplot.r script available on the TESS webpage (http://membres-timc.imag.fr/Olivier.Francois/tessplot.r). This script uses the admixture information for each sample and the geographic information to calculate and plot an interpolated distribution for each cluster.

Principle coordinate analyses (PCoA) were conducted in PAST (Hammer et al. 2001) to visualize the genetic structure of the microsatellites dataset. Dice similarity index was used (on a dataset where microsatellite loci were scored as 0/1 for each allelic) to calculate the PCoA. Analyses of molecular variance (AMOVA) based on pairwise distances, observed and expected heterozygosity values (H o and H e) and deviations from Hardy Weinberg expectations also were calculated in Arlequin 3.1. Population genetic analyses were conducted (i) on the whole European population including France, (ii) on the European population excluding the French isolates and (iii) on the French isolates alone as a sampling locality. A chi-square test was used to reveal whether the French population had a deviating distribution of VC groups compared to rest of Europe.
RESULTS

Population genetics equilibrium.—From the 14 microsatellites loci, 44 alleles were amplified (mean = 3.143, SD = 1.505, range = 2–7). One locus (Sl1) was monomorphic for all samples and dismissed from further analyses. Of the 76 samples analyzed, 61 unique multilocus microsatellite genotypes (MLG) were observed. Of these 54 were observed in one individual only, while seven microsatellite genotypes were shared among two or more samples. Pairwise linkage disequilibrium (LD) was significant \((P, 0.05)\) among 14 pairs of loci out of a total of 78 comparisons. Results from the calculations of population genetic equilibrium of the whole European population and the French sampling locality are summarized (Table I). The European population showed significant heterozygosity excess \((P, 0.05)\) at six microsatellite loci (Sl4, Sl5, Sl6, Sl7, Sl8, Sl15). Of these loci, four (Sl4, Sl6, Sl8, Sl15) also showed significant heterozygosity excess in the French sampling locality. Loci Sl9, Sl10 and Sl11 showed significant heterozygosity deficit in the total European population, whereas loci Sl5, Sl9 and Sl10 showed significant heterozygosity deficit in the French sampling locality.

Population genetic structure in Europe.—The STRUCTURE analysis of the microsatellite data did not reveal any distinct structuring of the analyzed European population. For all runs \((K = 2, \ldots, 10)\), the replicate runs produced different likelihoods and each individual had equal probability of belonging to all groups. Likewise, there was a lack of geographic structuring observed in the French population when run separately. However, for the TESS analyses, \(K_{\text{max}} = 3\) was observed (SUPPLEMENTARY FIG. 1), which indicates that the European samples can be subdivided into three genetic groups. The samples were split into two main genetic groups where the French population was genetically divergent from the rest of the European population (Fig. 1). The third group was admixed where no individual had its main belonging to this group (Fig. 1c). When the French population were compared to the populations from the rest of Europe, analysis of molecular variance (AMOVA) ascribed 9.2\% of the total variation to between-group variation, while within-group variation was 90.8\%.

Visualization of pairwise genetic distances between isolates in a PCoA plot showed that the French isolates, as well as isolates from Finland and Norway, largely clustered together in separate groups (Fig. 2). The isolates from Germany also clustered, but this clustering was less refined and overlapped with Belgium, Norway and England/Scotland. The first axis likely represents a latitudinal gradient. As opposed to genetic distances, the genetic variation, according to VC groups, failed to structure geographic groups (SUPPLEMENTARY FIG. 2), thus indicating a lack of correspondence between the multilocus genotype profiles and VC types. Among the 23 isolates (17 French, six European) sequenced, variable sites were not present in either the ITS (630 bp) or \(tub\) (435 bp) regions.

Distribution of VC groups.—Based on the vegetative compatibility reactions, the 76 isolates of \(S.\ lacrymans\) could be ascribed to six VC groups. Only four of these VC groups (A, C, D, E) were present in the French

<table>
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<th>Locus</th>
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<th>Europe</th>
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* Significant deviation from Hardy-Weinberg expectations \((P < 0.05)\).
population. The distribution of VC groups in France deviated significantly from rest of Europe (Fig. 3; chi square test, \( P < 0.01 \)). In the French population, the VC groups C and D predominated. All six isolates sampled throughout the British Isles belonged to VC groups E. Two or three VC types were observed in four of the seven MLGs that were present in more than one strain. For the other three non-unique MLGs only one VC type was observed.

**Multiple genets in one building.**—In both buildings where multiple isolates were available, two different genotypes were detected. In one building the two genotypes were variable at microsatellite loci Sl7 and Sl8, and in the other building the two genotypes possessed different alleles at five loci (Sl4, Sl6, Sl7, Sl10, Sl15). However, in both buildings only one VC group was present.

**DISCUSSION**

Together with northern Italy and Spain, France represents the southernmost distribution of *Serpula lacrymans* in Europe. Lower genetic variation often
occurs at the boundaries of species ranges, illustrated in other organisms (Ellstrand and Elam 1993, Hill et al. 2011). However, in this study we have shown that the same level of genetic variation exists in the French population as compared with the rest of Europe. An average of 3.1 alleles per locus was observed among the 76 analyzed isolates. This is low compared to a Japanese population of *S. lacrymans* but consistent with previously published results of European isolates of *S. lacrymans* (Engh et al. 2010). Low genetic diversity of the European population is supported by a lack of variation between the two sequenced regions.

The results from the STRUCTURE and AMOVA analyses demonstrate that little genetic differentiation is present in the European population, including France. However, the PCoA plot and the TESS analyses, although weak, indicated that some geographic structuring is present. While the French (and some of the Finnish isolates for the PCoA plot) separated from the others, isolates from Belgium, Germany, Norway and the UK largely grouped together (Figs. 1, 2). Further, a skewed distribution of VC groups was observed in France compared to the rest of Europe, also indicating that the French isolates deviate somewhat in genetic composition. One may speculate that, if the genetic structure of the European population had been shaped by aerial spread of basidiospores alone, a more distinct isolation-by-distance pattern would have been observed in the PCoA plot (Fig. 2). Rather, the observed pattern might reflect the level of transport of infected materials between countries. For example, trade traditionally has been high between Germany and Norway (e.g. the Hanseatic League); we found similar overlap in genetic diversity between these two countries (Figs. 1, 2). Less trade between France and Norway likewise mirrors the lack of genetic overlap documented here. It is notable that Germany overlapped with France, Norway, the UK and Belgium, as might reflect Germany’s central position in European trade. However, analysis of a larger sample pool from different parts of Europe is necessary to test this.

Most isolates (80%) had unique multilocus microsatellite profiles, which indicate that spread by sexual basidiospores dominates in the European population of *S. lacrymans*. Moreover, isolates that shared multilocus microsatellite (MLG) profiles often belonged to different VC groups. Our results showed that multiple genets of *S. lacrymans* might occur within single buildings. Hence, although there are some indications that trade and transport of infected materials might have contributed to shape the genetic structuring of *S. lacrymans* in Europe (as discussed above), it is also likely that meiospore dispersal rather than clonal dispersal dominated their spread. As such, we cannot rule out the importance of meiospore production.

The population-level genetic analyses revealed that deviations from Hardy-Weinberg expectations were present in several loci and that most loci had an excess of heterozygotes. This was the case both for the French population and Europe as a whole, which is in line with what was observed by Engh et al. (2010). It is known that heterozygosity can be decreased by a founder event (Balloux et al. 2003, Goyeau et al. 2007). This has not happened in the European *S. lacrymans* population, although it was established in Europe through a founder event (Kauserud et al. 2007, Engh et al. 2010). Although speculative, high heterozygosity could be due to non-random mating because of a limited number of mating types present in the European *S. lacrymans* population. The more successful mating of nonrelated isolates with different mating types could lead theoretically to more heterozygous loci. This effect would be very strong if some of the microsatellite loci were closely linked (data not shown) to the two MAT loci of *S. lacrymans* (JGI Genome Browser http://genome.jgi.doe.gov/, Eastwood et al. 2011).
The heterozygosity excess likewise could have been attributed to the existence of more than two nuclei in the secondary mycelia. This phenomenon has been observed in species with rare clamp connections such as *Heterobasidion annosum* (Johannessen and Stenlid 2004) or clampless species such as *Agaricus bisporus* (Raper et al. 1972) but, as far as we know, not in species regularly making clamp connections on dikaryotic hyphae such as *S. lacrymans*. However, during previous mating experiments with *S. lacrymans* we have observed that homokaryons that were unable to mate due to the presence of similar MAT alleles have formed a “chimeric mycelium” including two interwoven homokaryotic mycelia. This apparently happens because European isolates of *S. lacrymans* often share the same *vic* alleles, hence are unable to recognize self from nonself (Kauserud et al. 2006). Whether the high levels of heterozygotes are due to the presence of multiple nuclei in the *S. lacrymans* mycelia must be investigated more thoroughly (e.g. by use of microscopy).

Even though the European *S. lacrymans* population most likely was established over 100 y ago through a founder event (Kauserud et al. 2007), this study demonstrates that there is some genetic structuring within the European population. This supports the emerging view that geographic distances limit the dispersal of fungi.

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**COMPETING INTERESTS**

The authors declare no competing interests.

**AUTHOR CONTRIBUTIONS**

HK and GB designed the study. SM carried out the experiments. SM and IS performed the population genetic analyses. SM, IS and HK wrote the paper. All authors read and approved the final manuscript.

**LITERATURE CITED**


