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Fungal Genetics and Biology 43 (2006) 503–510

FUNGAL GENETICS AND BIOLOGY
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# Genetics of self/nonself recognition in *Serpula lacrymans*

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Received 13 June 2005; accepted 11 February 2006

## Abstract

This study provides an analysis of the vegetative incompatibility system in *Serpula lacrymans* (Basidiomycota), a genetic system used to recognize nonself in fungi. Seventy-five worldwide isolates could be grouped into eight vegetative compatibility (VC) types, some of them distributed on different continents. Mating studies combined with vegetative incompatibility analyses revealed that the vegetative incompatibility response between isolates mainly could be explained by two biallelic vegetative incompatibility (*vic*) loci. The frequency distributions of the interpreted *vic* alleles do not seem to support the idea of frequency-dependent or balancing selection acting on the *vic* loci. We find little genetic variation at the *vic* loci and in one of the loci there was a significant heterozyote deficiency among strains in the overall material. The results may be explained by a recent worldwide dispersal of a few *S. lacrymans* isolates and, correspondingly, only a few *vic* alleles are being maintained in these populations.

*Keywords:* *Serpula lacrymans*; Basidiomycota; Vegetative incompatibility; *vic*; Frequency dependent selection

## 1. Introduction

The normal basidiomycete life cycle includes a short-lived monokaryotic primary mycelium phase followed by a predominant secondary dikaryotic mycelium phase—in which the fruit bodies (basidiocarps) are produced. The mating compatibility system regulates the fusion between primary mycelia, while the vegetative incompatibility system regulates fusion between secondary mycelia or between primary mycelia with similar mating types (Rayner et al., 1984). Successful mating between basidiomycetes requires the override of vegetative incompatibility by the mating compatibility system. These two genetic systems operate in opposite ways: mating incompatibility is associated with genetic similarity in the mating type (MAT) loci, while vegetative incompatibility is associated with genetic dissimilarity in the vegetative incompatibility (*vic*) loci (Malik and Vilgalys, 1999). Vegetative incompatibility is thought to provide a protective mechanism to prevent transmission of infectious cytoplasmic elements, such as mycoviruses and senescence plasmids and avoid exploitation by 'aggressive' genotypes (Hartl et al., 1975; Milgroom, 1999; Worrall, 1997). Generally, fungi are thought to be vegetatively incompatible if they do not share alleles at all *vic* loci (Malik and Vilgalys, 1999). Based on this assumption, the vegetative incompatibility response has been extensively used for delimiting genets (genetically distinct individuals) and for studying the population biology of basidiomycetes. At the macroscopic level, two fungal strains are said to be vegetative incompatible if an interaction zone or barrage can be detected between them. Hyphal anastomosis between individuals who have alternative allelic specificities at any *vic* locus, usually results in compartmentation and death of the hyphal fusion cell. At the molecular level,

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doi:10.1016/j.fgb.2006.02.004

vegetative incompatibility is probably mediated by the presence of a heterocomplex composed of polypeptides encoded by the alternative *vic* alleles (Glass et al., 2000). In ascomycetes, genetic studies have shown that there are several unlinked *vic* loci that regulate the incompatibility response (Cortesi and Milgroom, 1998; Perkins and Davis, 2000; Saupe et al., 2000). Compared to ascomycetes, our knowledge of vegetative incompatibility systems in basidiomycetes is scanty and only a few studies have been conducted. Confrontation experiments have suggested that at least three to four loci are involved in the vegetative incompatibility reactions in *Collybia fusipes* and *Heterobasidon annosum* (Hansen et al., 1993; Marcais and Delatour, 2000). Unlike ascomycetes, which are haploid, the dominant mycelial phase of basidiomycetes is generally dikaryotic, which adds an extra layer of complexity to genetic studies of basidiomycetes.

Self-recognition systems of plants, animals and fungi share several general features, including the maintenance of large numbers of alleles at relatively even frequency over very long time periods (Richman, 2000). It has been hypothesized that the *vic* loci are under some form of frequency-dependent selection, favoring rarer alleles or genotypes (Muirhead et al., 2002; Wu et al., 1998). It is believed that this rare allele advantage is because strains with rare *vic* genotypes are less susceptible to infections by viruses, and they avoid exploitation by aggressive genotypes. Both theoretical and empirical data support the hypothesis of frequency-dependent selection acting on these systems (Brasier, 1988; Brasier and Buck, 2001; Muirhead et al., 2002; Wu et al., 1998). However, high levels of polymorphism can also be maintained by overdominance or other forms of balancing selection, as discussed in other self/nonself recognition systems (e.g., Nei and Hughes, 1991).

The present study deals with the vegetative incompatibility system in the dry rot fungus *Serpula lacrymans* (Boletales). This fungus is the most aggressive wood-rotting fungus that attacks houses and other wooden structures in temperate regions. It has been demonstrated that two sexually compatible varieties exist within *S. lacrymans*, one occurring frequently in houses worldwide (var. *lacrymans*), and one genetically distant ancestral group represented by individuals from forests in Northern California (var. *shastensis*) (Harmsen, 1960; Kauserud et al., 2004a). Kauserud (2004) observed that geographically widespread VC types occur among var. *lacrymans* isolates in Europe. AFLP analysis of European strains demonstrated that var. *lacrymans* in Europe is genetically extremely homogenous and that the few occurring AFLP polymorphisms are distributed independently of VC type boundaries, suggesting that the VC types do not represent clones. Rather, different genets of *S. lacrymans* frequently share similar *vic* alleles due to low genetic variation at these loci (Kauserud et al., 2004b).

The primary aim of this study was to decipher the genetic basis for the vegetative incompatibility response in *S. lacrymans* var. *lacrymans* and assign *vic* genotypes to the different VC types. This has been done by performing mating experiments between monokaryotic spore families of *S. lacrymans* coupled with vegetative incompatibility analyses. Further aims have been to perform population genetic analyses of the *vic* genotypes in order to (i) infer whether the observed *vic* loci are governed by frequency dependent selection, (ii) determine whether clonality or sexual propagation is predominant, and (iii) analyse the geographic distribution of *vic* alleles.

## 2. Materials and methods

Seventy-five dikaryotic isolates and 22 monokaryotic single-spore isolates of *S. lacrymans var. lacrymans* obtained from six dikaryons (six spore families) were included in the study (see Table 1). The isolates were obtained from various culture collections or obtained directly from buildings by the authors. Mating compatibility was studied in the 22 monokaryotic single-spore strains, crossed in all combinations. Two single-spore cultures were placed 2 cm apart on 9 cm petri dishes on 2% malt extract agar (MEA), incubated at 25 °C, and examined after four to five weeks. The crossings were judged as sexually compatible or incompatible based on presence or absence of clamp connections. Dikaryotic mycelium also showed a different morphology than monokaryotic mycelium, being less cotton-like and more yellowish with age. Vegetative incompatibility tests were carried out by placing three inocula 2 cm apart on 9 cm petri plates (2% MEA), incubating at 25 °C, and examining after four to five weeks (see Fig. 1). Both the mating tests and vegetative incompatibility tests were 100% reproducible when tested on a subset of isolates and self-pairings of dikaryons gave no vegetatively incompatibility reactions. Population genetic analyses of the interpreted *vic* genotypes were performed in Arlequin ver. 2.0 (Schneider et al., 2000).

## 3. Results

Based on vegetative incompatibility analyses (Fig. 1), the 75 field dikaryotic *S. lacrymans* var. *lacrymans* isolates could be grouped into eight VC types. All vegetatively compatible isolates were grouped into the same VC type and each VC type was given a letter from A to H ($VC_A$ to $VC_H$), according to Kauserud et al. (2004b). The geographic distributions of the VC types are shown in Fig. 2. $VC_A$ to $VC_E$ had wide distributions in Northern Europe, and $VC_A$, $VC_B$ and $VC_D$ included isolates from Australia, Canada and New Zealand as well. Two Japanese isolates constituted unique VC types ($VC_G$ and $VC_H$).

To uncover the genetic basis of the vegetative incompatibility response, six spore families (including a total of 22 monokaryotic isolates) obtained from six European dikaryons belonging to the VC types $VC_A$ to $VC_E$, were crossed in all combinations (231 crossings). Unfortunately, we did not possess monokaryons from $VC_F$, $VC_G$ and $VC_H$ for the crossing experiments. Compatible and incompatible crossings could readily be separated based on two criteria: