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Interfertility and genetic variability among European and North American isolates of the basidiomycete fungus *Chondrostereum purpureum*

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ABSTRACT

The conspecificity of Finnish and western Canadian isolates of the decay fungus *Chondrostereum purpureum* was investigated by several approaches, including the assessment of genetic variability, mating and progeny analysis, and the analysis of selected phenotypic traits. Eight second-generation single spore strains per fungal isolate pairing were investigated with specific genetic markers developed for both Finnish and Canadian parental isolates. Tests of linkage disequilibrium were used to analyze whether these markers assorted independently among single spore strains. This procedure was similarly applied to the third-generation spore progeny. Finally, global non-metric multidimensional scaling was used to analyze independent random amplified microsatellite marker data to assess the genetic variability of the parental Finnish and Canadian isolates, and their second- and third-generation progeny. Our results revealed that the parental isolates from Finland and western Canada were genetically divergent, but no interfertility barriers were identified between these geographically distant fungi. Furthermore, parental genetic markers used in mating studies demonstrated that second- and third-generation spore progenies underwent normal meiosis and genetic recombination without linkage disequilibrium. Based on this work, the studied *C. purpureum* isolates from Finland and Canada can be considered as belonging to a single biological species, although genetic and limited phenotypic differentiation was observed.

1. Introduction

Individuals belonging to the same species usually have a similar genetic background, but the different criteria used to determine species boundaries may not always provide the same conclusion. According to the biological species concept, organisms are considered to be conspecific if they have the ability to interbreed and produce viable progeny (Mayr, 2000). This is not a universal definition that may be applied to all species of fungi, as there are many that do not reproduce sexually and their species boundaries must be defined by other criteria (e.g., morphological, ecological, physiological and phylogenetic), with varying degrees of success (Cai et al., 2011; Giraud et al., 2008; Taylor et al., 2000). When following a morphological species concept, divergence in morphology is used as a species recognition criterion, whereas in ecological species criteria, adaptation to a specific ecological niche is cited, while for physiological species criteria, the organisms' function is considered (growth rate at a specific temperature or the ability to produce toxins), and in a phylogenetic species concept, nucleotide divergence (DNA sequences of selected genes or genomes) is the basis for determining organisms are different species.

In fungi, individuals belonging to the same biological species may undergo fusion of compatible mycelia followed by sexual events that produce recombinant genotypes containing novel combinations of parental genes. The development of barriers that limit these events and contribute to speciation is a temporally

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extended process that may have a number of contributing factors, including neutral or adaptive divergence, intersterility of genotypes observed as vegetative incompatibility, as well as morphological and ecological divergence related to fungal lifestyle (Cai et al., 2011). These factors may be encountered by both sympatric (i.e., local) and allopatric (geographically distant) fungal populations, with geographical isolation also contributing to reduced gene flow among disparate populations (Cai et al., 2011; Giraud et al., 2008). In allopatric populations, geographic barriers may cause speciation, whereas in sympatric populations, host specialization (i.e., individuals specialize to different ecological niches) may create barriers between the individuals of conspecific organisms. Among the plant pathogenic fungi, often only isolates growing within the same host tissue can mate which may, in theory, create conditions where different fungal genotypes become niche specialists in a sympatric population. For example, in *Heterobasidion annosum*, three intersterility groups are found in Eurasia and two in North America, and these groups are now formally described as species (Garbelotto and Gonthier, 2013).

During gametogenesis, meiosis allows the recombination of parental genotypes, producing progeny that are assumed to have a random assortment of parental genes (Mendelian inheritance). The nonrandom association of alleles at different loci can be investigated using linkage disequilibrium analyses, which are based on the occurrence of specific gene markers in DNA (Slatkin, 2008). The presence or absence of linkage disequilibrium is important in evolutionary biology as it reflects the population history, and may reveal the geographic subdivision of populations, selection pressures experienced by the population, gene conversion, gene linkage and mutation events (Slatkin, 2008) as well as conspecificity of fungi (Grillo et al., 2005).

The white rot basidiomycetous fungus *Chondrostereum purpureum* is a weak pathogen that can infect fresh wounds of deciduous trees causing silver-leaf disease; it can also grow saprophytically as an early-stage colonizer of dead wood (Becker et al., 2004; Butler and Jones, 1949; Gosselin et al., 1996, 1999a; 1999b; Lygis et al., 2012; Spiers et al., 2000). The fungus has both a broad host range and a wide geographical distribution in northern hemisphere temperate and boreal forest zones, occurring in North America and Europe, and in New Zealand as an introduced species (Ramsfield et al., 1996). The species disperses via wind-borne, meiotically-produced basidiospores, enabling gene flow among disparate populations (Becker et al., 2004; McDermott and McDonald, 1993). Although the majority of spores fall within the vicinity of a sporophore for *C. purpureum* (at maximum ca. 500 m from the source, De Jong et al., 1990) and other wood-decay fungi, some basidiospores may disperse by air over hundreds of kilometers (Edman et al., 2004a, 2004b; Garbelotto and Gonthier, 2013; Stenlid and Gustafsson, 2001). Furthermore, the species possesses a heterothallic, tetrapolar mating system that favors outcrossing (Nakasone, 1990), thus ensuring a high level of compatibility among homokaryotic mycelia derived from geographically distant populations (Becker et al., 2004; Wall et al., 1996). Successful matings of compatible homokaryons will produce heterokaryotic isolates that will typically form the founder population in an affected host tree (Spiers et al., 2000).

The species *C. purpureum* shows great potential as a bioherbicide agent for the suppression of re-sprouting by hardwood tree species; consequently, populations of *C. purpureum* has been investigated on a regional and continental scale to evaluate their mating system, morphology, variability in growth and virulence, mechanisms of pathogenicity, isoenzymatic profiles and population genetic diversity (Becker et al., 2004; 2005; Ekramoddoullah et al., 1993; Gosselin et al., 1999a, 1996; Ramsfield et al., 1996; 1999; Shamoun and Wall, 1996; Spiers et al., 2000; Vartiamäki et al., 2008; Williams et al., 2002). These investigations support a panmictic population for *C. purpureum,* with limited geographical or host specialization over its known range (Gosselin et al., 1996, 1999a; Ramsfield et al., 1999). The genetic diversity within populations has usually been high, accounting for most of the total genetic variation, while variation among populations has been small (Gosselin et al., 1999a, 1999b; Becker et al., 2004).

In this study, we investigated whether geographically distant Finnish and Canadian isolates of *C. purpureum* belong to the same biological species, or have become intersterile and thus unable to produce viable progeny. To investigate conspecificity, we paired homokaryons derived from Finnish and Canadian *C. purpureum* strains to determine the success of these matings. We also analyzed their sexual progeny to detect the occurrence of linkage disequilibrium among parental genetic markers. Different genetic markers were employed to allow the evaluation of phylogenetic species criteria and the assessment of genetic divergence between the isolates from Finland and Canada. In addition, phenotypic traits were assessed to compare these fungal isolates and evaluate conspecificity.

2. Materials and methods

2.1. Culture of fungal isolates, phenotypic and mating analysis

A total of 10 *C. purpureum* isolates, five Finnish (from southern and middle Finland) and five Canadian (from western Canada), were chosen for the study (Table 1). The phenotype of isolates was compared by measurements of laccase enzyme activity, mycelial growth rate, time to fruiting body development and the germination rate of basidiospores produced by those isolates. For the determination of laccase activity, fungal isolates were initially cultivated on Potato Dextrose Agar (PDA) Petri plates (24 g potato dextrose broth and 15 g agar in 1000 ml deionized water, Becton, Dickinson and Company, Sparks, US) at 20 °C for seven days. An agar plug (6 mm diam.) was transferred with a sterilized Pasteur pipette from the edge of the mycelium to the center of an ABTS [2.2′-azinobis (3-ethylbenzthiazoline-6-sulphonate)] plate to determine relative laccase activity of *C. purpureum* isolates, based upon a colorimetric reaction of the medium (see Hamberg et al., 2015). Two replicate plates per fungal isolate were incubated at 24 °C for four days, and the color reaction of the plates was determined using a relative scale; 0 = no color reaction (no laccase activity), 1 = slight green color reaction (low laccase activity), 2 = intermediate color reaction (moderate laccase activity), and 3 = strong green color reaction (high laccase activity).

The mycelial growth rate of both Finnish and Canadian isolates was determined at different temperatures in order to detect differences in phenotype. Each isolate was initially cultivated on PDA medium for seven days at 20 °C; and an agar plug (6 mm diam.) was then transferred with a sterile Pasteur pipette from the edge of the mycelium to the center of a new PDA Petri plate. Four replicates of each fungal isolate at each temperature (6, 12, 18 and 24 °C) were cultivated for 20 d, or until the hyphae were almost at the edge of a plate. Radial growth (mm) from the edge of the plug was measured in opposing directions at least twice per week (6 and 12 °C) or daily (18 and 24 °C) except on weekend days. The mean radius for each interval was calculated, and after plotting the values, the growth rate (mm d^{-1}) was calculated for the linear growth phase only.

In order to pair homokaryotic cultures derived from Finnish and Canadian isolates, each isolate was cultured on a PDA medium for about 3–4 weeks at 20 °C until it formed fruiting bodies (Table 1). A fruiting body was cut from a plate with a sterilized scalpel and transferred to a new PDA plate and attached to the underside of the lid. A drop of sterilized water was placed next to the fruiting body in