Thesis for the degree of Doctor, Sundsvall 2014

WOOD FUNGI AND FOREST FIRE

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ISSN 1652-893X
Mid Sweden University Doctoral Thesis 204
ISBN 978-91-87557-88-0

Akademisk avhandling som med tillstånd av Mittuniversitetet i Sundsvall framläggs till offentlig granskning för avläggande av filosofie doktorsexamen 03, 10, 14, klockan 10.00 i sal m111, Mittuniversitetet Sundsvall. Seminariet kommer att hållas på engelska.

I was made to play double bass
Tuomo Haapala once told me
Now im telling stories mute of sound
Ill try my best

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Printed by Mid Sweden University, Sundsvall, Sweden, 2014

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ISSN 1652-893X, Mid Sweden University Doctoral Thesis x; ISBN 978-91-87557-88-0

ABSTRACT
Forest fires have been the major stand-replacing/modifying disturbance in boreal forests. To adapt to fire disturbance, different strategies have evolved. This thesis focuses on wood fungi, and the effect of forest fire on this organism group. In many ways it is a study on adaptation to forest fire, in concurrence with adaptation to dry open habitats. In Paper I we study increased heat resistance in mycelia from species prevalent in fire prone environments. Fungi were cultivated on fresh wood and exposed to different temperatures. Species prevalent in fire affected habitats had a much higher survival rate over all combinations of time and temperature compared to species associated with other environments. Based on this results the competitiveness was tested after temperature stress (paper II), three fire associated species, were tested against three non fire associated species. All fire associated species had a clear advantage after heat treatment, conquering a larger volume of wood than its competitor. In paper III we studied the effect of heat shock on decomposition rate, 18 species was tested. Species were cultivated and monitored for CO_2 accumulation for 8 weeks and then heat shocked. All species including non fire associated species seemed to up-regulate decomposition after heat shock, this response was more pronounced in fire associated species. To look at the possible effect of forest fire on population structure (Paper IV), we developed 30 SNP for Phlebiopsis. gigantea. We amplified the marker containing fragments in 132 individuals of P. gigantea in 6 populations, 3 which were found in areas affected by forest fire and 3 in unaffected areas. We found no genetic structure in accordance to forest fire. However we detected geographic structure, which stands in contrast to earlier studies. This might be due to the method, using SNP´s and number of individuals in the study. Finally we collected cross-sections of decayed logs to evaluate the number of fungal species domains that are likely to be hit when drilling a saw-dust sample in a log. We used these estimates to simulate how many species that will be found by a certain number of samples. We found that in 99% of the simulations 4 or less species will be contained in a sample. We then tested if it would be possible to use T-RFLP to detect as many species at different DNA concentrations. We found that in most cases T-RFLP will be a reliable method (Paper V).

Keywords: Wood Fungi, forest fire, Genetics, Heat Shock, Population Genetics, Basidiomycetes, Interspecific competition, T-RFLP, Molecular methods, Carbon dioxide evolution

SAMMANDRAG

Bränder har historiskt varit vanliga i det svenska skogslandskapet, vilket gjort att arter har varit tvungna att anpassa sig och vissa arter har hittat en specifik nisch i förhållande till den här störningen. Den här avhandlingen handlar till stor del om detta, om hur en artgrupp; vedsvampar har anpassat sig till skogsbrand och hur frånvaron av skogsbränder under senare tid påverkar artgruppen. Våra resultat visar att mycel från arter som återfinns i brandpåverkade miljöer har högre värmetolerans än mycel från arter vilka återfinns i andra skogsmiljöer. Vidare gynnas dessa arter konkurrensmässigt av en värmechock som liknar den mycelet hypotetiskt utsätts för under en skogsbrand. Ett annat intressant resultat är att alla arter som vi testade uppreglerade nedbrytningen efter värmechock, detta mönster var extra tydligt hos arter som man återfinner i brandpåverkad skog eller liknande varma torra miljöer. Resultaten som beskrivs ovan tyder på att skogsbranden har en stor påverkan på artsammansättningen i skogslandskapet, och att frånvaron av densamma har förändrat förutsättningarna i de boreala ekosystemen.

För att studera om detta eventuellt går att detektera populationsgenetiskt utvecklade vi 26 genetiska markörer (SNP). Vi fann ingen genetisk struktur i förhållande till skogsbrand, däremot hittade vi viss rumslig struktur. Vilket står i kontrast till tidigare studier på vanliga vindspridna svamparter, där man inte har hittat någon tydlig struktur. I den sista studien testade vi en molekylär metod för att detektera arter i ved, detta är viktigt, då man annars är hänvisad till de arter som satt fruktkroppar. Det finns ett flertal sådana metoder men med olika användningsområden. Studien belyser både vilken provtagningsintensitet som behövs och analyserar metoden (T-RFLP) möjlighet att detektera de dominerande vednedbrytande svamparna i död ved. Resultaten visar att xx prov ur ett enskilt dött träd är tillräckligt för att täcka in de dominerande arterna och att T-RFLP kan identifiera upp till 5 arter ur ett enskilt prov.

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LIST OF PAPERS

This thesis is mainly based on the following five papers, herein referred to by their Roman numerals:
Paper I and II are printed with the consent of Elsewier Publishing Group

Paper I
Carlsson F, Edman M, Holm S, Eriksson AM, Jonsson BG (2012) Increased heat resistance in mycelia from wood fungi prevalent in forests characterized by fire: a possible adaptation to forest fire. Fungal Biology 116: 1025-1031

Paper II
Carlsson F, Edman M, Holm S, Jonsson BG (2014) Effect of heat on interspecific competition in saprotrophic wood fungi. Fungal Ecology 11: 100-106

Paper III
Carlsson F, Edman M, Holm S, Jonsson BG (2014)
Increased decomposition triggered by heat shock found in wood fungi.
Manuscript

Paper IV
Carlsson F, Olsson J, Holm S (2014)
Strong clustering in an SNP study of *Phlebiopsis gigantea* in Sweden.
Manuscript

Paper V
Carlsson F, Koch C, Edman M, Holm S, Testing the probability to find major decomposing Basidiomycetes in logs with T-RFLP – implications for field sampling
Submitted.

LIST OF ACRONYMS AND DEFINITIONS

SNP – Single nucleotide polymorphism
FST – Fixation index, measure of population differentiation
HSP – Heat shock protein
HSE – Heat shock element
XRE – Xenobiotic element
CBH - Cellobiohydrolase
Saproxylic – Dependent on dead wood
Saprophytic – Living on dead organic material
Sporocarp – Fruit body
Basidion – Fungal cells in which meiosis occurs
SMC – Small mycelia constituents
CWD – Coarse woody debris
PCR – Polymerase chain reaction
BLAST – Basic local alignment search tool

1. INTRODUCTION

1.1. Overview

This dissertation is about wood fungi and their adaptations to forest fire. First, it is an investigation of the physiological effects of forest fire on mycelial heat resistance, interspecific competition of saprotrophic wood fungi and wood decomposition. Second, an examination of the genetic structure of wood fungi populations in relation to forest fire. Third, it explores how to sample wood fungi in an efficient way, in relation to colonization patterns inside a piece of dead wood.

1.2 Wood Fungi

Wood fungi as a group consists of species from the whole fungal kingdom, and are therefore not a defined taxonomic group. Species from the fungal domains Basidiomycota and Ascomycota are the main constituents, and

the term is generally used for the fruit body producing Basidiomycetes and Ascomycetes, excluding molds and yeasts. In this thesis research is done exclusively on Basidiomycetes, so henceforth when the term wood fungi is used, it describes only species from this phyla.

Wood fungi are regarded as the main decomposers in boreal forests and play an important role in the functioning of forest ecosystems. As decomposers, they contribute to the turnover of nutrients and organic matter. Over time, the breakdown of fallen and standing trees will result in logs with different chemical and structural composition, providing a broad diversity of substrates on the forest floor and in the soil. From a biodiversity perspective, the presence of these fungal species contributes to the variability of dead wood, which is a prerequisite for many other saproxylic (dependent on dead wood) species (Jonsson et al. 2005). In this perspective, wood fungi could be viewed as ecological primers that modify woody substrates to become accessible for other organisms, in this context wood fungus has been referred to as "the engineers of dead wood" (Lonsdale et al. 2008). There are however substantial differences in dead wood breakdown rates depending on what species that are present in the substrate (Osono and Takeda 2002, Tanesaka et al. 1993) and different species cause cascade effects on the later forming community in the succession (Renvall 1995; Ovaskainen et al. 2010; Ottoson et al. 2014). In this regard it is important to conserve the fungal component of biodiversity in boreal communities. Many threatened and red-listed species are found among the wood fungi (Gärdenfors 2010).

There have been a number of laboratory studies of fungal interactions, both between different species of fungi and between fungi and other organisms (Boddy 2000, Toljander et al. 2006, Edman et al. 2014). These studies showed that fungal communities can be both founder and dominance controlled. The initial success of colonization and competition for space, i.e. the possibility to grow fast and gain a large volume of substrate, are main mechanisms in undisturbed systems, i.e. founder controlled systems. Ecological systems which are continuously disturbed are dominance controlled, where the determining mechanism for success is the ability of the fungi to handle disturbance (Schwilk 1997). Some fungal communities seem to react to disturbance in accordance to the intermediate disturbance hypothesis (Connell 1978; Toljander et al. 2003), while other species seems to be favored by

a lower frequency of disturbance (Schwilk et al 1997).

1.2.1 Wood fungi life cycle and reproduction

The life cycle of basidiomycetes is well described; a spore (n) land on the surface of a substrate and starts to grow into a single nucliec mycelium (n). This growth continues until the mycelium connect with mycelium from another individual with a compatible mating type, and a dicaryotic (n+n) mycelium is formed. The dicaryotic mycelium continue to grow until certain conditions are met, at which time it starts to form a fruit body called a sporocarp. In certain mycelial cells, called basidium, contained in the sporocarp, two meiotic divisions produces 2-8, spores (most species produces 4 spores) from one dicaryotic cell, and the spore is released and the cycle continues. What happens inside the wood substrate, from the start of mycelial growth to the production of a fruit body is less certain. There are no studies giving the complete picture of fungal interactions, or the relationship between di- and monokariotic mycelia, i.e. the length of the period of dikaryotic growth in relation to monokaryotic growth.

Figure 1 – Example of a fruit body, *Ischnoderma benzoinum*.
Photo by Mattias Edman.

Reproduction in wood fungi is a complex series of events, guided by several effectors. One hypothesis is that fungi starts to produce sporocarps when under stress from nutrient depletion, although fungi can utilize or modify a wide range of compounds (Boddy et al 2008; Moore 1998) and therefore will seldom be completely deficient in carbon sources. There is a consensus around wood decaying fungal fruiting that there needs to be some form of physiological trigger for the fungi to start fruiting (Moore 1998; Deacon 2006; Christia and Lockwood 1973). Also, certain triggers might favour fruiting, i.e. making the organism exert the intrinsic controls over metabolism (Hawker 1950). It has been shown that C:N-ratios (related to nutrient depletion) (e.g. Moore and Landecker 1993; Alejandro et al. 1993; Aschan 1954), humidity and temperature (Straatsma et al. 2001), as well as light conditions can affect the timing of fruiting (Kues and Liu 2000; Alejandro et al. 1993). An important chemical substance in this aspect seems to be small sugars such as glucose, which to a high degree inhibits fruit body production (Boddy et al 2008; Hawker and Chadhuri 1946). Fruiting conditions will differ between species with different ecology, which can ultimately lead to different reproductive strategies. However, after a potential lag phase of a varied number of years after colonization, most species reproduce at roughly the same time every year. This indicates that yearly variations in temperature, humidity and possibly irradiation regimes are enough to trigger the fruiting cycle. It also seems that most species responds to these similar triggers by fruiting at roughly the same time every year (Jenings 1995; Moore 1998; Scrase and Elliot 1998; Kues and Liu 2000). There might however be two parallel systems governing fungal fruiting. One system for non-acute situations where the aboitic/environmental cycle is the trigger, and one system where a disturbance overrules or coincides with other effectors that allow the organism to reproduce at otherwise non-optimal conditions (Moore et al. 2011).

The successful colonization and fruiting of an individual on a substrate is largely determined by succession, i.e. the time in order, at which a spore lands and is able to produce viable mycelium relative to other competitors for space (Fukami et al. 2012; Pentillä et al. 1995; Holmér&Stenlid 1985). There are however uncertainties concerning which factors that determine

fungal community structure in dead wood. Also, fruit body production may be a poor measurement of success regarding a colonization, since wood fungi can probably be present in a substrate for a very long time before producing a sporocarp. Indeed, there are examples of highly extensive mykorhizal mycelial networks on the forest floor probably 1900 - 8650 years old (Ferguson et al. 2003), which shows the importance of vegetative growth and gives an indication of the persistence of mycelial networks. Considering the restricted ”lifespan” of a piece of dead wood, wood fungal fruiting is more limited in time. Still, colonizations might be considered successful when a mycelium is able to survive and maintain its volume in the substrate.

When established in the substrate, the volume occupied by an individual is largely governed by the boundaries of the substrate and the interactions with other fungi. Saprophytic fungi get their nutrients by the breakdown of cellulose, hemi cellulose, starch and to some extent lignin. (See section 1.2.5) To access and detect feeding sources wood fungi has evolved a variety of responses. In homogenous material wood fungi treats the individual residues as part of a larger feeding source and grows homogenously through the substrate. However, in heterogeneous material fungi can relocate resources from one part of the mycelium to another and some species can form rhizhomorphs or mycelial cords which allow further allocation of resources like water and nutrients.

1.2.2 Effect of temperature on wood fungi

The plasticity of basidiomycete wood fungi is rather impressive, in regards to temperature tolerance. As an example Mawaka and Magan (1999) showed that the total growth range for both boreal and tropical species ranges from 0 °C (minimum for all species) to 56 °C (maximum for some of the included species). Moreover, some species can survive up to 95 °C for 4 h if growing in its natural substrate and some species can survive up to 2 weeks with highly elevated temperatures in comparison to their normal growth range (Schmidt 1995; 2006; Schmidt & Huckfeldt 2005). In one of our own studies (not included in the thesis) I found that some wood fungi could actu-

ally continue to decay the substrate below 0°C (Carlsson unpublished).

1.2.3 Genetic structure

In general our knowledge on the genetic structure of wood fungal populations is limited. However, there have been some studies on gene flow using microsatellite markers (eg. Stenlid 1985; Högberg et al. 1999; Vaino et al. 1998). It has been shown that there is generally very little genetic differentiation/structure between populations of common species, such as Trichaptum abietinum or *Fomitopsis pinicola* with Fst values close to or below 0.1. In contrast, higher Fst values have been estimated for populations of less common species. In *Fomitopsis rosea*, a Redlisted species in Sweden (Gärdenfors 2010) the low interconnectivity between populations has caused inbreeding effects with lower viability of the spores (Boddy 2008; Selosse 2011; Edman et al. 2004). One problem with these studies is the difficulty of delimiting the studied populations, or; where does the population start and end. (Rayner et al. 1984; Malik & Vilgalys 1999; Glass & Dementhon 2006) This is a problem in many areas of ecology, but emphasized with wood fungi where some species have spores that can travel at least 300 km (Rishbeth 1959). There are some studies on genetic structure utilizing snp analayzes of fungi (See ex. Amend et al. 2008; Zhang et al. 2013) but they are scarce and even fewer have been done on basidiomycete wood fungi (Heinzelmann et al. 2012; Kauserud 2006). Until recently no complete genome of any wood fungi have been sequenced, making the development of high amounts SNP´s a time consuming effort. This has improved over time and there are now genomes available for several fungi, recently including *P. gigantea*. This will probably boost research on population structure, and also on SNP´s related to functional genes.

1.2.4 Fungal interactions

A piece of dead wood on the forest floor hardly ever lacks fungal species; some is probably already present when the tree dies, either as parasites or as

small mycelia constituents in the bark (Boddy et al. 2008). In a newly felled piece of wood however, it can be argued that the substrate is un-colonized since the different species have not had time to develop large mycelium. This means that fungi at some point will have to compete for space, both with other species of wood fungi but also with other organisms such as bacteria and molds (Dowson et al. 1988; Donelly & Boddy 2001; Fukami et al. 2010).

During interaction, different antifungal chemicals are released in the interaction zone (Heilmann-Clausen & Boddy 2005), this influences the other present species, and can have one or several effects; antagonism at distance or by mycelia contact, and/or stimulation of growth.The result can be either that one species get the upper hand and outcompete its antagonist or a deadlock where both species holds on to their area. Combative interactions can also start with one species gaining volume and then end in a deadlock (Rayner 1978). However, volume in itself can affect competitive strength, as shown in interaction experiments where several species were combined with different percentage of the starting material colonized by each species (Holmer & Stenlid 1993). In Holmer and Stenlid's (1993) experiments fungi lost competitiveness with decreasing wood volume. The species composition in a log at a certain time is thereby determined by the relative ability of the different mycelia constituents to capture and defend the resource (Rayner & Webber 1984; Boddy 1993). These kind of interactions and replacements results in changes in species communities over time (Boddy 2001).

A modification of environmental conditions can change the inter-competitive balance in fungal communities; hypothetically, species present in low abundance may be able to expand when such a change occurs. There are several studies indicating that a log contains large amounts of (non-fruiting) mycelia comprised of many species, each being present at relatively low abundance (Ovaskainen et al. 2010; Kubartova et al. 2012). Hypothetically this could mean that it is increasingly more difficult for a "minor colonizer" to hold on to a diminishing volume of wood if the habitat is suboptimal (Fukami et al. 2010).
It has been shown that different species combinations yield different results,

i.e. the competitive strength of some species is increased against certain other species. For example, Trichoderma species has an specific antagonistic effect against some species of the *Ganoderma* genus (Grosclaude et al. 1990; Sariah et al 2005) and the inhibitory effect of *Phlebiopsis gigantea* on *Heterobasidium annosum* is used to control root rot (Meredith 1959).

1.2.5 Decomposition

Wood fungi utilize dead organic matter for growth and energy. The origin of these is mainly cell walls consisting of biopolymers, such as polysaccharides and cell wall polymers as well as proteins. Some of these like cellulose and hemi-cellulose are easily decomposed and used as a carbon source while lignin needs to be degraded to make other carbon source available. Wood fungi metabolism involve a range of enzymes and acids to decompose hemicelluloces, celluloses and for some species also lignin. They use both endo- and exo- types of hydrolases, while the specific enzymes varies between species almost all wood fungi contain some form of Endoglucanase (Highley 1988). Additionally, Cellobiohydrolase (CBH) is found in white rot fungi and possibly a few brown rot fungi (Nilsson & Ginns 1979; Schmidthalter & Canevasvini 1993). The possibility to use these rather inaccessible compounds gives them an advantage in relation to other organisms in the substrate.

The regulation of decomposition chemistry is still not well known, but there is evidence that carbon supply and availability of inorganic nitrogen has a role in up regulation of polysaccharide degrading enzymes (Rineau et al. 2013). For lignin degradation (Baldrian 2005, Hatakka 2001), regulation sequences in the lac and pox area (Kilaru et al. 2006; Larraya et al. 2000) of the genome are involved, including HSE, XRE and MRE derivate binding promoter regions (Piscitelli et al. 2011; Janusz et al. 2013). During decomposition, most carbon dioxide is released in to the surrounding air, while some is used in the synthesis of tissue and released when the mycelia is consumed or degraded (Boddy 1985).

The measurement of carbon dioxide release resulting from decomposition

by fungi and other microorganisms has been used to evaluate relationship between species diversity and decomposition rate. Species diversity has a strong impact on decomposition rate, some studies indicate that higher species diversity also increases the decomposition potential of microbial communities (Valentin et al. 1993) while it also is evident that other factors is also important, mainly the actual species composition (Hättenschwiler et al. 2005; Tiunov et al. 2008; Setäle & McLean 2004; Toljander et al. 2011). It has been hypothesized that the effect on different species on the total decomposition rate has to do with different combative species interaction regimes. There are a few ecological studies on actual decomposition rate on isolated species (ex. Edman et al. 2006; Heijari et al. 2005) but these contains very few species (1-3).

1.3 Forest fire

Forest fire is regarded as the main disturbance agent in boreal and hemiboreal forests (Linder et al. 1997; Östlund et al. 1997; Zackrisson 1977). In historical times, fires were shaping the species composition, and regulating biodiversity in several of the ecosystems in northern Europe and elsewhere. Non-anthropogenic fires were mainly caused by lightning and affected large areas (Wein & McLean 1983; Pyne et al. 1996; Ryan 2002). Early human influence in forests caused an increase in ignitions, but these fires were much smaller in size (Niklasson & Granström 2000; Wallenius et al. 2004, 2005). There is evidence of fires being frequent to the extent where certain forest types were likely to burn several times every century (Niklasson & Granström 2000; Hjalmarsson et al. in prep). This applies to forest where Scots pine (*Pinus sylvestris*) –dominated. Evidence of fire regimes generally comes from the study of fire scared wood samples taken from living trees, stumps or other dead wood, and such dendrochronological studies can provide chronologies including fire history stretching as far back as 1000 years (ex. McBride 1983). It is also possible to take cores from peat bogs, which will yield fire chronologies stretching further back in time, sometimes throughout all of the Holocene (last 10 000 years; Power et al. 2008; Marlon et al. 2008). Studies of fossilized coal remnants give an idea of fire regimes

in between the glacial periods. Taken collectively, these studies portray forest fires as a major disturbance factor in boreal forests, at least since the last glaciation and probably also in earlier interglacial periods (Harrison 1943; Stokland et al. 2013).

Species in boreal pine-dominated forests needs to adapt to frequent fires. It has been hypothesized that Scots pines needs forest fires at certain intervals to maintain its role in the boreal ecosystem (Zakrisson et al. 1977; Steijlen 1995; Zackrisson et al. 1995). If a pine is subjected to a moderate forest fire, it will start chemical processes that will increase the resilience towards infections of wood fungi and bacteria, thus the vitality in the affected trees (Mattson et al. 1988; Zobel et al. 2005). This is done by the accumulation of resins, hertzes and phenoles, which are all anti fungal/biotic. Furthermore, Norway spruce (*Picea abies*), is affected differently than pines and are often killed in forest fires. Spruce is a stronger competitor than pine. In the absence of fire, pine forest slowly transforms in to more closed stands dominated by spruce. In these systems pines are at a competitive disadvantage and as a shade intolerant species having considerable problems to regenerate (Steijlen et al. 1995). Over time, pines will eventually be outcompeted and lost from the stand. Fire regimes in spruce forests are generally different as these forests burn with a lower frequency. The fire severity in a spruce forest is however much greater with a greater mortality in the tree layer and consumption of woody substrates (Pentillä 2004).

Wood fungi are interlinked with forest fires in number of ways. Several species seems to have found a niche in relation to fire disturbance. Since forest fire creates a lot of new dead wood, it gives the opportunity for new colonization's, only paralleled in the boreal systems by large storm events and insect outbreaks (Stokland et al. 2012). Since there are some species that exclusively occur on burned or charred logs there have also been adaptations in their ability to colonize and grow on these substrates. A common feature in nature is that almost every niche is occupied by some form of organism.

Sometimes a disturbance or modification of the habitat is needed for some

organisms to survive. Some species are very poor competitors at some conditions, while they flourish under disturbed conditions. One example is the annual flower *Geranium langinosum* which needs the soil to be heated up to between 50 - 100 °C for its seeds to germinate (Risberg & Granström 2008). In boreal Scandinavia there are about 30 wood fungi species which can be associated with forest fires (Art-databanken), among them the extremely rare *Gloeophyllum carbonarium*, which only grow on charred wood surfaces. In other words; certain wood fungi species has found a niche defined by the temporal event – forest fire.

Since the birth of modern forestry, spurred by the industrialization (around 1850, not to be mistaken with early industrialization in England and to some extent Germany), forest fires have been actively suppressed. Today, natural fire regimes are virtually nonexistent. This has caused many fire-dependent species to decline in numbers. This is evident from the national Red Lists, which identify many fire-dependent species as threatened or near threatened (Rassi et al. 2001, Berg et al. 2002, Gärdenfors 2010). Wood fungi are overrepresented in the Red List compared to other organism groups. The main cause of their decline is the loss of dead wood in the forests, and the suppression of forest fires potentially adds to this problem. The absence of fire creates a different forest structure in comparison to forests with a natural forest regime, accordingly it has been shown that time since last fire effects the species composition (Gudrunsson 2014). Fires also creates very specific charred substrates, a prerequisite for some wood fungi species (MycoBank, Artdata banken,; Hallingbäck & Aronsson 1998; Larsson (Red.) 1997; Niemälä 1999). The effective fire suppression methods have also excluded fires from habitats normally very likely to burn, such as dry pine-dominated stands.

1.3.1. Restoration fire

Restoration burning is used as a conservation tool, with the main purpose of recreating forest structures that has been lost due to the absence of natural fire regimes. It has been shown that restoration fire can change the com-

munity structure of wood fungi, so that it will differ greatly from before the fire (Olsson 2008; Pentillä and Kotiranta 1996). To be effective it needs careful tending, so that not all substrates are consumed but at the same time becomes readily affected/charred (Eriksson et al. 2013). Thus, fire intensity can have a drastic effect on the success of the restoration fire, influencing the community structure of the wood fungi after the fire. A restoration fire initially has a major negative effect on the fungal community, comparing restoration burnings with different intensity (Olsson 2008; Pentillä et al. 2004; Junninen & Komonen. 20010 it is apparent that lower fire intensity allowed the fungi to regrow faster and new species to emerge. In contrast, high intensity fires cause extensive tree mortality and hence create more new substrates (Eriksson et al. 2013; Townsend 2001).

1.3.2 Adaptations to forest fire

Several studies have shown that some species of wood fungi only appear or become much more abundant after forest fire (e.g. Junninen et al. 2008; Olsson 2008). So how can wood fungi adapt to forest fires? To start understanding this question we need to begin with the different ways wood fungi can colonize wood after a fire. There are essentially three ways; (1) wood fungi can have a potent dispersal ability, where spores from the surroundings colonize the new substrates made available by the disturbance, (2) wood fungi can survive as mycelia inside the substrate, as long as the substrate is not completely consumed, and (3) some wood fungi can also grow in the ground, which could be viewed as a refuge, and after the fire they could re-colonize the original substrate or colonize newly created substrates (Figure2).

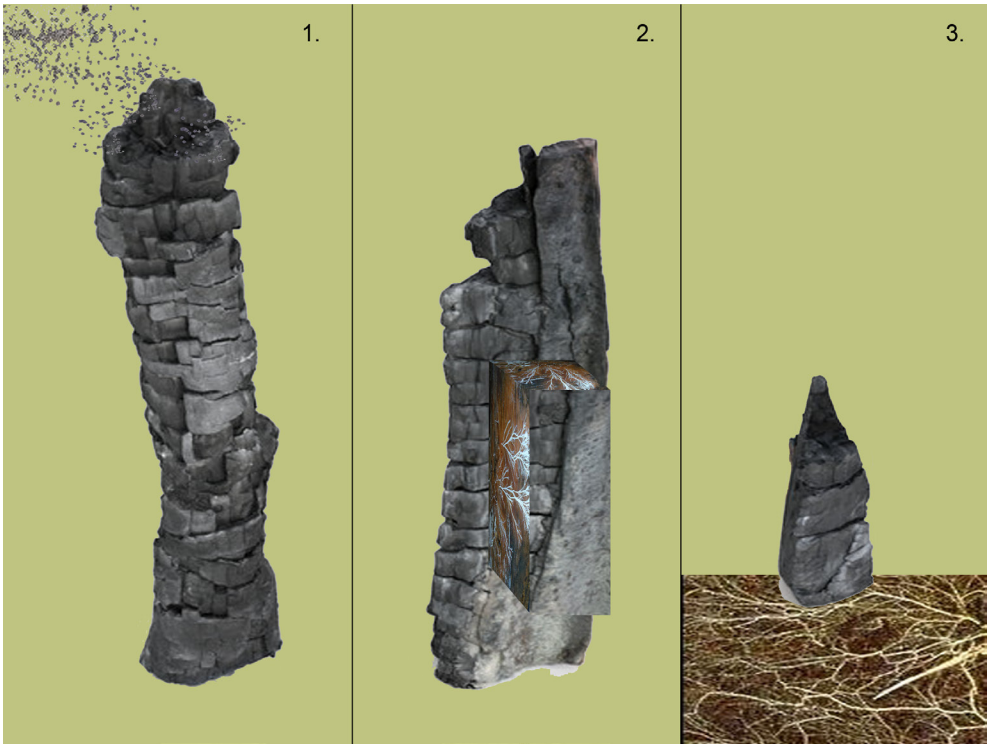

Figure 2 - Possible adaptations to forest fires 1) abundance of spores ready to colonize newly created substrates, 2) heat tolerant mycelia able to survive inside the substrate during the forest fire, and 3) mycelia life-boated in the ground.

The second and third strategy would require adaptation towards increased mycelial heat resistance, allowing the mycelium to survive inside the wood or soil during the elevated temperatures of the fire event.

1.4 Specific research questions

This thesis includes five papers all addressing the relation between forest fire and wood fungi and central methods to study fungal populations.

- In paper I and II we examine how fungi are adapted to forest fire. More specifically we examine if there has been any selection pressure on the ability of wood fungi to grow mycelia with high heat resistance. In paper I we test if there are any patterns that can be detected when comparing a group of species that is considered associated with forest fire and a group of species without such obvious relation.

Paper II uses the heat-resistance data that was obtained in the first paper to test if high resistance to heat can also allow for some species to gain in competitiveness. This paper is a continuation of the first paper, addressing questions that emerged from the initial study. Namely, we found that several species had an extensive resistance to heat in comparison to its natural growth range. When we tested how much the temperature increased inside a log during an experimental fire we found that it was not even close to the temperatures that the fungi could resist in a laboratory setting (figure 5, Paper I table 2). Even some of the non fire associated species could survive these temperatures. We therefore hypothesized that heat resistance in some species not only allowed them to survive the fire, but also allowed them to outcompete less heat resistant species in the aftermath of a fire disturbance.

- In paper III we examined the effect of heat on decomposition rates. In contrast to the two earlier studies on heat adaptation this study addressed the metabolic response of 18 wood fungi species to heat shock. There are several examples from other organism groups where heat triggers a shift in behavior. We were interested in seeing if heat can trigger contrasting response among different wood fungi. Increased decomposition after heat shock would indicate that the species can harvest newly available resources at a higher rate. This could give species surviving a fire inside a log an edge against other competitors after forest fire.

• In paper IV we investigated if forest fire has any impact on the genetic population structure of one species of wood fungi *Phlebiopsis gigantea*. We chose a species that is relatively common even in the absence of forest fires, but reported to increase in numbers after a forest fire. We used a common species mainly due to the problem with obtaining a sufficient number of individuals using very rare species in a population study on a genetic level (Hartl & Clark 2007). To do this we scanned the genome of two individuals to detect single nucleloid polymorphisms (SNP). In addition to burned and unburned sites, the sampling included a wide geographic gradient as well as individuals collected from both spruce and pine wood.

• Paper V is a method development paper. Inventories of wood fungi in forests have presented problems, since they have generally been performed by detection of fruit bodies. This only represents a small part of the population, since fungi can be present as mycelia, and in fact some recent studies indicates that fruit bodies only represent a minor part of all species present. Several solutions now exist to detect wood fungi with the help of molecular methods, and we tested one of these. In the fourth paper we evaluated the use of T-RFLP to detect fungi in wood. This study was performed simultaneously with another study where we scanned a log for all major fungal colonizers. This way we could see how the restrictions, in regards to species number and DNA concentrations, presented by the sampling method itself would influence the applicability of the method. Our results provide a framework for the T-RFLP method, providing details about its accuracy and how much species data it can obtain.

2. MATERIALS AND METHODS

2.1 Methods summary

In general we used methods previously developed by other researchers and published accordingly. Materials and methods are described in each paper, so in this section I will only give a short overview of the respective methods and then elaborate some on the findings and failings that we experienced.

2.1.1 Collection of wood fungi species and study areas

Basidiomycete species has been added to the Mid Sweden Fungal collection continuously, but most of the species used in the papers presented in this dissertation were collected during the Autumn of 2007 and 2008 by Anna-Maria Eriksson and Mattias Edman (both at Mid Sweden University, Sundsvall), with exception of the individuals which was used in the population study (paper IV) which was collected during the autumn of 2012 by Jörgen Olsson (Swedish Agricultural University, Umeå) and Helene Bjurström (Swedish Agricultural University, Uppsala. All species were collected in the northern/middle part of Sweden (figure 3) with the exeption of two individuals of Dichomitus squalens from eastern Finland.
All fungi were collected as fruit bodies (no direct cultivation from mycelia was done). After field sampling, the fungi were put in a laboratory cooler before isolated on artificial medium (agar). Generally, isolation was done in less than one week and in most cases in a few days, after the collection date.
Considering that we studied some really rare species, it is also worth mentioning the effort put in to collecting the fruit bodies

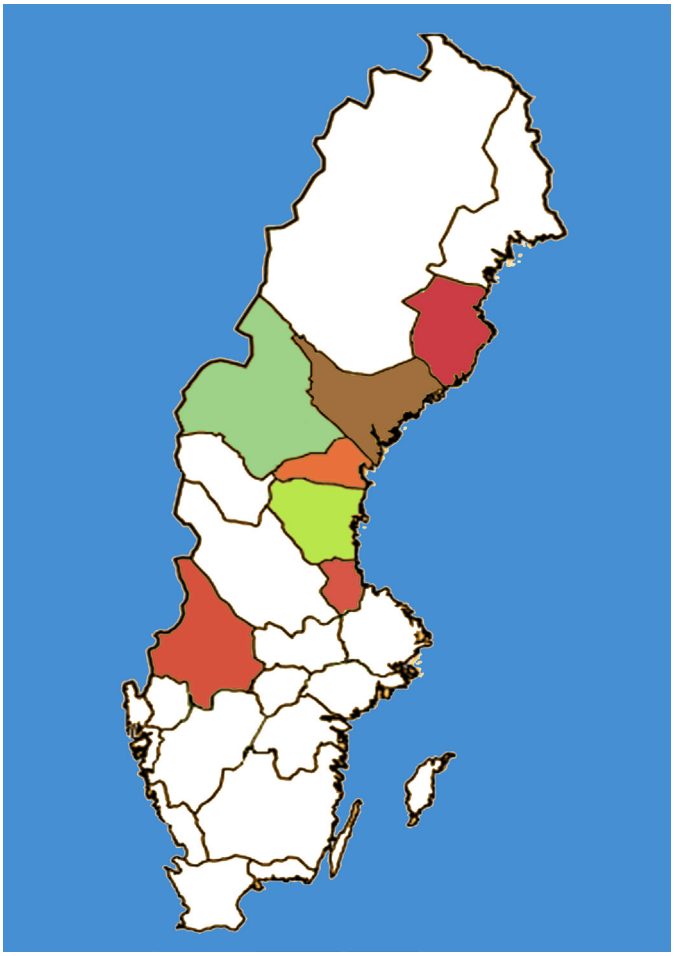

Figure 3 – Map of the areas in which fungi was collected for various experiments. Collection was done in the regions highlighted in color, from the north – Västerbotten, Jämtland, Ångermanland, Medelpad, Hälsingland, Gästrikland, Värmland.

2.1.2 Inoculating and growing wood fungi in the lab.

The isolation and cultivation of a large set of fungal strains presents a large undertaking.. The laboratory procedure after collecting fruit bodies was always performed in the same way. Generally isolation was done in less than one week and in most cases in a few days after the collection date. It needs to be stressed that the success of isolation was largely related to the storage time of fruit bodies before isolation. As growth medium we used modified Hagem agar as described by Stenlid 1985, with the addition of benomyl (4 mg), thiabendazol (575 mg), penicillin (75 mg), streptomycine (75 mg), and tetracycline (75 mg) * L-1, to limit the growth of moulds and bacteria. Isolation was done as soon as possible, by inoculating a small piece of the fruit body on agar plates. When possible we tried to take material from inside of the fruit body without surface contact. This was mostly possible with the larger fruit bodies, but problematic when it came to resupinate or semi-resupinate species like *Antrodia sinuosa* or *P. gigantea*. When needed we took mycelia from inside the substrate just under the bark where the fruit body was situated. Notably, even in species with very large fruit bodies like Leptoporus mollis we had sometimes problems obtaining material free from contamination.

These methodological issues had some implications. It made us sequence many of the fungal species targeting the ITS-region and blasted against NCBI to secure the species identity See (2.1.4).

When we obtained cultures that were pure and identified by sequencing, we allowed them to grow in the dark in room temperature. Several of the cultures were also stored in agar filled test tubes in a laboratory cooler, for long-term storage. The cultures stored in room temperature were re-inoculated every 4th months. This is necessary to keep the specimens alive, since the nutrients in the agar plates will eventually run out. Moreover, we found that some of the species produced extremely potent acids. In nature these acids will diffuse out in the nearby substrate, but in the lab they will continue to accumulate. In an unpublished study (Ali and Carlsson Unpublished) we found that some of the *Antrodia* species could lower the pH to around

1 when cultured in liquid media. Our laboratory experiences shows that long-term inoculation will affect the wood fungi, and stresses the need for constant re-inoculation .

It is possible that the accumulated acids are a part of the defense system against bacteria or/and used in intercompetive interactions between different species or individuals of the same species. The acids are also part of the cellulose, hemi-cellulose and lignin degrading process, included in the non enzymatic fungal decay system (Boddy et al. 2008; Stokland et al. 2012). In our interaction experiments, a lot of chemicals accumulated in the interaction zone coloring the agar, and occasionally these zones contained large extractive globules (Figure 4).

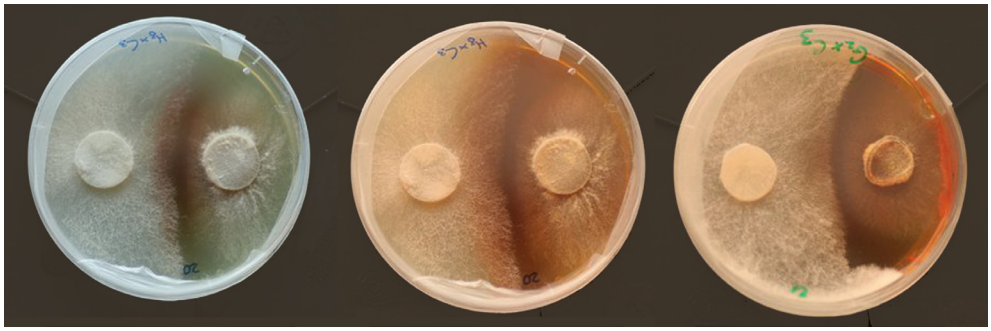

Figure 4 – Three examples of extractive substances diffusing in to the agar during combative interactions photo Melanie Wagar.

For all experiments presented in this dissertation, we re-inoculated the fungi on autoclaved wood discs 1 x 9 cm in paper I and II, 2 x 3 cm in paper III. After we obtained pure cultures, the re-incoluation procedure was much easier and we had almost no contamination of the wood discs. This was due to the usage of already purified cultures, but also the usage of wood discs as a growing medium. Long–term incubation should preferably be done on wood discs instead of agar for two reasons (1) wood discs functioned as a selective medium favouring wood decayers over other contaminating fungi and bacteria and (2) wood fungi grows slower on the medium which means that

fewer re-inoculations were needed. Moreover, fungi seemed to survive in wood discs for a very long time even when the substrate was almost entirely consumed. In some cases, we re-invigorated cultures from wood discs which had been left unattended for 5 years.

We had to discard one species from paper I since we could not properly identify it (later identified as a *Phlebia sp*.) using BLAST (see definition list). At the time of this publication, it was not possible to cross reference it with another individual of the same species from the gene bank, and it didn't group satisfactory in the phylogenetic tree. The lack of fungal sequenced material in the gene bank remains a problem, and other researchers have also argued that the material obtained through the gene bank can sometimes be wrong (Ovaskainen 2010). Other data bases are available, such as UNITE, but to have an efficient system all sequences needs to be stored at the same place and a standard procedure regarding database handling and the submission of sequences needs to be implemented among the mycological research community.

2.1.3 Characterization of species

In several of the papers (I,II and III) we a priori divide wood fungi species in to two groups; Fire associated and Non Fire Associated. The species classified as fire-associated are mainly found in natural forests with a history of fire influence with most of them being rare in managed forests. Four of the species (*Dichomitus squalens, G. carbonarium, Antrodia xantha* and *Antrodia sinuosa*) are clearly more frequent on pine logs associated with recent forest fires (Junninen et al. 2008, Olsson 2010) while others (*Gloeophyllum protractum, Junghuhnia luteoalba, Oligoporus sericeomollis* and *A. infirma*) are not as clearly associated with recent fires, but still appear to be more frequent in pine forests with a history of fire influence (personal observation M. Edman). The two remaining species in this group *Phlebiopsis gigantea* and *Gloeophyllum sepiarium*, are included because they are predominantly found in open, dry habitats which resemble the microclimatic situation in forests subjected to fire (Hallingbäck & Aronsson 1998; Larsson (Red.) 1997;

Niemälä 1999). Species in the non fire associated group has no specific relation to forest fire, or open dry habitats; the group consists of species with differing habitat requirements, many common in moist and closed spruce forests.

Although in general there is an ecological contrast between the two groups, it needs to be stressed that the characterization of species in the respective groups are not completely straight forward. For example the generalist F. pinicola has been shown to increase in numbers after forest fire (Junninen 1995) and *J. luteoalba*, here classified as fire-associated, are also sometimes found in rather moist forests on spruce.

2.1.4 Statistical analyses

The experiments in papers I, II and III are all replicated laboratory experiments with one or several distinct treatments. Hence analysis of variance has been used assuming normal distribution in the response variables. Analyzes have been performed in Minitab and R. In paper IV we used PHASE for haplotyping, and Fst values were calculated with STRUCTURE

2.1.5 Species confirmation by sequencing

All species studied in this dissertation are maintained in the Mid Sweden Fungal Collection and have been confirmed by sequencing. This was done by sequencing their ITS regions, which is widely used as a region to separate strains at species level. The ITS region was targeted by primers ITS1F and ITS4B, and in some cases ITS4. Extraction of DNA was done according to a modified protocol of Taylor et al. (1979) (See paper V) or by using the MoBio Power soil® #12888-50 DNA isolation kit (See paper IV). In the latter case, extractions were performed according to the manufacturer's instructions. PCR´s where done using the AmpliTaq Gold kit (Applied biosystems) in which case the PCR protocol was based on Jasalavich (2000) or the Kapa Robust PCR kit (Kapa biosystems) in the latter case the PCR-

protocol was based on the manufacturer recommendations. The sequencing reaction was done using Big dye 3.1 (Applied bio systems) according to the manufacturer's instructions. Column electrophoresis and detection was carried out at UGC Uppsala, Sweden.

2.2 Paper-specific methods

2.2.1 Paper I methods

The aim of paper I is to evaluate if wood fungi prevalent in forests charachterized by fire can survive elevated temperatures in relation to species with no such distinction.

In paper I fungal mycelia from 15 species were grown on wood disks and subjected to heat treatments. To control mycelial growth uniformity among the replicates and throughout the single wood disc, total DNA was extracted with a modified protocol from Taylor et al. (1979). 0.5 cm3 of material from each corner of the wood disc was extracted. DNA concentration in the extracts was determined on a 1.2 % (wt/v) agarose gel with λDNA (Roche) after staining with ethidium bromide. When mycelia covered the surface one wood disc from each species and batch were controlled by sequencing the ITS region. Wood discs were used at an approximate concentration of 5ng/mm3 mycelial DNA. Very small variations in mycelial content were detected at different parts of the wood discs.

The inoculated wood discs were cut in four equally sized sections under sterile conditions. Discs were treated with heat in four series (new discs for each series) with different temperatures. The temperature was set to 100 °C, 140 °C, 180 °C and 220 °C. For each temperature, three replicates from each species were treated (one for each individual with the exception of D. squalens and A. infirma for which all replicates were taken from the same individual) at 5 different time intervals, 5, 10, 15, 20 and 25 minutes. Conditions were chosen as to resemble those in a forest fire. In total 960 repli-

cates for heat treatment and 42 controls for untreated re-growth (1 for each individual) were used. After heat treatment the wood samples were placed on new agar plates sealed with parafilm and re-grown in darkness at room temperature for 12 days. Fungal re-growth were checked every second day by visually scanning the surface of the wood disc and the agar for mycelia.

2.2.2 Paper II methods

The aim of paper II is to see if there is any effect of heat shock on interspecific competition

In paper II the effect of heat treatment on competitive interactions were tested. To mimic heat stress during a forest fire, the temperature treatments were calibrated according to measurements obtained from a single log during an experimental fire event. The temperature change during the fire event was used to calibrate the heat treatment of the wood discs in the laboratory experiment (Figure 5). To obtain the same temperature in the laboratory experiment as under field conditions, the temperature inside the experimental wood discs were calibrated (n=6) by adjusting oven temperature and the time wood disks were placed in the oven.

Mycelia from six species (three fire-associated, *Phlebiopsis gigantea*, *Dichomitus squalens*, *Gloeophyllum sepiarium* and three non-fire associated *P. Pini, I. benzoinum,F. pinicola*,) were inoculated and grown on wood disks. The heat shock treatment was done as follows; the temperature was set to 100 °C, the wood discs were contained in sterilized tin foil packages. The packages were put in the oven and treated for 25 min, corresponding to an inner wood temperature of 56°C (st.dev.±3°C) (Carlsson et al. 2012), which roughly equates to the temperature at 3 cm depth under the bark recorded in the in situ tests (Figure 5).

After heat treatment wood discs were, cut in half and placed on agar plates in combinations so that all the individuals of the fire-associated species were combined with all the individuals of non fire-associated species (Supple-

mentary Table 1). Each combination of strains was replicated 5 times (interactions with *Dichomitus squalens* were replicated 15 times to compensate for the lack of different strains) and once for the unheated control, resulting in 405 replicates of the heat treatment and 81 controls. The statistical significance of the competition outcomes was tested with a two factor analyses of variance (ANOVA). All pairs of interactions were also tested with a two sample t-test, assuming unequal variance.

Figure 5

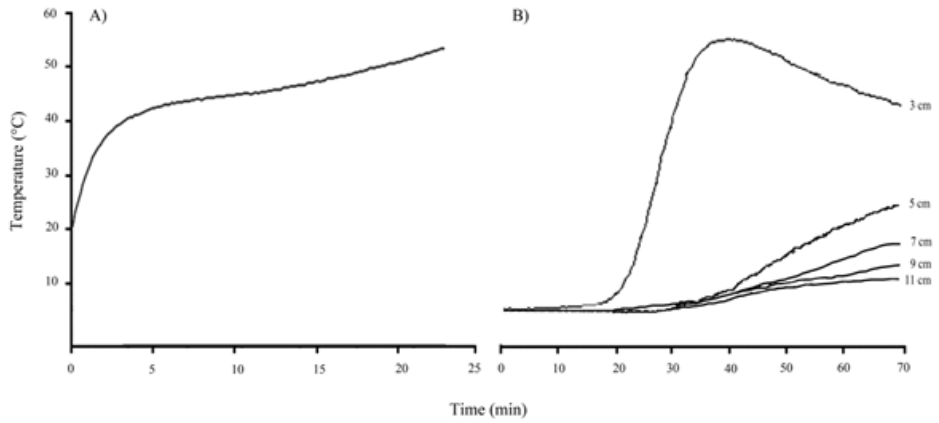

Figure 5 - (A) Temperature regime inside the wood discs during the heat treatments. The diagram shows mean values of temperature. The temperature was recorded six times; standard deviation values range between ± 0.6 °C. B) Temperature regime inside a log during burning: temperature was recorded at different distances from the surface (measured from beneath the bark).

2.2.3 Paper III methods

The aim of paper III is to look at decomposition rate at the onset of colonization and after heat shock.

In paper III changes in decomposition rate after heat treatment was analyzed for 18 different species. After pure cultures had been attained, mycelia were transferred to normal Hagem-agar plates. From these plates 0,5 cm^2 of mycelia was inoculated on sterilized wood cylinders (2 x 3 cm). Before Inoculation the wood cylinders were dried in a Memmert 500 oven at 30°C for 3 days.. The wood cylinders were soaked in sterilized water for 2 hours, and autoclaved for 20 min. The wood cylinders were placed in 3,5 x 6 cm autoclaved glass containers with resalable lid To keep humidity during the experiment these containers were supplemented with a 0,5 cm layer of water agar. In total we used 60 wood cylinders for each treatment, resulting in 180 samples in total. All containers were kept in a sterile bench during the whole experiment. One set of the experiment (60 wood pieces) where inoculated on charred wood. Wood was charred over a benzene burner at 5 cm distance from the apparatus at maximum possible gas flow, 1 min on each side of the wood cylinder, yielding a homogeneous coal layer covering the surface.

To measure CO_2 evolution, we used an EGM-4 Environmental CO_2 gas monitor (PP systems©). We started measuring the day after inoculation, then measurements were carried out each 3,5 days for 5 weeks and then 1 measurement each week. At the start of a measurement the container was cleared of accumulated CO_2 by opening the lid an waiting for 30s, after this a lid mounted to a syringe with connection to the detector was used to close the container (Figure 3). The CO_2 evolution was then measured for 1 min, the initial and last value was taken, giving the CO_2 accumulation for each species during 1 min, i.e. providing a proxy for the decomposition rate at that specific time.

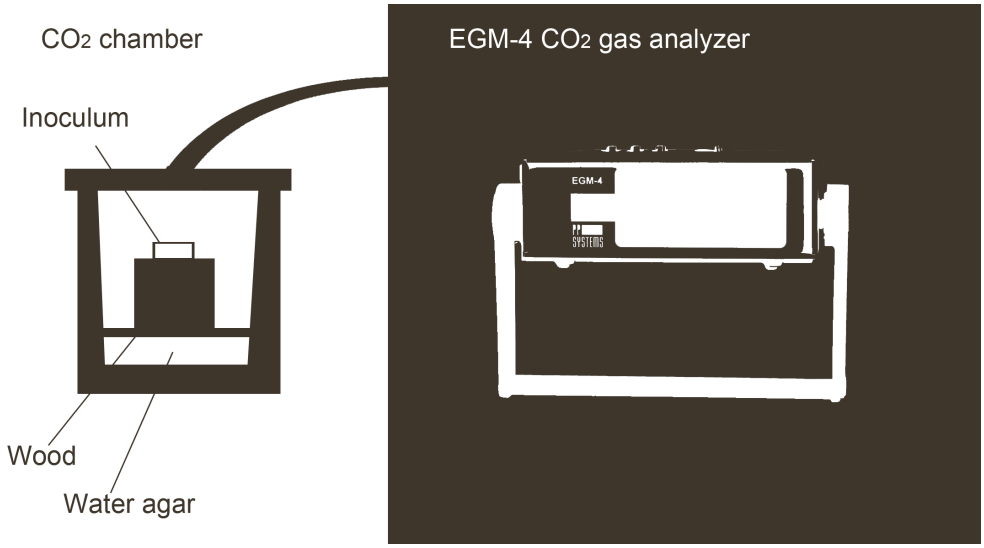

Figure 6 – Experimental set up of the CO_2 measurements,The container (left) containing inoculated wood fungi was connected to a EGM-4 Environmental CO_2 gas monitor (PP systems©) at the onset of measurement.

After 8 weeks of measurement the samples inoculated on un-charred wood were heat shocked. All samples were put in sterilized packages of tin foil in the sterile bench. The tin foil packages were put in a Memmert© 500 oven. The temperature was set to 100°C and samples were treated for 25 min. The glass containers were cleaned and autoclaved and new water agar was added before the wood cylinders were put back (matching wood cylinders so that they were put back in the same container) and the lids were sterilized with 70% EtOH. Measurements were resumed directly after the heat shock treatment, and continued for 8 weeks (1 measurement per week).

2.2.4 Paper IV methods

The aim of paper IV is to look at population structure of *P. gigantea* using SNP´s. To evaluate if there is any structure that can be related to forest fire in a relatively common species

In paper IV the population genetic structure of *P. gigantea* was studied by means of SNPs. Two strains of *P. gigantea* were isolated from fruit bodies collected at two sites in Västernorrland and Västerbotten counties (Figure 7). Strain Pg.V2008ME was collected during the autumn 2008 and strain P.g A2009FC was collected during the autumn 2009. These two strains were used to construct the snp-library (supplementary table 3).

For the actual population study, initially 180 individuals where collected from 6 different populations. Some of these were later lost during cultivation, leaving 132 individuals for the analysis.The populations where chosen pair-wise so that two populations where sampled in roughly the same geographic area, with an internal distance ranging from ~10 km – 70 km. These pairs consisted of one population from a forest fire-influenced area and one control population from an non-fire influenced forest . The southernmost pair was situated in Uppland County, the central pair in Västernorrland County and the northernmost pair in Västerbotten County (Figure 7).

The cloning, -reactions, and sequencing was done using well established methods, and the procedure is thoroughly described in paper IV. All sequence data where analyzed in Codon Code Aligner, from the start we used the built in Polymorphism detector, but we ended up checking all sequences manually, due to background traces giving false results, and we wanted to be completely sure about all polymorphisms used. Subsequently, our sequences may contain some polymorphisms which we discarded because of their uncertainty. All polymorphisms used in the population structure analysis had a very low or no background disturbance (Figure 8) (supplementary Table 2, Table 3).

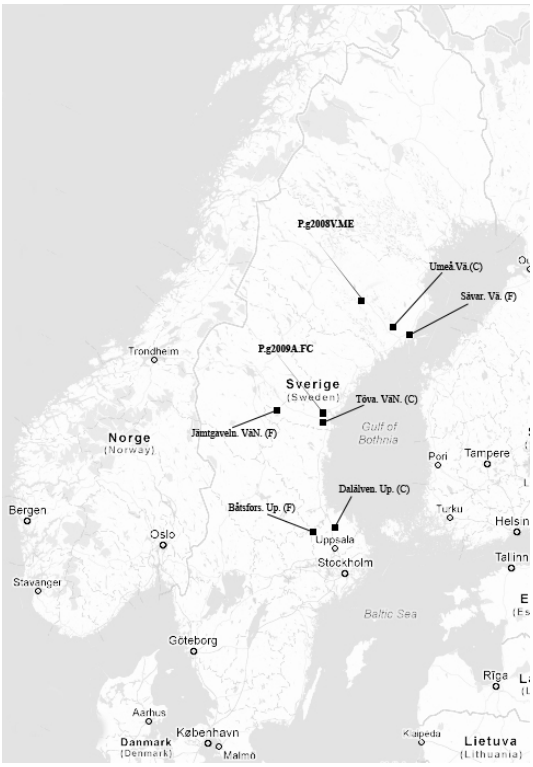

Figure 7. Map of Sweden with locations of the populations used to create SNP markers and the six populations used in the population study.

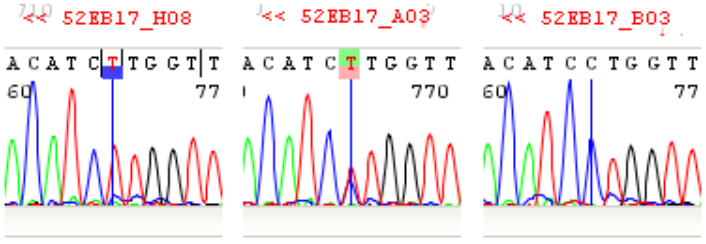

Figure 8 – Examples of traces that were determined valid for analyzis, only traces with very low or no background noise were used.

At the start of this project, no complete genomes of basidiomycetes were sequenced. Now when complete genomes are available, other methods might be more efficient. Such as alignment of large stretches of nucleotides, possible with the access to genomes from several individuals of the same species. We started by cloning fragmented DNA from *Phlebiopsis gigantea* in to *E. coli*. All clones containing fragments were sequenced, from these sequences we created primers and used those on another individual of the same species. All loci were aligned and SNPs were detected by the use of Codon code aligner.

In paper IV the two individuals that were used for the detection of SNPs were not used in the latter population study. This is because we we had to change the study areas, due to lack of fruit bodies in one of the initial populations. The occurrence of wood fungi depends on both spatial and temporal variables, and some species form fruit bodies in a very short time window during the succession of fungi on individual logs. Thus, when planning for population studies, one should take into account that the availability of fruit bodies may drastically change between years and sites, which makes planning and sampling efforts potentially more time consuming.

2.2.5 Paper V methods

The aim of Paper V is to address the problem of sampling wood fungi based on mycelia and includes two steps;

First we analysed the number of species that is possible to obtain in one or several samples of wood material when using a normal drill; a common practice when taking live samples. This was done by taking 131 cross sections from 66 decaying *Picea abies* logs, and delineating the interaction zones between different fungal individuals to visualize the occurrence of fungi in the sampled cross section. Then we did a sampling simulation. Sampling was simulated with 16 evenly distributed lines representing drill holes along the log, with hole number one at the top, corresponding to the position of the

cross-section in the field (Figure 9). Each interaction zone was considered to represent one fungal species. To estimate the number of species that would be detected by a certain number of drill holes, we used PC-ORD (McCune et al. 2011) to generate species accumulation curves.

After the simulation, we evaluated if the T-RFLP (See Dickie & Fitzjohn (2007). for a review of T-RFLP) method could be used to detect all the species present in live wood samples and to which extent it was possible to pool samples.We also wanted to see if there was a species effect on the sensitivity of the method, i.e. if the actual species composition affected the success of the initial PCR-reaction. We did this by both mixing DNA solutions of 8 species of wood fungi using different DNA concentrations, and also altering the DNA concentrations between pair wise combined species. PCR and restriction enzyme cutting was done using well established protocols (Paper V).

There are several molecular methods available to detect wood fungi. The most common are RFLP, DGGE, T-RFLP and pyrosequencing. Pyrosequencing is increasingly used and considered to be the method with the best potential. It has been evaluated in a series of recent papers (e.g. Ovaskainen et al 2010; Kurbatova et al. 2012). The T-RFLP and pyrosequencing methods both are limited by things such as primer bias, and it can be argued that with pyrosequencing there are problem with too high sensitivity. Pyrosequencing will amplify fragments from extremely low concentrations of DNA, such as those present in spores and highly fragmented mycelia, and studies shows that as much as 389 species can be detected in sawdust from one piece of dead wood (Kubartova et al. 2012). It is highly unlikely that such high amounts of species can be functionally present in a log. In this context, "functionally present" is defined as a colonization that results in a persistent body of mycelia which significantly contribute to the decomposition of the log, or the survival of the individual. As a comparison, it is estimated that there are around 1000 wood fungal species in Sweden. Although the results from the pyrosequencing studies are very interesting, and generates questions about spore availability and the value of minor mycelial content, it seems unlikely that more then 1/3 of all potenal wood fungi species in Sweden are present and active in the decay in one log at one time. There

might be ways in the future to make quantifications on how much fungal material that is present in the wood based on of the amount of fragments that are amplified, but until this problem is properly solved other methods might be preferable.

So, it is still possible that T-RFLP is a potentially useful method when the community of active decayers is addressed. In our paper we wanted to examine the restrictions of this method, which had earlier been addressed more generally by for example Avis et al. 2006 and more specifically in relation to fungi by Allmér et al 2004, and relate it to the amount of dominant colonizers that can be found in a log. Our approach was that T-RFLP could fill the gap as a cost-efficient alternative to traditional fruit body inventories and complete scanning by pyrosequencing.

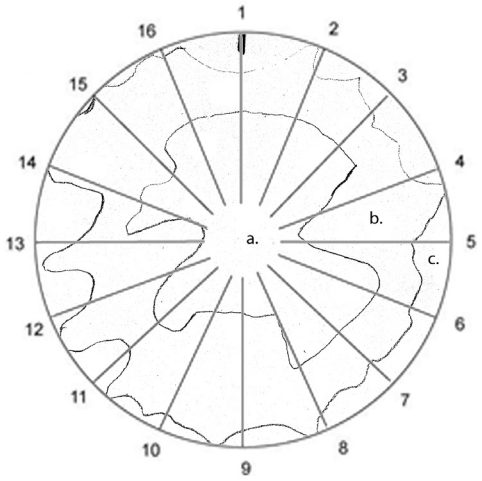

Figure 9 - Simulated drill holes and their position on cross-sections. Number 1 is located at the upper side and number 9 at the underside of the logs as positioned in the field. Letters a-c denote different species separated by interaction zone lines. The three zones in the cross section represents occurrence of different fungal individuals.

3. RESULTS

3.1 Paper I results

In paper I the main results were that wood fungi associated with forest fires, or conditions found in open, dry and warm habitats, can survive higher temperatures than fungi without such association. The results were really pronounced, as all species in the fire-associated group survived up to 220 °C for 5 min (corresponding to an inner wood temperature of 73 °C) and some species (*A. infirma, D. squalens, P. gigantea, G. sepiarium, G. protractum*) survived 180 °C for 10 min (79°C inner wood temperature). By contrast, in the non fire-associated group none survived any of these treatments and only one of the species (*F. pinicola*) survived 180 °C for 5 min (66°C inner wood temperature) (figure 10). The maximum temperature is probably not the only important factor determining fungal survival. We also noted that the speed of the temperature increase was important for mycelia survival.

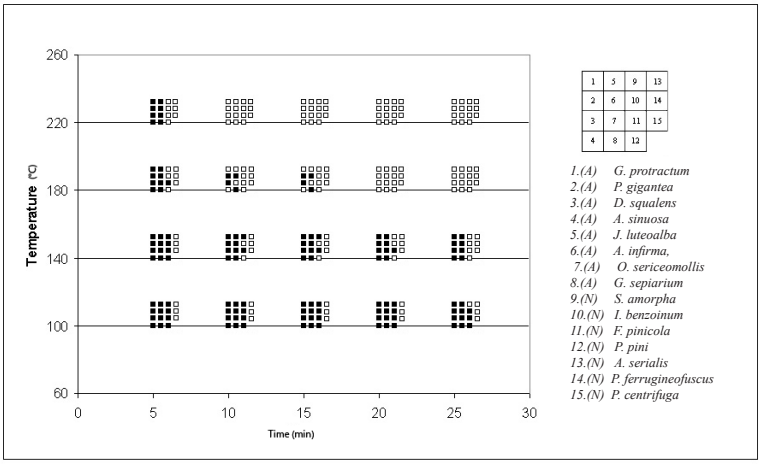

Figure 10 - Survival of 15 different species of wood fungi at different temperature treatments. The 8 leftmost dots represent forest fire associated species and the 7 to the right represent species with no such fire association. A black dot indicates that the species has survived that specific treatment.

3.2 Paper II results

In paper II the species patterns in heat resistance from paper I were used to select species for a competition experiment. We used three species from each group, fire-associated and non-fire associated species and allowed them to compete after heat treatment. All fire-associated species had a clear advantage after heat treatment, conquering a large volume of wood from its competitor (Figure 11). There were also clear differences in competitive strength when comparing the individual species, both in the fire-associated and the non-fire associated group. The controls remained mostly at a stalemate, where both species generally held on to their area. However, I. benzoinum showed a tendency to expand its mycelium in the controls against all fire-associated species, and this was also the case for *D. squalens* (with the exception when combined with *I. benzonium*). However, the standard deviations were rather high in these samples. *D. squalens* was arguably the strongest competitor in the fire-associated group after heat treatment; it conquered large areas from all competitors so that it held 78%, 95%, and 94% of the total wood volumes. The mycelia of *D. squalens* was even more competitive than *F. pinicola* (78%), which was the strongest of the non-associated species. *F. pinicola* only lost ~10% of the total wood volume to *G. sepiarium* and *P. gigantea*. In short, all fire-associated species had higher mycelial competitive ability after the heat treatment compared with non-associated species. This pattern was statistically significant in comparison to the controls (Paper II, table 3).

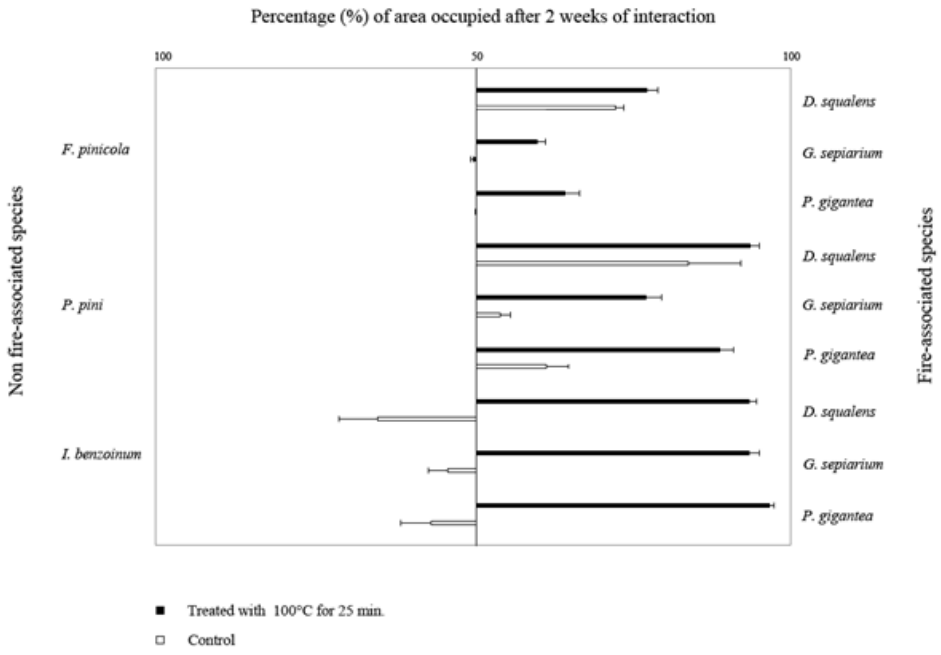

Figure 11 - Interspecific competition in wood discs after heat exposure. Before heat treatment the competitors each occupied 50% of the wood disc area (middle line of the diagram). The results were recorded after 2 weeks of re-growth. The black bar shows heat treated samples and the white, untreated controls. Standard error values range between ±0.24 and ±8.66. All pairs of interactions had a significant difference between heat treatment and control. The species on the left side of the diagram are non-fire-associated and the species on the right side are fire-associated.

3.3 Paper III results

In paper III we measured the decomposition rates of the 18 species of wood fungi. The decomposition followed a general pattern over time after inoculation as their decomposition rates increased over time until a plateau was reached, after which decomposition rates leveled off. The plateau seemed to be reached when the whole substrate unit was colonized. The actual level of this plateau varied considerably among species and substrate types (figure 12 supplementary figure 1.) and different species reached their plateau at different times. There were large differences in decomposition rates among species, once the fungi had colonized the whole substrate (Paper III, Figure 3.a, 3.b, 4). Species that had a high decomposition plateau also reached the plateau earlier, and early successional species found on fresh wood. *H. annosum, G. sepiarium and D. squalens* were among the first seven species to reach their asymptote in decomposition rates, while late successional species like O. sericiomollis and P. nigrolimitatus were among the last species to reach their asymptote, when ranked according to their decomposition rates (Paper III, Table 2, Figure 3).

All species except *I. benzoinum*, a non fire associated species, had a significantly higher decomposition rate after heat shock treatments. This increase was greater in the fire-associated group, even though the greatest difference between the two groups was the drop in decomposition rates of the non-associated species after heat shock (Paper III, Figure 1). After heat shock the decomposition rate generally started to decline after a maximum was reached.

Two major differences in decomposition rates emerged when comparing the fire-associated species with the non-fire associated species. Firstly, there was a significantly greater decline in decomposition rates in the non-fire associated group after heat shock. Secondly, increase in decomposition rate after heat treatment was higher in the fire-associated group. There was however great variation within the groups and the characterization of species (See 2.1.3) in the respective groups were not completely straight forward. A clearer result would plausibly been obtained if the control group would have

contained species only found in moist habitats, and if the fire associated group would only have included species strictly related to charred wood.

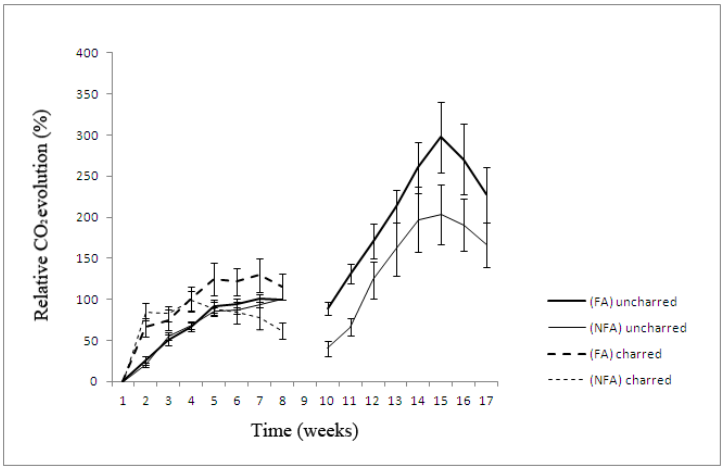

Figure 12 – Decomposition rate over time in wood fungi. Species have been grouped according to their fire association, Fire associated species (FA) and Non Fire Associated (NFA). Data has been normalized, values are relative to the maximum decomposition rate (100%) at normal conditions

3.4 Paper IV

In paper IV we identified 26 SNP and in 6 sequenced DNA-fragments from each of two individuals of *Phlebiopsis gigantea*. High LD was found between SNPs on the same DNA-fragment in all cases and analyses includes 6 loci. Best haplotype was inferred using PHASE and 119 unique genotypes were indentified among the 132 individuals from the 6 different sampling locations. We found deviations from the Hardy-Weinbergs equilibrium, both on sampling locations and over all populations, we did a test for genetic structure departing from sampling locations. Analyses in STRUCTURE showed 5 clusters (Pr[K5=1]) with clusters having individuals from several sampling locations, both close and far away on a geographical scale. Also, some sampling locations belonged to several clusters. No correlation asso-

ciated with areas associated with forest fires was found (Table 1).

Table 1. Proportion of memebership in each pre-defined populations of *Phlebiopsis gigantea,* to the 5 clusters calculated with STRUCTURE. Pre-defined populations are sampled from three pairs of location in middle to northern Sweden.

Predefined pop (n)	Inferred Clusters				
	1	2	3	4	5
1A, Umeå (10)	0.066	**0.723**	0.002	0.000	0.209
1B, Sävar(17)	**0,514**	**0.438**	0.003	0.005	0.040
2A, Jämtgaveln(28)	*0.000*	*0.016*	*0.015*	*0.001*	***0.968***
2B, Töva(22)	0.174	0.046	**0.383**	0.007	**0.390**
3A, Båtsfors(24)	**0.342**	**0.376**	0.000	0.111	0.171
3B, Dalälven(31)	**0.392**	0.004	0.001	**0.595**	0.007
He	0.3012	0.5558	0.4403	0.3441	0.3395
Fst, mean value	0.2805	0.0697	0.3939	0.3010	0.2020

3.5 Paper V

In Paper V five study species (*P. leucomallella, D. squalens, S. amorpha, P. gigantea, P. tephroleuca*) were included. All species were possible to detect in artificial species mixtures based on their genomic DNA. A 20-fold concentration difference between two species was found to be the critical lower value for detection. When five species were present within a sample, the peak height of the restriction cut products was comparable to samples with two species at a 1:5 content ratio. There was no indication for interference

between species as DNA concentrations of different species in a mixture had a strong influence on the species detection ability using T-RFLP.
When combining these results with data from sampling simulations it was however apparent the level of detectability in most cases is sufficient for an efficient field sampling protocol. The simulations showed that the number of major fungal decomposers found in sawdust from one sample will rarely exceed 5. The number of species found in one cross section of the log seldom exceeded 5 (< 25%) (fig. 2) and 80% of the species in the cross section were collected with 5 samples (Figure 13). This suggests that it is possible to pool samples from one location on a log, since the amplification tests showed that in most cases 5 species were detectable in the same reaction. The accumulation curves were inversely exponential, which means that it is hard to obtain 100% of the species in a single cross section. For example, with 5 species present 16 samples were required (figure 13).

Implications for field sampling

If the aim is to also identify species with small amounts of mycelia, taking more samples might not yield better results since the additional material would only represent a small fraction of the total DNA. One way to overcome this problem might be to take several samples with an increment corer instead of a drill. Mycelia generally extend in the same direction as the wood fibres, which makes the areas occupied by the different individuals longitudinally directed (Boddy et al. 2008). This means that the samples should be taken perpendicular to the longitudinal direction of the log. However, our results showed that pooling 5 samples from the same location on the log should in most cases work since the average number of functionally important species found on a single cross section very rarely exceeded 5.

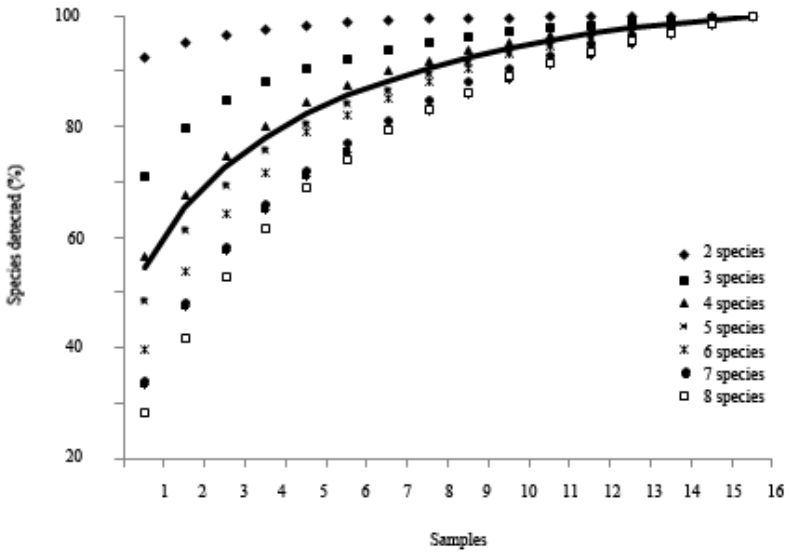

Figure 13 - Results of the simulation of the species detection probability depending on the number of samples taken. The different curves represent cross-sections with ≤ 3, 4, 5 and ≥ 6 fungal species. Cross sections with 3 or less species and cross sections with 6 or more species were pooled, due to low sample size.

4. DISCUSSION

Wood fungi are a magnificent example of the high plasticity that can be found within a species group. This has been evident during the whole project in different contexts, and is perhaps the most lingering impression from working with these fascinating organisms. We have seen individuals survive pH drops below 1 in liquid cultures (Carlsson & Ali in prep.), and some species could decompose wood at minus degrees whilst others survived temperatures above the boiling point of water.

4.1 Major findings

The major findings in my thesis were that several wood fungi can survive extreme temperatures for extended periods of time, and this has probably been a very important adaptation to allow the different species to survive. We are not the first research group to have reported this, (See for example Humphrey & Siggers 1933; Miric & Willeitner 1984; Schmidt 1995, 2006; Boddy et al. 2008) but we extend the current understanding of heat resistance in wood fungi by linking the species' heat resistance traits to their associations with forest fire. Wood fungi occupying a log are not necessarily situated in a stable environment. The temperature in a piece of dead wood on the forest floor can vary greatly within a single day and over the season. Furthermore, as the forest goes through succession the environment will change, i.e. gap dynamics which can cause a sudden shift in irradiation (Runkle 1982; Kuuluvainen 1998). Our results show that wood fungi situated in areas with high frequencies of fires can survive temperatures over the boiling point of water for short periods of time (Paper I). Considering that areas that are likely to burn frequently are also the driest and warmest places in the forest, species associated with these environments probably experience high temperatures also during periods without fires.

The growth on a charred surface on a southern slope in an open forest might actually present a physiological challenge in parity with surviving a forest fire. When we tested how high the temperature actually rises in a log during a forest fire, we found that three cm from the surface the temperature only

rose to about 56 °C and in the interior of the log the change was hardly noticeable. This condition was survivable by several of the species without any specific association to forest fire, implying that community responses of wood fungi to forest fire more complex (see below).

4.2 Approaches and hypotheseses

After publishing paper I, our initial hypothesis was that some fungi survive the fire and that these species take over the wood volume that was previously colonized by more heat-intolerant species. We needed to partly revise this hypothesis, given that several of the non fire associated species could survive temperatures typical for forest fires. This was the starting point for the second paper (II), which showed that increased heat tolerance in some wood fungi allowed them to expand their mycelium after a forest fire. This was not possible because the earlier dominant colonizers died during the fire, but caused by f temperature influenced changes in the competitive balance between species (figure 14).

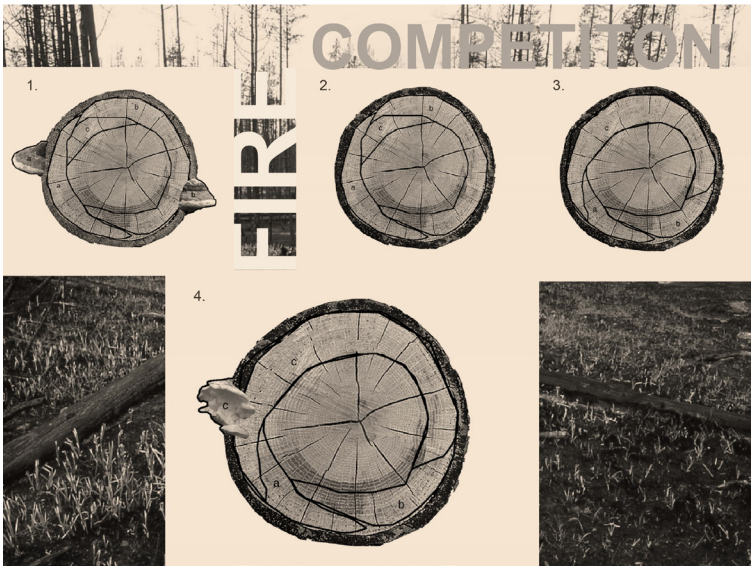

Figure 14 – Hypothetical visualisation of combative interactions after a forest fire. 1) A log before disturbance, containing a number of major colonizers. 2) Forest fire affects the substrate, the intercompetetive balance is changed, favouring those species with higher heat tolerance. 3) Favoured species gains wood volume on the expense of earlier dominant colonizers. 4) Forest fire favoured species are able to produce fruiting bodies.

4.3 Community structure and combative interactions

Earlier studies have shown that wood fungal communities are founder controlled, and that intermediate to low frequencies of disturbance is optimal for species diversity. However, our results showed that fungal communities under certain circumstances can be dominance controlled. Without heat shock the controls remained at a stalemate or near a stalemate in several of our interaction experiments (Figure 9). This response/behaviour is also in accordance with the founder control hypothesis, which is also shown by Holmér and Stenlid 1985. However, after the heat treatment we found that

the fire-associated species outcompeted their antagonists. The fire-associated species used in this study all had high heat-resistance (could handle the same temperatures, paper1, figure1). The non-fire associated species studied in paper II, were among the strongest in their category i.e. had high heat tolerance in relation to other non fire associated species, and could readily survive the temperature applied in the competition experiment.

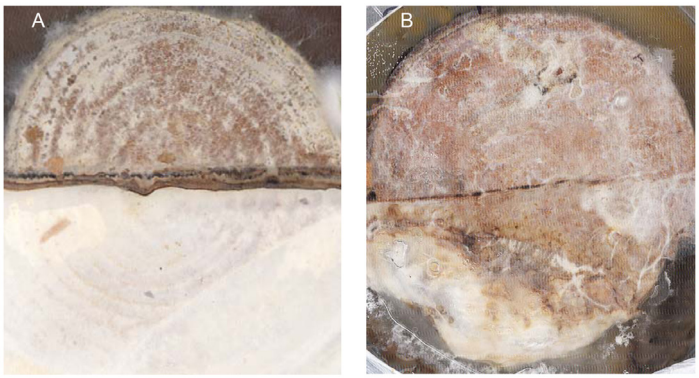

Figure 15 – Example of combative interactions between D. squalens and F. pinicola after two weeks at different conditions; a) with no disturbance, b) disturbed by heat shock.

All our competitive experiments showed the same general pattern. However competitiveness varied among species. Our revised hypothesis was that wood fungal communities are generally founder controlled, that the time at which the fungi colonize the substrate is critical for its success (see introduction for a definition on success in this context). After the "founder phase" the log slowly transforms due to the decomposition of the substrate This will have a long-term effect on the species community. The succession is largely dependent on the change in chemical properties of the log, where mid and late succession species depends on modifications in cellulose, hemicellulose and lignin content caused by earlier colonizers (for an extensive

list of wood fungi related to succession see Renvall (1995)). However, after a disturbance, such as forest fire, the community shifts rapidly in accordance to a dominance-controlled system, where the stronger competitors gains ground and outcompetes the earlier dominant species (figure 7).

Ultimately, there will be a change in the species community a couple of years after the disturbance, and the log will once again transform due to the long term effects listed above. As decomposition proceeds the fire-associated species will eventually lose their competitive edge, and successors will take their place. However, the post-fire succession may well go in a different direction compared with the successional pathway in a forest not affected by fire. An ongoing long-term experiment has shown that the effect of fires extend up to at least 5 years after the disturbance (Eriksson et al. in prep.). Also, the species community differs in logs that have been affected by fire, in comparison to unaffected logs (Berglund et al. 2011; Olsson 2010). This can be the effect of different primary colonizers as well as the chemical and physical traits of the log. The fire-associated species are important for biodiversity, not only directly, but also indirectly when they create substrates habitable for a wider variety of wood fungi , i.e. the composition of the major colonizers at a certain time has cascade effects for the following species succession. The hypothesis that fungi can survive forest fire inside logs was strengthened by the fact that several of the species studied have been shown to decline the first year after a fire, only to return after four years (Olsson 2008). These species have also been detected on substrates strongly affected by restoration fires, and some had fruit bodies on the charred surface of wood (Junninen et al. 1995; Pentillä 1995).

4.4 Decomposition

In our decomposition rate experiments (paper III) it is interesting that all species except *I. benzoinum* had a significantly higher decomposition rate after heat shock; since it indicates that heat shock induces some form up-regulation of fungal metabolism. The increase was also found to be greater in the fire-associated group, even though the biggest difference between

the two groups is the significant drop in decomposition rate in the non fire-associated species after heat shock (Figure 12). As discussed above the studies on competitiveness and survival after heat shock showed that fire-associated species were found to be more competitive after heat shock. It is not clear, however, whether this was simply due to the increased survival at those temperatures or whether there is some other mechanism involved. For fire-associated species, an increase in decomposition rate would help them expand after a forest fire. If the increased tolerance to higher temperatures is truly an adaptation to forest fire, then there is a possibility that some of the fire-associated species utilize some sort of "sit and wait" strategy (Thompson & Rayner 1983). The triggering of increased metabolism, which our results indicate, could help some species get the competitive edge after fire seen in paper II (figure 11).

The decomposition rate in the 18 species of wood fungi used in this study follows a general pattern after colonization/inoculation. The decomposition rate increases until a plateau is reached. This probably has to do with the colonization of the substrate i.e. the fungi colonize the substrate and expand, so that more wood begins to be decomposed. This will continue until the whole substrate is colonized at which point the increase in decomposition rate levels out and a plateau is reached for that specific substrate–species combination. It would be interesting to examine what happens to the decomposition rate when the substrate volume limit is set by a competitor. Metabolism has been looked at from a population perspective before and it has been shown that communities with high biodiversity also have an increased decomposition rate. Our approach would make it possible to look at different decomposition rate in single species in relation to antagonism. It is possible that increased decomposition rates could be due to up-regulation of metabolism rather than the compatibility of the community (Dickie et al. 2012; Fukami et al 2010).

Different species reach this plateau at different times, indicating that the colonization of the substrate takes different lengths of time for different species. This has been hypothesized before (Boddy et al. 2008) and is related to the strategy and mycelial extension rate of individual species. Somewhat

more surprising are the large differences in decomposition rates after the fungi have colonized the whole substrate (Figure 2, Figure 3). Species that achieved a high decomposition plateau also reached the plateau earlier.

4.5 Summary of paper I II, III

The results in papers I, II and III clearly indicates that wood fungi already present in dead wood will survive forest fires, and that some species will be favoured due to higher heat tolerance and mycelial competitiveness. Thus, forest fires clearly affect fungal species composition and diversity in boreal forests. In addition, a forest fire will also create new substrates available for colonization. Which of these two processes that will constitute the main driver of fungal diversity will likely differ for specific forest fires or restoration burnings? A forest fire with high intensity will likely create a large volume of new substrates and consume already present dead wood (cf. Eriksson et al. 2013), while a low intensity fire to a higher degree will modify already present dead wood and create less amounts of new substrates. When planning for restoration burning, these variables need to be taken in to consideration. It should be possible to make species not earlier detected as fruiting individuals in the area to appear by burning with low intensity in areas with a lot of dead wood. However, the intensity needs to be high enough to actually modify the substrates, and considering our temperature measurements (Paper II, figure 1), the present dead wood needs to be readily charred to yield a substantial change in species composition.

4.6 Population structure in relation to forest fire

We found (paper IV) a genetic structure based on 5 clusters among the 132 investigated individuals that deviates geographically from the 6 sampled "populations". This shows a similar result, with more or less panmictic structure, as other studies with long distance dispersal ability and high spore production in this species and other related species (Rizhbeth 1959; Stenlid 1985; Boddy 2008; Selosse 2011). In the present study (paper IV) several

clusters covered the whole investigated range. Results also indicate a more complex pattern with several clusters represented in the same sampling area. The study did not show genetic structure association with forest fire areas. It would however be interesting to look at functional genes involved in heat resistance to see if some polymorphisms related to heat protection areas in the genome (for example the HSE region) has an allele frequency structure depending on forest fire associated environments. As earlier studies have shown there is a very high level of gene flow between populations, in air dispersed non ecto-mychorizal species, our study shows a less clear pattern with a detectable genetic structure and consequently our Fst values was higher than those obtained in earlier studies (Stenlid 1985; Högberg et al. 1999; Vaino et al. 1998), but also indicating long distance dispersal with clusters covering large areas. This might be due to the use of SNP´s instead of microsatellites, but can also be accounted to the fact that we used a higher number of individuals in our study. Out of the 132 individuals in the study 119 had its own genotype, showing a high level of genetic variation. Number of detected SNP per 100 nucleotides differed between amplified fragments, from 0,26 – 1,01, showing an uneven distribution of polymorphism over the genome, with some parts highly variable. In total 29 000 base pairs were sequenced and used in the first step to find polymorphism. All of the loci used in the study can be considered as neutral, since no known functional genes were attached to them (Paper IV). The underlying ecological process which gives this structure is hard to hypothesize about, but it is possible that this structure could be due to earlier and geographic overlapping dispersal and colonization events. Knowledge concerning the genetics of wood fungi is increasing rather quickly and the first complete genomes are under construction and that will increase the possibility to address more specific genetic/ecological questions to a the group of species living in dead wood.

It was apparent in paper I, that spruce species had very low heat tolerance. This might be because of several reasons;

- Since spruce forests burns less frequently, but with higher intensity, there is no need for species generally confined to spruce to adapt to forest

fire.

- The high mortality of living spruce makes naturally fire prone ecosystems pine dominated
- Since dead spruce decompose faster than pine, and substrate consumption during fire is dependent on the decay stage (Eriksson et al. 2013), individuals already present in dead spruce wood will have little chance of surviving the fire inside the substrate.

4.7 Sampling

At any given time in a log, there are only a certain number of species that are being able to reproduce, even though there might be many more species present. The discrepancy in observed fungal diversity between new molecular studies and fruit body inventories is large, and give different pictures of the wood fungal community structure. We found that in a cross section of a log there were between two and 12 species which were detectable by delineating growth/interaction zones (Paper IV). This corresponds rather well with data from field studies of fruit bodies (Olsson et al. 2008, Eriksson et al. 2014). Hence, it seems that the amount of dominant mycelia at any given time is somewhat related to the amount of species producing fruit bodies. Considering that mycelia spread along the wood fibres, a cross section of point samples can be considered representative of a larger piece of wood.

A weakness with our study was that we did not conduct a total inventory of the species which were present in the log. Nonetheless, we assume that fruit bodies on our specific study log were produced by the species detected in the cross section. To reproduce, the fungus must gather enough resources to produce and maintain a fruit body structure (Boddy et al. 2008). This means that a certain amount of the wood needs to be colonized to allow the fungi to fruit. With T-RFLP up to 80% of the dominant colonizers were detected.

The importance of small mycelial domains (SMD) should not be underestimated and it is fascinating to hypothezise around how as much as 300 species could fit in a log (Ovaskainen 2010; Kubartova 2012). Future research in this area may change our view on fungal interactions. Combative

interaction studies (Rew by. Boddy et al. 2008; Paper II), however, indicates that only one species is present in a colonized wood volume, and that this volume is surrounded by an interaction zone where the fungi strives to gain the upper hand on its competitor through the extraction of secondary compounds. This has also been our experience when working with fungi in the laboratory. The only exception is cultures infested by parasitic fungi such as species from the *Athelia* genus.

4.8 Conclusions

In conclusion, individual species of wood fungi are probably more widespread than previously known, and the amount of species present in a forest stand might be 10-fold of those that can be detected by fruit body inventories (Ovaskainen et al. 2010; Kubartova et al. 2012). Small mycelial domains in logs might be of great importance for the population dynamics of wood fungi, and the "sit and wait" -strategy (Boddy et al. 2008) may prove to be important for a large number of species. According to our population study (paper IV), as well as other studies, this is also apparent in populations of some wood fungi with high genetic diversity and little genetic structure. At a certain time the dominant mycelial constituents remains relatively few, constituting perhaps > 99% of the volume in a log (paper V). The dynamics of these mycelial constituents, the SMDs and the dominant colonizers, is governed by colonization, succession and disturbances like fire (paper I and II). At the onset of a disturbance the fungal community shift in to a drastic dominance-controlled phase, where some species are more competitive and out-compete other species, in some cases earlier dominant colonizers. Hypothetically, a disturbance is the only way for some species to attain enough wood volume to produce a fruit body, and they are subsequently dependant on disturbance for their survival, e.g. truly fire dependent.

The loss of forest fires from boreal ecosystems pose a great threat to biodiversity. Although potentially important, restoration fires needs to be carefully designed and evaluated in order to be effective. Burning only clearcuts will not fill the functions of natural forest fires, since no or very little

fungi are present in the substrates of such areas, and it will provide very little new dead wood. It is possible that dispersal and colonization from the surrounding landscape is important after a restoration fire with very high intensity However, in areas affected by low intensity fires, the major type of re-colonization will likely be from species already present in the log prior to the fire. , In areas affected by very severe fires, the main re-colonization will likely be by spores. In a study by Olsson et al. 2008, it was shown that some species disappear as fruiting individuals immediately after fire but fruit on the same log again after a few years. This fire was of much lower intensity than a high-intensity fire studied by Pentillä et al. 2004, where it took much longer time for the amount of wood fungi to rebound at levels attained before the restoration burning, although in time significantly surpassed pre-fires species richness..

Our research expands the current understanding of forest fire effect/importance, mainly concerning the physiological impacts in wood fungi, but also on the community dynamics in dead wood, and the related genetical population structure.

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6. APPENDICES

6.1 Acknowledgements

Moa, Edvin, Jussi and Bella
My family who has lived with me during this whole time. To you three small tricksters; it is amazing what has become of you, even more considering your Phd-ing father, im so proud of you all three.

Elisabeth, Maria and Kristina
My mother and amazingly talented sisters, I love you so much.

Ove and Daniel
I will miss you always.

Barbro Sjögren
My everlasting Grand Mother please become at least 100 years, not so far to go! (Min föreviga mormor snälla bli minst hundra år)

Mattias Edman
My friend, I am so glad I have gotten to know you, it will be all about studies on concurrence in the future!

Bengt-Gunnar Jonsson
Also my Friend, thank you for being what I am not, or becoming what I am not, and having very much patience with it! I might even get in to Bridge, that's how thankful I am!

Svante Holm
Friend, thank you for all the discussions in the lab, in the office in the pizzeria…..basically everywhere, about science and other things.

Grand Barker
I don't think you guys know how much being in this band means to me, it

has been a life saver to go on our Monday out lashes some times. And playing on motor cycle clubs are not so bad after all.

Ida Fällström
I miss you sincerely in the lab, thank for alerting me about the inhaling of sandwiches during “lunches”.

Anna-Maria Eriksson
Thank you so much for beeing a wonderful friend and room mate, i enjoy talking to you a little bit to much. You are also one of the coolest women i know!

Tessa Pocock
You taught me so much about being a scientist. You are a great teacher and a friend; we had so much fun in the lab! There are still some super secret messages that need to be handed over, keep a look out for a Pez-dispenser.

Marie Jönsson
Thank you for helping me out, with the language in this thesis, and also during that awfully snowy autumn.

Erika, Ann, Carlos, Samson, Kristin, Mattias, Fredrik, Tara, RenYun, Miriam, Sara, Jenny, Jennie, Lisbeth, Joel, Amelie, Jörgen, Andreas, Maria, Boel, Torborg, Håkan, Hugo.
Give me a hug you all!!! It has been an honor to work with you!

Arne, Daga, Anders & Anita
Thank you! You were the first and possibly, the only people who thought that I would do science.

My Clan - Gunnar, Olov, Elsa, Göran, Erik, Jonas, Frida, Lena, Lennart, Oskar, Signe, Inez, Annika, Tone, Sanna, Josef, Valdemar, Selma, Daniel, Ingrid.
You have always been important to me, you shaped me as a person, this amazing extended family is also something I am proud of being part of.

This my excavation and today is Qumran
Everything that happens is from now on
This is pouring rain
This is paralyzed

Justin Vernon

6.2 Supplementary tables and figures

Supplementary Table 1 - Table of results from a two way ANOVA with replication, showing the statistical significance of the competition outcomes. The Sources of variation tested are within and between the respective groups of species. The explanatory variables are heat treatment and non-fire associated species, Ischnoderma benzonium, Phellinus pini and Fomitopsis pinicola.

D.Squalens

Source	DF	SS	MS	F	P			
Treatment		1	0.428852		0.428852		80.45	<0.001
Non Fire A. species		2	0.128071		0.064036		12.01	<0.001
Interaction		2	0.25601	0.128005		24.01	<0.001	
Error	12	0.063965		0.00533				
Total	17	0.876898						

S = 0.07301 R-Sq = 92.71% R-Sq(adj) = 89.67%

G.sepiarium

Source	DF	SS	MS	F	P	
Treatment		1	1.116	1.116	230.71	<0.001
Non Fire A. species		2	0.24893	0.12446	25.73	<0.001
Interaction		2	0.39896	0.19948	41.24	<0.001
Error	48	0.23219	0.00484			
Total	53	1.99608				

S = 0.06955 R-Sq = 88.37% R-Sq(adj) = 87.16%

P.gigantea

Source	DF	SS	MS	F	P	
Treatment		1	1.68239	1.68239	201.54	<0.001
Non Fire A. species		2	0.32399	0.162	19.41	<0.001
Interaction		2	0.49594	0.24797	29.71	<0.001
Error	48	0.40068	0.00835			
Total	53	2.903				

S = 0.09136 R-Sq = 86.20% R-Sq(adj) = 84.76%

Suplementary Table 2 - Combination of treatments, replicated 5 times for heat treatments but only once for the non heat treated samples. D.sn= D. squalens individual A, F.pn = F. pinicola, G.sn = G. sepiarium, P.gn = P. gigantea, I.bn = I. benzoinum, P.pn = P. pini

D.s1-F.p1	D.s1-F.p2	D.s1-F.p3	D.s1-P.p1	D.s1-P.p2	D.s1-P.p3	D.s1-I.b1	D.s1-I.b2	D.s1-I.b3
D.s1-F.p1	D.s1-F.p2	D.s1-F.p3	D.s1-P.p1	D.s1-P.p2	D.s1-P.p3	D.s1-I.b1	D.s1-I.b2	D.s1-I.b3
D.s1-F.p1	D.s1-F.p2	D.s1-F.p3	D.s1-P.p1	D.s1-P.p2	D.s1-P.p3	D.s1-I.b1	D.s1-I.b2	D.s1-I.b3
G.s1-F.p1	G.s1-F.p2	G.s1-F.p3	G.s1-P.p1	G.s1-P.p2	G.s1-P.p3	G.s1-I.b1	G.s1-I.b2	G.s1-I.b3
G.s2-F.p1	G.s2-F.p2	G.s2-F.p3	G.s2-P.p1	G.s2-P.p2	G.s2-P.p3	G.s2-I.b1	G.s2-I.b2	G.s2-I.b3
G.s3-F.p1	G.s3-F.p2	G.s3-F.p3	G.s3-P.p1	G.s3-P.p2	G.s3-P.p3	G.s3-I.b1	G.s3-I.b2	G.s3-I.b3
P.g1-F.p1	P.g1-F.p2	P.g1-F.p3	P.g1-P.p1	P.g1-P.p2	P.g1-P.p3	P.g1-I.b1	P.g1-I.b2	P.g1-I.b3
P.g2-F.p1	P.g2-F.p2	P.g2-F.p3	P.g2-P.p1	P.g2-P.p2	P.g2-P.p3	P.g2-I.b1	P.g2-I.b2	P.g2-I.b3
P.g3-F.p1	P.g3-F.p2	P.g3-F.p3	P.g3-P.p1	P.g3-P.p3	P.g3-P.p3	P.g3-I.b1	P.g3-I.b2	P.g3-I.b3

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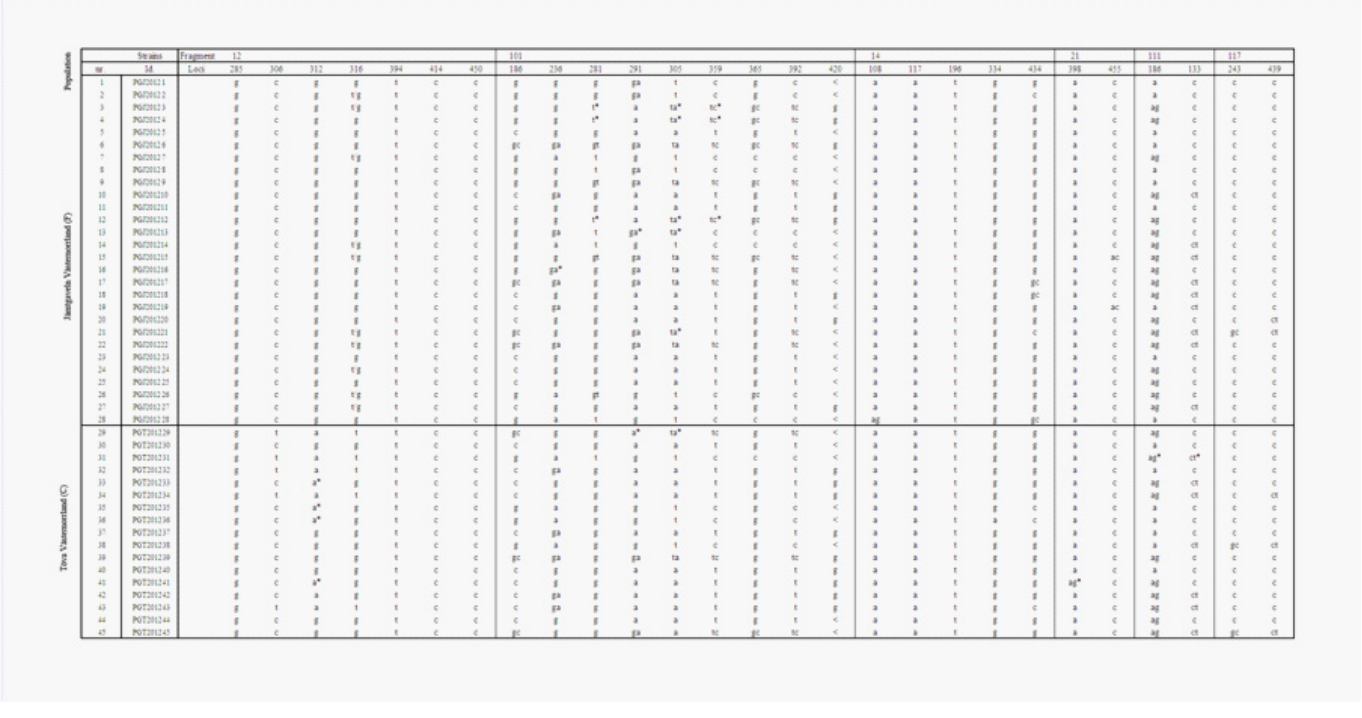
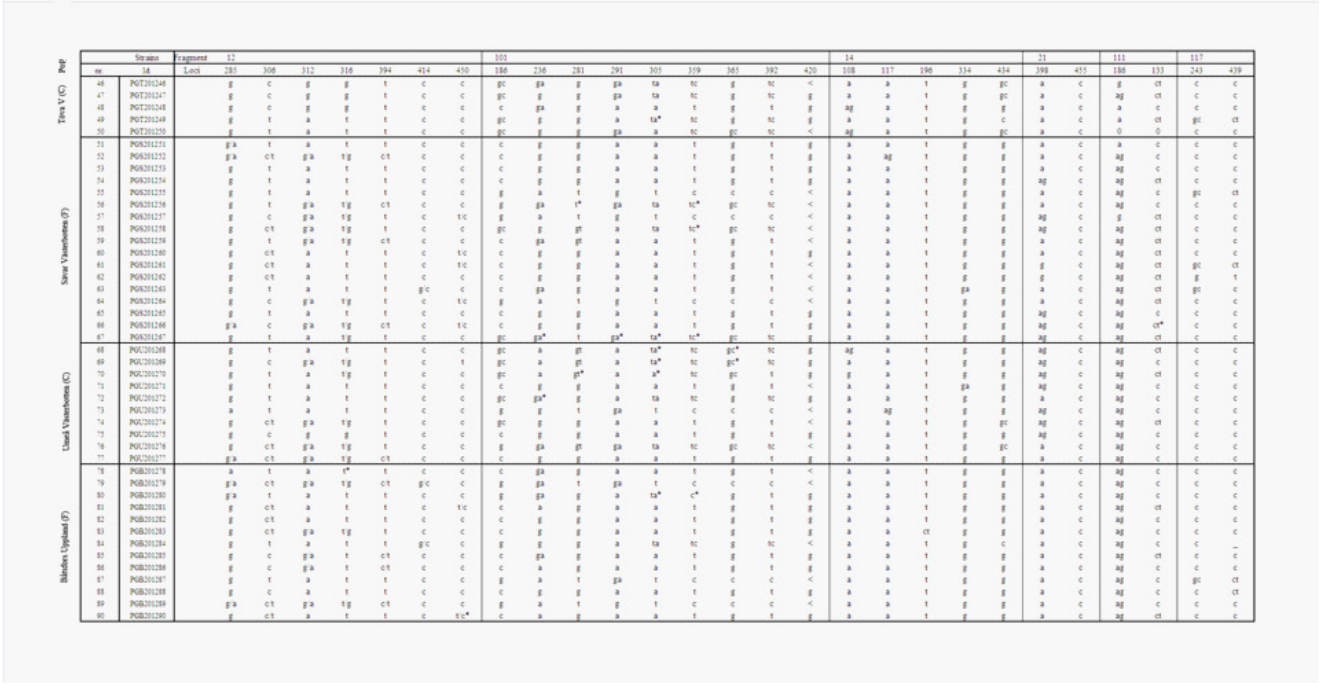
Supplementary table 3 – All primers pairs that amplified fragments in *P. gigantea*

Primer nr.	Oligo name	Sequence (5'-3')	Tm (°C)	Product size
1	Pgf10	GCTACCCGTCATTGAGAGAAG	52.4	166 bp.
2	Pgr10	ACAGACGTTCATCGTGACC	52.6	
3	Pgf12	CCTCCAACCTTCAACGTCAC	59.4	744 bp.
4	Pgr12	TACAAGTCTAGCAGCCTCGG	59.4	
5	Pgf14	ACAACTCTGGTAGGTATGAAGC	58.4	642 bp.
6	Pgr14	GCCTCTGGTAACAGTCCAAC	59.4	
7	Pgf17	TCGCATACGCCACTATATTTAC	56.5	209 bp.
8	Pgr17	AGTTATAGTATGGGCCAGTCAC	58.4	
9	PgfA21	TGACCCACTGCTCAACTAC	56.7	780 bp.
10	PgrA21	AGATACGATGAGAACAGACTCC	58.4	
11	Pgf26	GTATCTTGCAGCAGTGACG	56.7	225 bp.
12	Pgr26	CTTACATTTGCCCAGGAGAC	57.3	
13	Pgf27	ATTTGAGGGTCAGCACTG	53.7	178 bp.
14	Pgr27	AGCGTCTTGATAAATTCACCC	55.9	
15	Pgf30	TGCCAAATCTCATAGCAGAAC	55.9	125 bp.
16	Pgr30	TTGCTAGAGAGCATTTCGATAC	56.5	
17	Pgf31	CGTCTTCGTCTTCAGACTCAG	59.8	164 bp.
18	Pgr 31	CGAGTACAAGAAGGAACGAGAG	60.3	
19	Pgf36	CATACCCACGCCAATTTCTC	57.3	100 bp.
20	Pgr36	TCATTAGCGTTCGTTGACTAC	55.9	
21	Pgf38	AAACCCGGAGCTATAGGAATC	47.6	655 bp.
22	PgrA38	CGTCCTCTCTCTCTACAAGTC	52.4	
23	Pgf41	TCTCAGTCGATGGGATTGTG	57.3	113 bp.
24	Pgr41	CCCTACTAGTCTAGAGTTCGC	59.8	
25	PgfA46	ATGAACCTAGAAAAATCTGCCC	56.5	714 bp.
26	PgrA46	AACCCGTATACTTGCCTCC	56.7	
27	PgfB46	GCACGAAGATTACGATGGTAAG	58.4	773 bp.
28	PgrB46	ATTAAAGGATAATGCTCCGGC	55.9	
29	Pgf49	TTCCCACTCACCTCTTTGCG	59.4	280 bp.
30	Pgr49	TCAGACGGAGAAGACGACAC	59.4	
31	Pgf52	AACTTCCTCCCCTCCTTTG	56.7	135 bp.
32	Pgr52	CTTGTCGTTCCCTGATCTTG	57.3	
33	Pgf53	TGTTCGTCCGTTCTAAACTG	55.3	326 bp.
34	Pgr53	TTACAACGTCCGCAACTTC	54.3	
35	Pgf55	CAAAGTCTCATAACGCTACTCC	58.4	126 bp.
36	Pgr55	GAATTCGCGTAGAGCTTGG	56.7	
37	Pgf56	CCGCATGTTGTTTTACGCATAC	58.4	203 bp.
38	Pgr56	GGACAAGTGAGTGGGCTAAC	59.4	
39	Pgf60	TTTGCACAATAGTCTGCCTC	55.3	157 bp.
40	Pgr60	GGAAATGCGAATTGCAGC	53.7	
41	Pgf67	CACAATGATGGAGTCGCAAG	57.3	437 bp.
42	Pgr67	GCAACCATAGACAAGGGAAG	57.3	
43	Pgf68	GCAAAATAGCCTCCGAAGTC	57.3	250 bp.
44	Pgr68	GGTAACTAGCTTCCTTGCAAAC	54.8	
45	Pgf73	GCGGCGAACCAAATGATAAG	57.3	168 bp.
46	Pgr73	GGAGATGACGAGAACAATGGG	59.8	
47	Pgf75	CCTTTTCAACCCCTTTTGATTG	56.5	201 bp.
48	Pgr75	GCAGGCTTTACCCTTATCTAAC	58.4	
49	Pgf76	TTCTCCGTCTGCGTCTCTATCC	62.1	99 bp.
50	Pgr76	GGGAGCGTGATATTCGCTCATC	62.1	
51	Pgf78	GAATGCTTACGGGACAGTG	56.7	200 bp.
52	Pgr78	GATAAGCACCAGATGACTAACC	58.4	
53	Pgf81	ACTAGGACGAAAGACTTCGG	57.3	282 bp.
54	Pgr81	GCAGCTTAGCAGTTATACCAC	57.9	
55	Pgf89	AAGTCGCCAAAGCAAGACC	56.7	232 bp.
56	Pgr89	TCGTGAAGACTAGGGACCAC	59.4	
57	Pgf101	ATGGGATACGCGGCATCTTCAG	56.9	895 bp.
58	Pgr101	AGCGATCCTCTCGTGATGTACC	55.8	
59	Pgf107	TGGGAAGGAGAGTTTGCTG	51.1	123 bp.
60	Pgr107	GTTCGCATTGCAAAGAAACTAC	51.3	
61	Pgf 111	CCAAGTCAACGATGAAGTCAAG	51.9	550 bp.
62	Pgr 111	CAGAACATCCAATTCAACCCC	51.3	
63	Pgf112	ACCTCTCAACTCACTGAAGG	50.7	397 bp.
64	Pgr112	GATGCCCCAGACAATAAGC	50.3	
65	Pgf 113	GTTTGCGACTACCACATCC	50.6	849 bp.
66	Pgr 113	GGTCCGATTTTATGGCTGAG	50.7	
67	Pgf114	ATGGTTCCGGTAGCTTTCC	51.3	330 bp.
68	Pgr114	TATTGCCTACACCAGACCC	50.3	
69	Pgf117	ACGAAAGTCAAACGGGATG	54.5	586 bp.
70	Pgr117	CCCGAACAGACGATTTCAG	56.7	
71	Pgf120	ACTTTGGACCCCCTAAGAC	56.7	123 bp.
72	Pgr120	GTAGATGGAGATGATTTCTGGC	58.4	
73	Pgf122	TATGCCCATGTTCCTGGCCTC	59.4	203 bp.
74	Pgr122	CAAAATCTGCAAGGGCACGC	56.7	
75	Pgf129	TAGAATGGTGGCACAGCTC	56.7	115 bp.
76	Pgr129	CCACGATCACATTGCAACC	56.7	
77	Pgf134	AGGATAGTTAGTCGGAGACAC	57.9	148 bp.
78	Pgr134	GTCACACGACTCGAAACAG	56.7	
79	Pgf138	CGTTTACGAAGAAAAAAACCGC	56.5	634 bp.
80	Pgr138	TGCAGGAATTCGATATCAAGC	55.9	
81	Pgf142	CGCTCTCCTTCTTGATCTGC	59.4	234 bp.
82	Pgr142	TGGTGCAAATAGAGCAAGATG	55.9	
83	Pgf145	TGTAAGCATCACATGCCTAGC	57.9	86 bp.
84	Pgr145	AGTCGGCCTGGATCATGAAC	59.4	
85	Pgf146	GGTAGAAAGCGCCCAGTATATC	60.3	94 bp.
86	Pgr146	AGAAACTATGGCTGGCGTCC	59.4	
87	Pgf147	CGTATGTCTTCTCACAATCGTC	58.4	148 bp.
88	Pgr147	CGGTATAGAAGAGGTACGGTG	59.8	
89	Pgf149	CTGGCGAATGGGTTCTGTTG	59.4	145 bp.
90	Pgr149	ATGGAGGAGAGTGAGGTCATGC	62.1	
91	Pgf151	AGGGAAGACCTTGAGAGTTG	57.3	85 bp.
92	Pgr151	TGTATTAGCGGATTCACG	54.5	
93	Pgf153	CATCCAAGAGAAACGCGAC	56.7	306 bp.
94	Pgr153	CCTGGAAGGAGGTTATCAGC	59.4	
95	Pgf154	GGTAGCCACAGAGGTATCAG	59.4	188 bp.
96	Pgr154	CGCATATCCGTGCTCAAAC	56.7	
97	Pgf155	ACTCAAATAGATTCGAGCGTC	55.9	254 bp.
98	Pgr155	TGTAGGTCTGAAAATTCTGAGC	56.5	
99	Pgf156	TGTCGGTCAAGGTTGTCAG	56.7	155 bp.
100	Pgr156	ACACTCACCAGCCTAACAC	56.7	
101	Pgf157	ACTTCCGAAGAGGAGGATTAC	57.9	234 bp.
102	Pgr157	GAAGTTACCCTGGCTTTGTG	57.3	
103	Pgf158	TGCCACTGCCACCAATCAAC	59.4	215 bp.
104	Pgr158	TGGCGAGCAGGCCAAATAAC	59.4	

Supplementary table 4 - List of polymorphisms in all individuals listed by population.



Cont.



		Strains	Fragment	12						101								14					21		111		117	
		nr.	Id	Loci	285	306	312	316	394	414	450	186	236	281	291	305	359	365	392	420	108	117	196	334	434	398	455	186	133	243	439
PoP	Tiva V (C)	46	PGT201246		g	c	g	g	t	c	c	gc	ga	g	ga	ta	tc	g	tc	<	a	a	t	g	gc	a	c	g	ct	c	c
		47	PGT201247		g	c	g	g	t	c	c	gc	g	g	ga	ta	tc	g	tc	g	a	a	t	g	gc	a	c	ag	ct	c	c
		48	PGT201248		g	c	g	g	t	c	c	c	ga	g	a	a	t	g	t	g	ag	a	t	g	g	a	c	a	c	c	c
		49	PGT201249		g	t	a	t	t	c	c	gc	g	g	a	ta*	tc	g	tc	g	a	a	t	g	c	a	c	a	ct	gc	ct
		50	PGT201250		g	t	a	t	t	c	c	gc	g	g	ga	a	tc	gc	tc	<	ag	a	t	g	gc	a	c	0	0	c	c
Silver Waterbottom (F)		51	PGS201251		g/a	t	a	t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	a	c	c	c
		52	PGS201252		g/a	c/t	g/a	t/g	c/t	c	c	c	g	g	a	a	t	g	t	g	a	ag	t	g	g	a	c	ag	c	c	c
		53	PGS201253		g	t	a	t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
		54	PGS201254		g	t	a	t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	ag	c	ag	ct	c	c
		55	PGS201255		g	t	a	t	t	c	c	g	a	t	g	t	c	c	c	<	a	a	t	g	g	a	c	ag	c	gc	ct
		56	PGS201256		g	t	g/a	t/g	c/t	c	c	g	ga	t*	ga	ta	tc*	gc	tc	<	a	a	t	g	g	a	c	ag	c	c	c
		57	PGS201257		g	c	g/a	t/g	t	c	t/c	g	a	t	g	t	c	c	c	<	a	a	t	g	g	ag	c	g	ct	c	c
		58	PGS201258		g	c/t	g/a	t/g	t	c	c	gc	g	gt	a	ta	tc*	gc	tc	<	a	a	t	g	g	ag	c	ag	ct	c	c
		59	PGS201259		g	t	g/a	t/g	c/t	c	c	c	ga	gt	a	a	t	g	t	<	a	a	t	g	g	a	c	ag	ct	c	c
		60	PGS201260		g	c/t	a	t	t	c	t/c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	ct	c	c
		61	PGS201261		g	c/t	a	t	t	c	t/c	c	g	g	a	a	t	g	t	<	a	a	t	g	g	g	c	ag	ct	gc	ct
		62	PGS201262		g	c/t	a	t	t	c	c	c	g	g	a	a	t	g	t	<	a	a	t	g	g	g	c	ag	ct	g	t
		63	PGS201263		g	t	a	t	t	g/c	c	c	ga	g	a	a	t	g	t	<	a	a	t	ga	g	a	c	ag	ct	gc	c
		64	PGS201264		g	c	g/a	t/g	t	c	t/c	g	a	t	g	t	c	c	c	<	a	a	t	g	g	a	c	ag	ct	c	c
		65	PGS201265		g	t	a	t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	ag	c	ag	c	c	c
		66	PGS201266		g/a	c	g/a	t/g	c/t	c	t/c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	ag	c	ag	ct*	c	c
		67	PGS201267		g	t	a	t/g	t	c	c	gc	ga*	t	ga*	ta*	tc*	gc	tc	g	a	a	t	g	g	ag	c	ag	ct	c	c
Umrd Waterbottom (C)		68	PGU201268		g	t	a	t	t	c	c	gc	a	gt	a	ta*	tc	gc*	tc	g	ag	a	t	g	g	ag	c	ag	ct	c	c
		69	PGU201269		g	c	g/a	t/g	t	c	t	gc	a	gt	a	ta*	tc	gc*	tc	g	g	a	t	g	g	ag	c	ag	c	c	c
		70	PGU201270		g	t	a	t/g	t	c	c	gc	a	gt*	a	a*	tc	gc	t	g	g	a	t	g	g	ag	c	ag	ct	c	c
		71	PGU201271		g	t	a	t	t	c	c	c	g	g	a	a	t	g	t	<	a	a	t	ga	g	ag	c	ag	c	c	c
		72	PGU201272		g	t	a	t	t	c	c	gc	ga*	g	a	ta	tc	g	tc	g	a	a	t	g	g	a	c	ag	c	c	c
		73	PGU201273		a	t	a	t	t	c	c	g	g	t	ga	t	c	c	c	<	a	ag	t	g	g	ag	c	ag	c	c	c
		74	PGU201274		g	c/t	g/a	t/g	t	c	c	gc	g	g	a	a	t	g	t	<	a	a	t	g	gc	ag	c	ag	ct	c	c
		75	PGU201275		g	c	g	g	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	ag	c	ag	c	c	c
		76	PGU201276		g	c/t	g/a	t/g	t	c	c	g	ga	gt	ga	ta	tc	gc	tc	<	a	a	t	g	gc	a	c	ag	c	c	c
		77	PGU201277		g/a	c/t	g/a	t/g	c/t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
Bilinden Upptand (F)		78	PGB201278		a	t	a	t*	t	c	c	c	ga	g	a	a	t	g	t	<	a	a	t	g	g	a	c	ag	c	c	c
		79	PGB201279		g/a	c/t	g/a	t/g	c/t	g/c	c	g	ga	t	ga	t	c	c	c	<	a	a	t	g	g	a	c	ag	c	c	c
		80	PGB201280		g/a	t	a	t	t	c	c	g	ga	g	a	ta*	c*	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
		81	PGB201281		g	c/t	a	t	t	c	t/c	c	a	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	ct	c	c
		82	PGB201282		g	c/t	a	t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
		83	PGB201283		g	c/t	g/a	t/g	t	c	c	c	g	g	a	a	t	g	t	g	a	a	ct	g	g	a	c	ag	c	c	c
		84	PGB201284		g	t	a	t	t	g/c	c	g	g	g	a	ta	tc	g	tc	<	a	a	t	g	c	a	c	ag	c	c	_
		85	PGB201285		g	c	g/a	t	c/t	c	c	c	ga	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	ct	c	c
		86	PGB201286		g	c	g/a	t	c/t	c	c	c	a	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
		87	PGB201287		g	t	a	t	t	c	c	g	a	t	ga	t	c	c	c	<	a	a	t	g	g	a	c	ag	c	gc	ct
		88	PGB201288		g	c	a	t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	c	ct
		89	PGB201289		g/a	c/t	g/a	t/g	c/t	c	c	g	a	t	g	t	c	c	c	<	a	a	t	g	g	a	c	ag	c	c	c
		90	PGB201290		g	c/t	a	t	t	c	t/c*	c	a	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	ct	c	c

Cont.

Population	Strains		Fragment	12							101									14					21		111		117	
	nr.	Id	Loci	285	306	312	316	394	414	450	186	236	281	291	305	359	365	392	420	108	117	196	334	434	398	455	186	133	243	439
Båtsfors Uppland (F)	91	PGB2012 91		g	c/t	g/a	t/g	t	c	c	c	ga	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	gc	ct
	92	PGB2012 92		g	t	a	t	t	c	c	c	a	g	a	a	t	g	t	<	a	a	t	g	c	a	c	ag*	c	c	c
	93	PGB2012 93		g	t	a	t	t	g/c	c	g	ga	t	ga	t	c	c	c	<	a	a	t	g	g	a	c	ag	c	c	c
	94	PGB2012 94		g	c/t	g	t/g	t	c	c	c	ga	g	a	a	t	g	t	<	a	a	t	g	g	a	c	ag	c	c	c
	95	PGB2012 95		g	t	a	t	t	c	c	g	ga	g	ga	ta	tc	g	tc	<	a	a	t	g	g	a	c	ag	ct	c	c
	96	PGB2012 96		g	c/t	g/a	t/g	t	c	c	g	g	g	ga	ta*	c*	g	c	<	a	a	t	g	g	a	c	ag	c	c	c
	97	PGB2012 97		g	c/t	g	t/g	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	ct	c	c
	98	PGB2012 98		g	c/t	g	t/g	t	c	c	g	a	t	ga	t	c	c	c	<	a	a	t	g	g	a	c	ag	c	c	c
	99	PGB2012 99		g/a	c/t	g/a	g/t	c/t	c	c	g	g	g	ga	ta*	c*	g	c	<	a	a	t	g	g	a	c	ag	c	c	c
	100	PGB2012100		g	t	a	t	t	c	c	g	a	t	ga	t	c	c	c	<	a	a	t	g	g	a	c	ag	c	c	c
	101	PGB2012101		g	c/t	a	t	t	c	t/c	g	ga	t	ga	t	c	c	c	<	ag	a	t	g	g	a	c	ag	ct	c	c
Dalälven Uppland (C)	102	PGUK2012102		g/a	t	a	t	c/t	c	c	g	ag	g	a	t	t	g	t*	g	a	a	t	g	g	a	c	ag	c	c	c
	103	PGUK2012103		g	c/t	g/a	g/t	t	c	c	g	ag	g	ga	ta	tc	g	0	<	a	a	t	g	g	a	c	ag	c	gc	ct
	104	PGUK2012104		g/a	c	a	g/t	c/t	c	c/t	g	ag	g	ga	ta	tc	g	0	<	a	a	t	g	g	a	c	ag	c	c	c
	105	PGUK2012105		g	c/t	g/a	g/t	t	c	c	g	ag	g	a	t	t	g	t*	g	a	a	t	g	g	a	c	ag	c	c	c
	106	PGUK2012106		g	c/t	a	t	t	c	t/c	g	ag	g	a	t	t	g	t*	g	a	a	t	g	g	a	c	ag	c	c	c
	107	PGUK2012107		g	t	a	t	t	c	c	g	ag	g	a	t	t	g	t*	g	a	a	t	g	g	a	c	ag	c	c	c
	108	PGUK2012108		g	t	a	t	t	c	c	g	a	g	g	t	c	g	c	<	a	a	t	g	g	a	c	ag	c	c	c
	109	PGUK2012109		g	t	a	t	t	g/c	c	g	g	g	a	a	0	g	t	g	a	a	t	g	g	a	c	ag	c	c	ct
	110	PGUK2012110		g	c/t	g/a	g/t	t	g/c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	ct	c	c
	111	PGUK2012111		g	t	a	t	t	g/c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
	112	PGUK2012112		g	t	a	t	t	c	c	g	g	g	a	a	t	g	t	<	a	a	t	g	g	a	c	ag	c	c	c
	113	PGUK2012113		g	c/t	a	t	t	c	t/c	g	a	g	g	t	c	g	c	<	a	a	t	g	g	a	c	ag	c	c	c
	114	PGUK2012114		g	t	a	t	t	c	c	g	g	g	a	a	0	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
	115	PGUK2012115		g/a	t	a	t	t	c	c	g	g	g	a	a	0	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
	116	PGUK2012116		g	t	a	t	t	g/c	c	g	a	g	g	t	c	g	c	<	a	a	t	g	g	a	c	ag	c	c	c
	117	PGUK2012117		g	c/t	g/a	g/t	t	c	c	g	a	g	g	t	c	g	c	<	a	a	t	g	g	a	c	ag	c	c	c
	118	PGUK2012118		g	t	a	t	t	g/c	c	g	a	g	g	t	c	g	c	<	a	a	t	g	g	a	c	ag	ct	c	c
	119	PGUK2012119		g	t	a	t	t	c	c	g	ag	g	ga	ta*	tc	g	c	<	a	a	t	g	g	a	c	ag	c	c	c
	120	PGUK2012120		g	t	a	t	t	c	c	g	ag	g	ga	ta*	tc	g	c	<	a	a	t	g	g	a	c	ag	c	c	c
	121	PGUK2012121		g	c/t	g/a	g/t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
	122	PGUK2012122		g/a	t	a	t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
	123	PGUK2012123		g	c	a	t	t	c	t	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	gc	ct
	124	PGUK2012124		g/a	t	a	t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
	125	PGUK2012125		g/a	c/t	a	t	t	c	c	c	g	g	a	a	t	g	t	<	a	a	t	g	g	a	c	ag	c	c	c
	126	PGUK2012126		g	c/t	g/a	g/t	t	c	c	g	ag	g	ga	ta*	tc	g	c	<	a	a	t	g	g	a	c	ag	c	c	c
	127	PGUK2012127		a	c	g	g	c	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
	128	PGUK2012128		g	c/t	a	t	t	g/c	c	g	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	a	c	c	c
	129	PGUK2012129		g	t	a	t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	c	c
	130	PGUK2012130		g	c/t	g/a	g/t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	c	ct
	131	PGUK2012131		g	c/t	g/a	g/t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	ag	c	gc	c
	132	PGUK2012132		a	c/t	g/a	g/t	t	c	c	c	g	g	a	a	t	g	t	g	a	a	t	g	g	a	c	a	ct	c	c

Supplementary table 5 . Allele frequency in 6 SNP loci among 6 population of Phlebiopsis gigantea.

	Pop					
	Sävar	Umeå	Jämtgaveln	Töva	Båtsfors	Dalälven
Loci						
pg12						
1	0,088	0,200	0,821	0,455	0,125	0,129
2	0	0,050	0	0	0	0
3	0	0	0,179	0	0,042	0
4	0	0	0	0,227	0	0
5	0,029	0	0	0	0,063	0,032
6	0,147	0,050	0	0	0,063	0,081
7	0	0	0	0	0,042	0
8	0	0	0	0	0,021	0
9	0,059	0	0	0	0,021	0
10	0	0	0	0	0,042	0
11	0,029	0,050	0	0	0	0
12	0,529	0,500	0	0,318	0,417	0,500
13	0,029	0	0	0	0,042	0,0,097
14	0	0	0	0	0	0,016
15	0,059	0,050	0	0	0,021	0,032
16	0	0	0	0	0,021	0,016
17	0,029	0,100	0	0	0,083	0,065
18	0	0	0	0	0	0,016
19	0	0	0	0	0	0,016
pg14						
1	0,942	0,650	0,860	0,705	0,875	1,000
2	0	0,100	0,130	0,250	0,083	0
3	0	0,150	0,010	0,045	0,021	0
4	0,029	0,050	0	0	0	0
5	0,029	0,050	0	0	0	0
6	0	0	0	0	0,021	0
pg21						
1	0,706	0,650	0,964	0,979	1,000	1,000
2	0,294	0,350	0	0,021	0	0
3	0	0	0,036	0	0	0
pg101						
1	0	0	0,054	0,023	0	0
2	0	0	0,018	0	0	0
3	0	0	0	0	0,042	0
4	0	0	0	0,045	0	0
5	0	0,050	0,036	0,045	0,063	0
6	0	0	0	0	0	0,065
7	0	0	0	0	0,021	0
8	0	0	0	0	0	0,048
9	0	0,100	0,054	0	0,042	0,194
10	0	0,050	0,054	0	0	0
11	0,029	0,050	0,071	0	0,063	0
12	0	0	0,018	0	0	0
13	0	0	0,018	0	0	0
14	0,029	0	0,036	0	0	0
15	0	0	0,054	0,182	0,021	0,242
16	0	0	0	0	0,021	0
17	0	0	0	0	0	0,065
18	0,206	0,050	0,143	0,045	0,167	0
19	0,029	0	0	0	0	0
20	0	0,100	0,018	0	0,063	0
21	0	0,050	0	0	0	0
22	0,588	0,350	0,375	0,568	0,250	0,387
23	0,059	0	0	0	0	0
24	0,059	0,200	0,054	0,091	0,250	0
pg111						
1	0,500	0,500	0,660	0,643	0,500	0,516
2	0,177	0,150	0,140	0,190	0,125	0,032
3	0,324	0,350	0,180	0,119	0,375	0,435
4	0	0	0,020	0,048	0	0,016
pg117						
1	0,853	1,00	0,964	0,909	0,932	0,919
2	0,117	0	0,018	0,068	0,045	0,032
3	0	0	0,018	0,023	0,023	0,032
4	0,029	0	0	0	0	0,016