Assessing local adaptations in a widespread forest fungus by *in vitro* growth experiments

Tor Arne Justad

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Department of Bioscience

UNIVERSITY OF OSLO

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Tor Arne Justad

http://www.duo.uio.no/

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Abstract

Climate change is altering many aspects of local selection regimes. Which aspects of changing environments causing the strongest selective force, differ widely across species. Strong climatic and topographical heterogeneity makes Norway an ideal area to study the relative importance of environmental factors potentially decisive for local adaptation. The main aim of this study is therefore to investigate to which extent climatic variability and habitat (substrate) requirements lead to evolution of local adaptation in the widespread and ecologically important wood-decay fungus *Fomitopsis pinicola*.

By testing specific hypotheses aimed to evaluate the ability for local adaptation in *F. pinicola,* this study found that different responses to abiotic factors can be linked to local climate. Further, sporocarps sampled from the two most common hosts, *Alnus incana* and *Picea abies* revealed significantly different rate of decay when comparing mass loss of wood chips made from *A. incana* and *P. abies.* The relationship between growth and decay was negatively correlated, indicative of an underlying trade-off. Thus, this study suggests that climatic variation and substrate requirements affects the phenotype of *F. pinicola*.

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1 Introduction

Local natural selection is partly driven by climate (Pecl et al. 2017). Consequently, a change in climate can alter local selection regimes by affecting timing of growth and reproduction among species across a manifold of ecosystems (Hughes 2000, Radchuk et al. 2019). A consequence causing substantial redistribution of species at both local and regional scales (Pecl et al. 2017). How species are affected by changing environmental conditions are largely dependent on their ability to adapt or migrate (Olson-Manning et al. 2012). Adaptation to changing selective regimes, depends on pre-existing genetic variation and new mutations that confer advantageous alleles when spread across the population by natural selection (Olson-Manning et al. 2012). If an organism lack the ability to adapt, local extinction may be prevented by migration to new habitats with similar ecological niches as their original habitat (Aitken et al. 2008). However, the speed and magnitude of climate change affect species differently (Hendry et al. 2008). Understanding how species locally adapt to their current environment, is therefore crucial for predicting the magnitude of the ongoing redistribution of species (Hendry et al. 2008). Local adaptation has been studied mainly for plants through laboratory, greenhouse, field common garden and reciprocal transplant experiments, revealing, in many cases, strong evidence of local adaptation (Baughman et al. 2019). However, for plant-associated organisms, like fungi, very little is known about their ability to adapt to the changing climate.

Fungi influence almost every aspect of terrestrial ecosystem functioning (Treseder and Lennon 2015). As the dominant decomposers of organic plant material, fungi have a direct effect on the global carbon and nutrient dynamics (Baldrian et al. 2014). Considering the estimation of more than 70 Pg carbon to be stocked in dead wood worldwide (Pan et al. 2011), understanding relationships between basidiomycete community dynamics and wood decay is crucial for predicting future ecosystem functions under environmental change (Baldrian et al. 2014). Given that different fungi process organic matter at different rates, the composition of fungi in an area can affect the overall functioning of the ecosystem (Treseder and Lennon 2015). This composition of fungal communities is in part driven by climate, as well as other environmental characteristics (van der Wal et al. 2006). Hence, a change in climate can potentially affect the local-scale fungi community, which in turn may alter the overall functioning of the ecosystem. For example, different moisture and temperature conditions, are argued to potentially prolong autumn seasons and reduce snow cover in winter

seasons, which directly affects the rate of annual decomposition and production of sporocarps (Piao et al. 2008). However, different functional groups of fungi are expected to have different responses to these environmental changes (Crowther et al. 2014).

In temperate regions the red-belted bracket fungus *F. pinicola* (Fig.1) is widespread and ecologically important for decomposing logs or standing dead trees (Ryvarden and Gilbertson 1993). In Norway, as well as other parts of Europe, it has a continuous distribution and as one of few wood-decay fungi, it grows on both conifers (e.g. *Picea abies* and *Pinus sylvestris*) as well as deciduous trees (e.g. *Alnus incana* and *Betula pubescens*) (Högberg et al. 1999). A widespread distribution combined with large and perennial sporocarps make the species a perfect target for wide sampling efforts. The strong climatic and topographical heterogeneity (Fig. 1) makes Norway an ideal study area for assessing the relative importance of environmental factors potentially decisive for local adaptation. Although previous studies have shown that *F. pinicola* is a fully outcrossing species with little genetic differentiation among Scandinavian populations, these studies do however only consider neutral genetic variation. Hence, in traits and loci selected for (adaptive) the situation might be different. To investigate whether the heterogeneity in the study area has led to adaptation to varying climatic conditions, specific hypotheses on climate-related adaptations will be tested in a series of common garden experiments. The main aim of this study is therefore to investigate the extent climatic variability and habitat (substrate) requirements lead to evolution of local adaptation in *F. pinicola*.

Previous studies have shown that there is an underlying trade-off between growth rate, stress tolerance and competitive abilities linked to different environment (Maynard et al. 2019, Lustenhouwer et al. 2020). For instance, habitats characterized by relatively warm and rainy seasons, like the tropics, typically select for fungi with low stress tolerance but rapid growth enhancing competitive abilities (Maynard et al. 2019). On the contrary, in more temperate climates, selection will typically favor dense hyphal growth, enhancing stress tolerance at the expense of competitive abilities. These trends have been primarily studied at broad geographical scale, however, the extensive variation in temperature and precipitation in Norway give reasons to assume that similar patterns can occur on local scale as well. Although conceptual frameworks for categorization of trade-off between life history traits, such as Grimes C-S-R theory, are historically more frequently applied by botanists than mycologists, Grime himself suggested life history traits for fungi to follow similar patterns as

plants (Grime 1977). Here: rapid growth and effective rate of decay align with R-selection (Ruderal strategy), dense mycelium and prevalent rhizomorphs for C-selection in low stress environments (Competitors strategy), and slow growth with high stress tolerance for S-selection in highly fluctuating environments (Stress tolerant strategy) (Grime 1977). Hence, approaching potential for local adaptation, may benefit from considering general trade-off patterns, as varying environmental factors may represent different degrees of selective force amongst different strategist. Thus, the climatic variation in the study area may affect the costs and benefits associated with life history trade-offs differently. I therefore hypothesize that the climatic variation in the study area has led to local adaptations in growth rates and stress-tolerance in *F. pinicola* (Hypothesis1; H1). To test this, dikaryotic strains isolated from sporocarp tissue of *F. pinicola*, sampled from *P. abies* across a variety of climatic locations in Norway, will be used to study whether growth rates at different conditions of temperature and water potential varies across populations and to which extent this can be linked to local climate.

Most wood-decay fungi occur strictly on either coniferous or deciduous host trees and hence, have adapted to the biochemical composition and wood structure of their group of host trees. However, as one of few wood-decay fungi, *F. pinicola* can decay both coniferous and deciduous hosts. I therefore hypothesize that there is an adaptation towards effective decay of either conifer or deciduous wood substrates (H2). To identify adaptation to substrate, strains isolated from sporocarp tissue sampled from *A. incana* and *P. abies* will be set to decay wood chips made from: *A. incana* and *P. abies*, the two most common hosts.

Several studies have shown a relationship between growth and decay, either positively or negatively correlated (Zheng et al. 2018, Lustenhouwer et al. 2020). At the genetic level, functional genes regulating decomposition (specifically breakdown of cellulose and lignin) are shown negatively associated with genes promoting stress tolerance (Treseder and Lennon 2015, Zheng et al. 2018). Hence, the correlation between growth and decay, may shift either way, dependent on how stress-tolerant a given fungi may be. Given that fungi in temperate regions are shown more stress-tolerant than in the tropics (Maynard et al. 2019), I hypothesize that: *F. pinicola* is trading-off between vegetative growth and substrate decomposition (H3).

Since trade-off between stress-tolerance and competitive abilities is environment-dependent (Maynard et al. 2019), I hypothesize that the competitive ability of *F. pinicola* varies between locations (H4). The competitive ability of *F. pinicola* will be compared across different

populations, by confronting the mycelia of *F. pinicola* against four polypore species (*Pycnoporellus fulgens, Antrodiella citronella, Fomitopsis rosea* and *Phellopilus nigrolimitatus)*, that co-exist with *F. pinicola* in boreal forests.

By measuring growth rates at different temperatures and water potential levels, wood decay rates on different substrates, competitive ability, and estimating subsequent correlations between growth and decay. I aim to reveal, whether local adaptions affect the phenotype of *F. pinicola* in a common garden experiment.

2 Materials and methods

2.1 Sampling

I collected field samples in nine different locations in Norway (Fig. 1) representing a wide climatic range, from the wettest/warmest to the driest/coldest areas, where both hosts *A. incana* and *P. abies* are present. The sampling locations were selected based on differences in annual mean temperature and precipitation, downloaded from WorldClim database (Hijmans et al. 2005) at 2.5 min resolution.

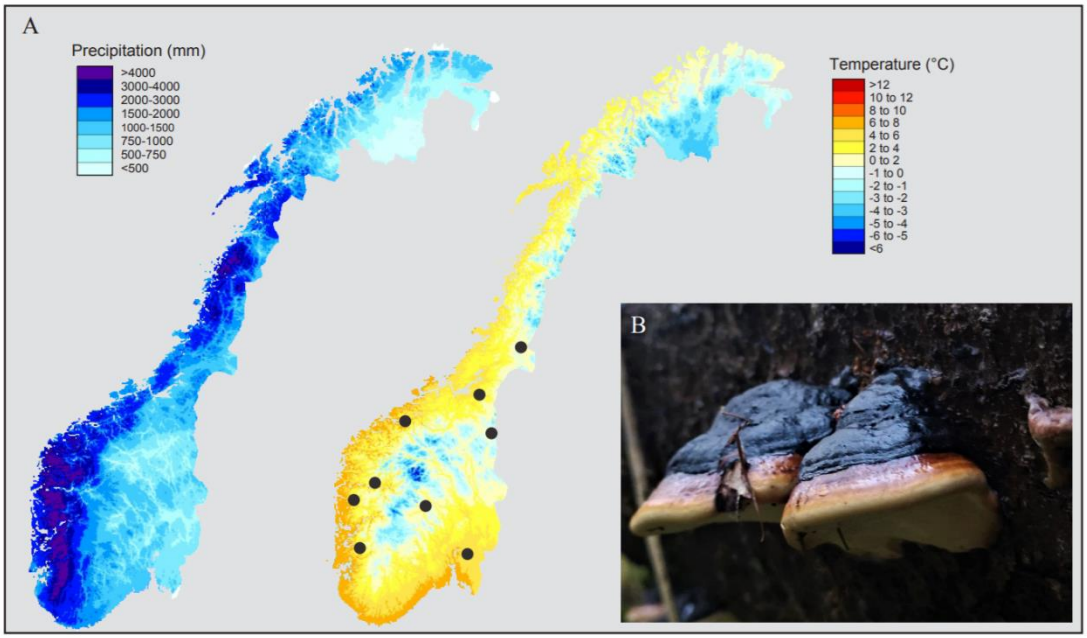

Figure 1: (A) Average annual precipitation (mm) in Norway is shown on the left-side map and annual mean temperature (ºC) on the right-side map. The black dots indicate the nine sampling locations. (b) Sporocarps of *Fomitopsis pinicola*.

In each location, eight sporocarps were sampled within an area of 200 m^2, four on *A. incana* and four on *P. abies*, altogether 72 sporocarps. When possible, sporocarps were collected on different logs. In locations with insufficient logs, two sporocarps were sampled on the same log as far apart as possible. All sampled sporocarps were stored in paper bags at 4 °C until processing (i.e. culturing), then dried for long-term storage.

Table 1: Coordinates and climatic variables for the nine studied locations. For each location, a unique 2-letter abbreviation is specified in parentheses which are used in the figures. Climate data were obtained from Horvath et al. *(Horvath et al. 2019)*.

Locations	Latitude	Longitude	Annual mean temperature (°C)	Annual precipitation (mm)
Vinterbro (Vi)	59.75219	6.99402	5.84	945
Voss (Vo)	60.6696	6.332595	1.58	2061
Valdres (Va)	61.07567	9.11683	2.03	733
Brekke (Br)	61.37271	5.37271	3.01	2805
Loen (Lo)	61.78089	6.99402	4.14	1438
Beverfjorden (Be)	62.97926	8.88054	3.79	1410
Haltdalen (Ha)	62.89283	11.18758	0.57	787
Orkanger (Or)	63.27966	9.7181	4.30	1270
Gartlandet (Ga)	64.54513	12.379303	2.40	1343

2.2 Culture isolation

Cultures were isolated by transferring a small piece of tissue from inside of the sporocarps to 9 cm Petri dishes containing 2% malt extract agar (MEA) with antibiotics (ampicillin 33 mg/l, streptomycin 8.3 mg/l, tetracyclin 3 mg/l) and the fungicide benomyl (1 mg/l). After initial growth on this media, axenic cultures were transferred to new Petri dishes with only MEA and grown at 20 °C. To ensure that sporocarps collected on the same log were not originating from the same individual, the corresponding cultures were confronted through a vegetative incompatibility test. No cultures were compatible, indicating that each culture represents a different genet. In further growth experiments, mycelial plugs of diameter 6 mm were used to inoculate new MEA plates.

2.3 Growth at different temperatures

To investigate whether populations have different growth rates at different temperatures, isolates were grown at four temperatures: 5 °C, 20 °C, 28 °C, and 34 °C. Only the 36 isolates collected from *P. abies* were used in this experiment. Each condition was replicated four times, hence, 144 Petri dishes were established and monitored. Prior to incubation at different temperatures, all isolates were incubated at 20 °C for four full days, to ensure initial growth for all the isolates. The temperatures were selected based on preliminary tests, where a few isolates of *F. pinicola* were grown at minimum and maximum temperatures. At 4 °C and 37 °C, no growth where observed during a 14-day period, while minimum growth was observed at 5 °C and maximum at 34 °C. According to previous studies, optimal growth for *F. pinicola* was expected to be around 29 °C (Marković et al. 2011, Maynard et al. 2019). Hence, the selected temperatures cover the entire expected growth response curve (minimum, optimum and maximum).

2.4 Growth at different moisture levels

The same 36 isolates used in the temperature experiment were re-cultured and used to test potential differences in growth at variable moisture levels. MEA plates were amended with three levels of potassium chloride (KCl) to simulate different water potential (MPa) regimes: -0.5 MPa (regular MEA without KCl), -1.5 MPa (5.4 g KCl/300 ml MEA) and -3.5 MPa (16.1 g KCl/300 ml MEA). The relationship between KCl concentration and water potential was based on previous studies (Molloy 2004). These three values of MPa were chosen based on previous studies demonstrating minimum and optimum fungal growth to fall within this range (Maynard et al. 2019). Given that -0.5 MPa is the default water potential in standard MEA, and that growth at different moisture levels were monitored at 20 °C, the same data for growth at -0.5 MPa as for growth at 20 °C, was used. Hyphal growth at different conditions of temperature and water potential were visualized using box plots (Figure 2) (Wickham 2016).

2.5 Decay rate at different substrates

Substrate preference was tested by inoculating wood chips of *A. incana* and *P. abies* with cultures originated from both tree species, followed by measurement of mass loss. The wood chips of size 1 x 1 x 0.5 cm^3 were made from dried planks of *A. incana* and *P. abies.* The

chips were individually numbered, then dried for 72 h at 78 °C in a dryer before weighting. The wood chips were then autoclaved three times at 120 °C for 20 min, thus minimizing the risk of contamination.

Eight individuals from each of the nine localities (four collected from *A. incana* and four from *P. abies*) were cultured in 15 cm Petri plates (72 cultures) with MEA for 14 d at 20 °C. Thereafter, four wooden chips from each tree species were placed on top of the mycelia for inoculation. After seven weeks of incubation at 20 °C, the wood chips were removed from the cultures, any surface mycelium was scraped off the wood chips, before drying for 72 h at 78 °C. The chips were again weighted and resulting mass loss calculated. As both tree species (*A. incana* and *P. abies*) and the number of wood chips (2 x 4) were confounded in the same Petri dish, the data obtained from the decay experiment was not independent from each other and therefore did not meet the criteria for further statistical analysis to address local differences. This was established due to a high number of wood chips with unchanged mass after seven weeks incubation alongside a relative few wood chips with considerable mass loss.

2.6 Competitive ability

To test whether local differences in competitive ability of *F. pinicola* towards other wood-decay fungi exist, the four isolates from *P. abies* in all nine locations were confronted pairwise with four polypores: *Pycnoporellus fulgens*, *Antrodiella citronella*, *Fomitopsis rosea* and *Phellopilus nigrolimitatus*. These four species are all know to co-exist with *F. pinicola*. Inoculum of each species were placed 1 cm from the edge of the Petri plates. Over 14 weeks, the mycelial confrontations were monitored to determine whether *F. pinicola* from the nine localities demonstrate diverging competitive abilities. Confrontation were scored either as deadlock or win, the latter if mycelia from one species overgrew the other.

2.7 Statistical analysis

All statistical analyses were done using the R version 3.6.1 (R Core Team 2019). Analysis of variance (ANOVA) and Tukey's test was performed to reveal whether significant differences in growth rates and decay ability could be found across populations. To reveal if annual mean temperature and annual precipitation (Table 1) could account for variation in growth rates observed between populations, a linear mixed effect model (LMe) (Bates et al. 2015, Pinheiro

et al. 2020) was used. To do this, the packages “lme4” and “nlme” were used (Bates et al. 2015, Pinheiro et al. 2020). A linear model with daily growth as response and climate variables as predictor served as fixed effects in the LMe model whilst locations served as random effect. Daily growth rates (mm/d) at 20 °C for all isolates (36) sampled from *P. abies* and the corresponding mass loss (mg) of *P. abies* chips were used to assess the relationship between growth and decay and correlated using a linear regression.

3 Results

3.1 Local adaptation to abiotic factors

The mean daily growth was 0.07 mm at 5 °C, 0.79 mm at 20 °C, 0.89 mm at 28 °C and 0.97 mm at 34 °C. Isolates from two locations (Voss and Orkanger) showed maximum growth at 28 °C, while seven localities demonstrated a maximum growth at 34 °C (Figure 2A). As expected, growth increased with a rise in temperature.

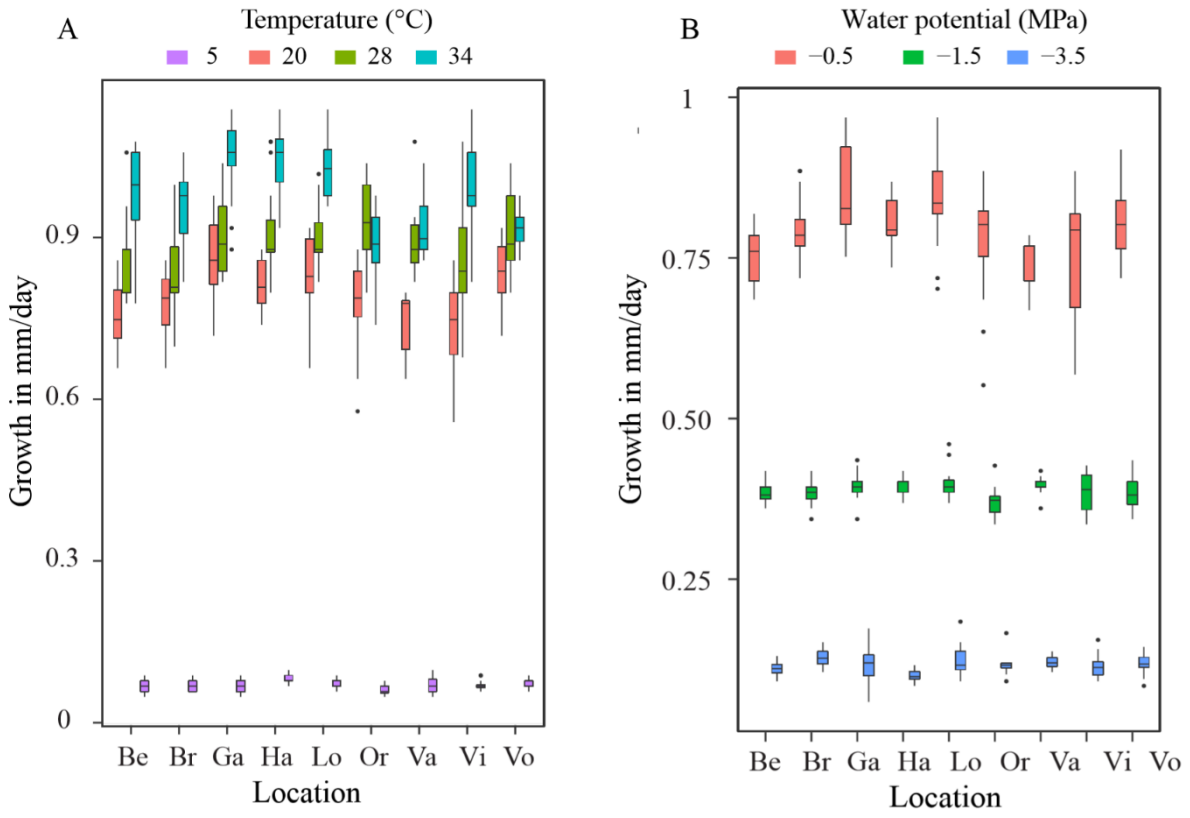

Figure 2: The box plots summarizing daily growth of *Fomitopsis pinicola* (mm) at different temperatures (A) and water potentials (B).The y-axes show daily growth (mm) and the x-axis show nine locations from where the isolates are collected. The full names of the locations can be found in Table 1. Growth at each location is represented by measurements of four replicates of four isolates. Horizontal lines represent the median. Each box represents the 25th and 75th percentile, with whiskers 1.5 times the interquartile range above 75th percentile and below 25th percentile respectively.

A significant difference in growth rate was observed between locations at temperatures 20 °C, 28 °C and 34 °C (ANOVA, $p < 0.05$). Whether the variation in growth rates can be attributed to local climate differences were assessed by implementing local climate variables for each location (LMe, Table 1). Annual precipitation at the different locations was significant to the intercept between growth at 34 °C relative to 20 °C indicating that growth was precipitation dependent (LMe). The difference in growth at 20 °C and 34 °C, declined with increased annual precipitation (LMe). Hence, growth rates between locations vary significantly, and this variation is linked to the local climate.

The growth rate was strongly influenced by the water potential (Figure 2B), where minimal growth was obtained at -3.5 MPa (≈ 0.1 mm), intermediate growth at -1.5 MPa (≈ 0.35 mm), and maximum growth at -0.5 MPa (≈ 0.8 mm) across all populations. At -3.5 MPa and -1.5 MPa there was no significant difference in growth between the locations (ANOVA, $p > 0.05$).

3.2 Local adaptation to biotic factors

When comparing decay of *A. incana* and *P. abies* in respect to which wood species the strains originated from, decay was shown significantly affected by substrate origin (ANOVA $p < 0.05$). Isolates collected on *A. incana* demonstrated significantly more effective decay (39%) of *A. incana* than mycelium from *P. abies* (ANOVA $p < 0.05$). Isolates from *P. abies* demonstrated significantly more effective decay of *P. abies* than *A. incana* (85.31%) (ANOVA $p < 0.05$). *P. abies* had a significant higher mass loss across all populations compared with *A. incana*, without significant difference between host origins (Table 2, Figure 3) (ANOVA $p < 0.05$). This indicates that *P. abies* may be the preferred substrate regardless of phenotype. However, the 39% difference in mass loss in *A. incana* is indicative of certain phenotypes adopted toward more effective decay of *A. incana* to exist.

The relationship between daily growth (mm) and mass loss (mg) had a correlation coefficient $r = 0.5$ (Figure 4). The regression line illustrated a negative correlation between growth and decay. Hence, relationship between daily growth and observed decay, indicate a trade-off between growth rate and ability to decompose.

Table 2: A summary of the mean mass loss of wooden chips after seven weeks of decay by *Fomitopsis pinicola*. Wood chips of *Picea abies* had a significant higher mass loss across all populations compared to wood chips of *Alnus incana*. Isolates collected on *Alnus incana* had significantly higher mass loss (39.4%) of *Alnus incana* than isolates from *Picea abies*. Isolates collected on *Picea abies* demonstrated significantly higher mass loss of *Picea abies* than of *Alnus incana* (85.31%). P-values for each category was calculated using ANOVA. 144 wood chips were used in each category, except one outlier of an isolate that originated from *Alnus incana* and decayed *Picea abies* that was removed.

	A. incana mean mass loss (%)	*P. abies* mean mass loss (%)	Difference in mass loss (%)	P-value
Isolate originated from *A. incana*	9.9 (N=144)	14.46 (N=143)	37.4	$p<0.05$
Isolate originated from *P. abies*	6.64 (N=144)	16.52 (N=144)	85.3	$p<0.05$
Difference in mass loss (%)	39.4	13.3		
P-value	$p<0.05$	$P>0.05$		

The combative experiments between *F. pinicola* and four other polypore species showed little variation across populations (Figure 5). After 14 weeks of confrontation the outcome was either deadlock, or that *F. pinicola* was overgrowing the confronted polypore species. When *F. pinicola* was confronted with *Antrodiella citronella* and *Phellopilus nigrolimitatus*, 34 out of 36 Petri dishes with confronting mycelium (94.4%), were in both cases won by *F. pinicola*. On the contrary, when confronted with *Fomitosis rosea* and *Pycnoporellus fulgens*, the mycelial interactions ended in deadlock for all plates.

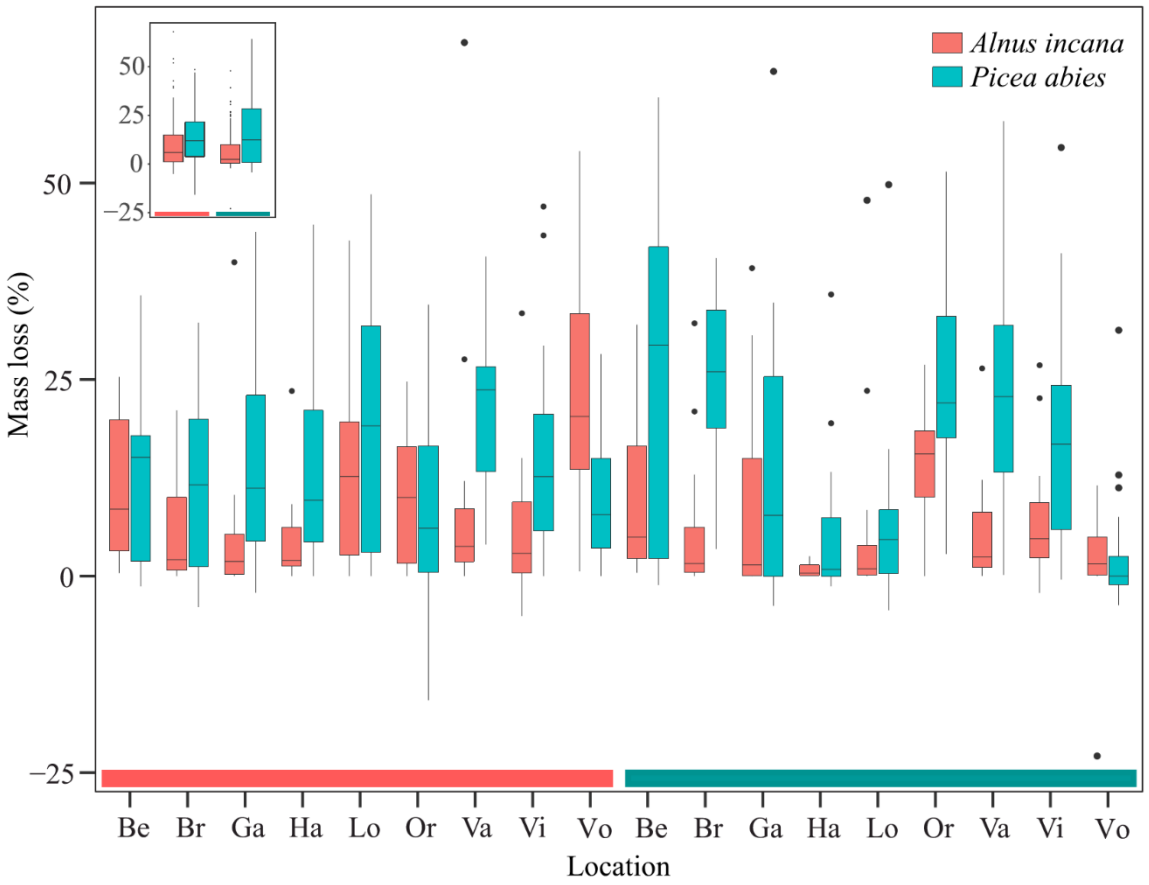

Figure 3: Box plot summarizing the mass loss of *Picea abies* (blue) and *Alnus incana* (orange) wood chips after wood decay by *Fomitopsis pinicola* from nine locations. The orange bar at the base of the plot indicates strains collected on *Alnus incana*, while the blue bar indicates strains collected on *Picea abies*. Horizontal lines represent the median. Each box represents the 25th and 75th percentile, with whiskers 1.5 times the interquartile range above 75th percentile and below 25th percentile respectively. The black dots represent data falling outside the interquartile range. The small panel at upper left corner indicates mean mass loss for both substrates combined for all locations. Statistical differences between the different categories were evaluated using an ANOVA test with p-value < 0.05.

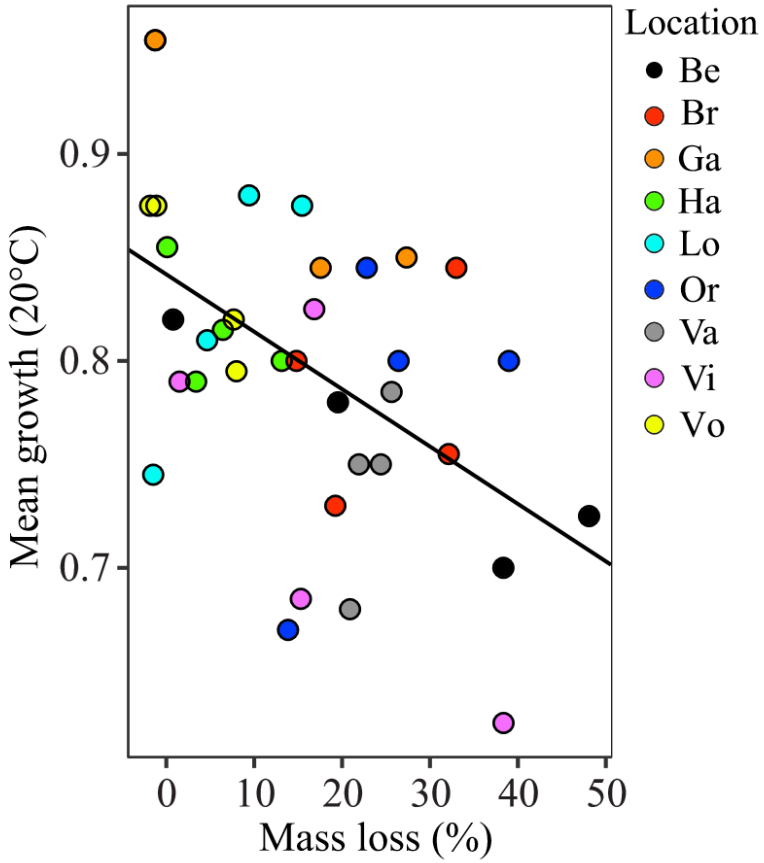

Figure 4: Biplot indicating the mass loss (x-axis) versus growth (mm/day) rates (y-axis) for 36 isolates isolated from *Picea abies*. Each dot represents average mass loss/growth rate of four replicates. Each location is indicated by a different color, nine in total. Mass loss (%) of 144 chips of *Picea abies* was estimated after seven weeks of decomposition at 20 °C. The regression line (black) illustrates the negative correlation between x and y, with a correlation coefficient of 0.5.

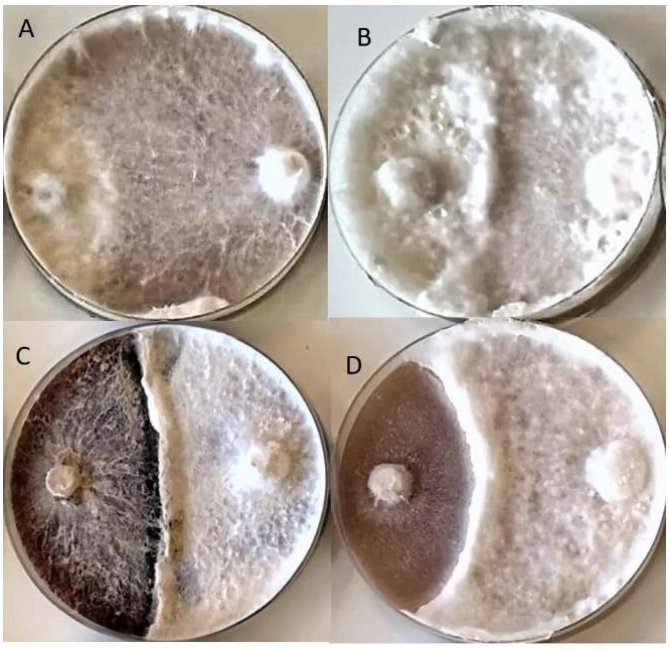

Figure 5: Combative experiments in Petri dishes (9 cm) after 14 weeks of growth when *Fomitopsis pinicola* was paired with four other polypore fungi. In A and B, *Fomitopsis pinicola* has overgrown the cultures of *Antrodiella citronella* (A) and *Phellopilus nigrolimitatus* (B). C and D shows deadlock between *Fomitopsis pinicola* and *Pycnoporellus fulgens* (C) and *Fomitosis rosea* (D). *Fomitopsis pinicola* is growing on the right side of each plate in these images.

4 Discussion

In this study, the adaptive ability of the wood-decay fungus *Fomitopsis pinicola* collected at nine localities with a variation of climatic conditions was investigated. A diversity of experiments was set-up in order to understand which factors affect phenotypic variation in this species. Different life history traits and characteristics were evaluated, including differences in temperature and moisture tolerance, the ability to decay two different substrate types, and finally, the combative ability of the populations.

4.1 Local adaptation to different temperatures

The growth of *F. pinicola* at different temperature conditions varied significantly between locations, suggesting local adaptation to different environmental conditions. Mean annual precipitation, which varied from 733 mm to 2805 mm in the study area, significantly explained parts of the observed variation in growth rates between temperature conditions. The difference in growth rates between 20 °C and 34 °C was negatively correlated with mean annual precipitation. This result indicates less responsiveness to rise in temperature among isolates from locations with high annual precipitation levels. Similar patterns have been observed in other fungi. For instance, in the barley pathogen (*Rhynchosporium commune*), selection for high growth rate is suggested weaker in populations with stable climate, while rapid growth is favored by selection to compensate for shorter growth periods governed by climate fluctuations (Stefansson et al. 2013). Given that annual mean precipitation is an order of magnitude more variable than temperature in the study area, isolates from locations with high annual precipitation levels e.g. Voss and Brekke, can be argued less dependent on rapid growth, as these locations in general offers longer periods with stable moisture levels above what is needed for continuous growth. On the contrary, isolates from locations with low annual precipitation levels e.g. Valdres and Haltdalen, may benefit from increased responsiveness to temperature as they may experience less time where both temperature and moisture conditions enables growth.

Interestingly, populations of *R. commune* populations from habitats with high climatic variability is observed to have less genetic variation than populations from more stable climatic habitats (Stefansson et al. 2013). The same trend has been found in common garden experiments with insects, *Callosobruchus maculatus* (Hallsson and Björklund 2012)

and *Drosophila melanogaster* (Pélabon et al. 2010). Thus, isolates from locations with short growth periods (corresponding to locations with low annual precipitation in my study), might have been under stronger directional selection for more rapid growth, than isolates from locations with longer growing seasons (locations with high annual precipitation). Whether genetic variability among *F. pinicola* populations correlates with climatic variability is therefore an interesting research perspective to investigate further. Given the vast difference between seasons in the study area, and that only two factors (annual temperature and precipitation) was accounted for in this study, further research addressing other climate factors, or the interactions between them, may also provide further insight into how growth relates to temperature and precipitation. However, my results support the hypothesis that climatic variation in the study area has led to local adaptations in growth rates in *F. pinicola* (H1).

4.2 Local adaptation to different moisture levels

The difference in growth at -3.5 MPa and -1.5 MPa were not significant between locations. Stress-tolerance to different moisture levels is dependent on various life-history traits. Under highly favorable conditions, characterized by e.g. warm and rainy seasons, genotypes with low stress tolerance but rapid growth enhancing highly competitive abilities, may be selected for. In more stressful conditions, selection might rather favor dense hyphal growth, enhancing stress tolerance at the expense of competitive abilities (Maynard et al. 2019).

Stress-tolerance is historically known to negatively correlate with growth rate in plants, such as described in Grimes C-S-R triangle (Grime 1977). Several studies, including Grime himself have suggested that fungal life strategies are comparable to plants (Rogers 1989, Maynard et al. 2019, Grime 1977). One study found that fungal species with high optimum temperature and rapid growth generally show a narrow moisture-niche width i.e. range of moisture levels, exhibiting ≥ 50% of their maximum growth rate (Maynard et al. 2019). They found that a moisture niche width, where 50% of maximum growth rate was obtained within the range of -0.5 MPa to -2.0 MPa is considered wide, while a narrow moisture niche is between -0.5 MPa and -1.0 MPa (Maynard et al. 2019). Further, they found that optimum temperature around 28 °C with a daily growth rate around 0.7 mm/day was typical for most of the 22 saprotrophic fungi species sampled from a broad geographical range of environments (Maynard et al. 2019). Given that *F. pinicola* demonstrates an approximate

moisture niche ranging between -0.5 and -1 MPa, *F. pinicola* can be characterized as fungi with a relative narrow moisture niche width. Further, our observation of 1 mm growth per day at 34 °C, supported by an optimum growth temperature around 29 °C (Marković et al. 2011), *F. pinicola* is arguably a species with relative rapid growth at a relatively high optimum temperature. In Grimes C-S-R triangle, this means that *F. pinicola,* can be placed toward the R-selected (Ruderal strategy). Consequently, evolutionary constraints may explain why stress tolerance across different moisture levels did not vary between the locations, as the values tested may have been outside the moisture niche width, and hence too extreme to reveal local differences. The moisture width tested in this study were more appropriate to reveal adaptive divergence for fungi closer to the S-selected end of Grimes C-S-R triangle. To reveal local adaptation to varying moisture levels, I suggest that the moisture niche width of the given fungi is first defined before conducting the experiment. Even though no difference between isolates collected at different localities were observed in the moisture experiments, growth at different temperatures was dependent on precipitation. Thus, some dependency on moisture levels was observed, and if grown at different temperatures as well as different moisture levels, one might see a different result.

4.3 Adaptation to effective decay of either conifer or deciduous woody substrates

When comparing the decay efficiency of *F. pinicola* growing on *A. incana* and *P. abies* in respect to which wood species the strains originated from, I found that decay is apparently affected by substrate origin. This finding is in line with previous studies showing that *F. pinicola* strains sampled from deciduous and conifer hosts exhibit variation in both growth and wood decay rate (Dresch et al. 2015). *Alnus incana* wood chips showed 39% more mass loss when decomposed by isolates collected on *A. incana* than isolates collected on *P. abies*. In opposite, isolates from *P. abies* demonstrated 85% more mass loss of *P. abies* wood chips than *A. incana* wood chips. Unfortunately, the experimental set-up lead to non-independent mass loss measurements, thus the significance toward local differences could not be calculated. However, it is highly unlikely that these clear patterns occurred at random. Hence, these results indicate that effective decay, of either conifer or deciduous wood substrates, is an adapted trait. Thus, this result supports H2, that *F. pinicola* adapts to different substrates.

Since the obtained data did not meet the criteria for statistical analysis addressing local differences, I suggest a different approach for future experiments, using one wood chip per Petri dish, instead of eight wood chips, is more likely to generate independent data. By doing so, local climate can be implemented as a factor, enabling testing of local differences in wood decay efficiency. Moreover, decay rate of *F. pinicola* is previously shown to vary between temperatures, and humidity levels (Venugopal et al. 2016). Also, optimal temperature for growth is shown to differ when *F. pinicola* originated from *P. abies* are being compared with *A. incana* (Dresch et al. 2015). Hence, the results may have provided further insight if decay was measured at several temperatures and water potentials.

Quality of wood has been shown significant in terms of decay rate (Venugopal et al. 2016). In one study, decomposition from *F. pinicola* was shown significantly affected by the quality of the wood, defined as the number of annual rings per cm^3. Hence, a fast-growing *P. abies*, may decompose faster than a slow growing *A. incana* and vice versa. Based on field observations, I expected that *A. incana* would decompose at a faster rate than *P. abies*. Surprisingly, the opposite was observed. Given that the wood chips from *P. abies* used in the decay experiment had considerably fewer annual rings per cm, than the chips from *A. incana*, the wood used in this study represented rather different growth rates. Hence, the wood quality used in this study was different, which may influence the results. A noteworthy difference, as global warming is likely to induce faster growth of trees, lowering the wood quality, which may in turn lead to faster decomposition (Venugopal et al. 2016). Hence, further research regarding substrate preferences in *F. pinicola* can potentially provide valuable insight to how climate change affects the global carbon cycle through altered decomposition rates. The results from my study, as well as observations made by others (Dresch et al. 2015, Venugopal et al. 2016), suggest that *F. pinicola* is likely to thrive at higher temperatures, and might be capable to outcompete other wood-decay fungi, as other temperate wood-decay fungi often are less competitive than *F. pinicola* (Venugopal et al. 2016). Thus, if global warming proceeds as predicted, general wood quality may decrease, and consequently *F. pinicola* can be even more widespread.

4.4 Trade-off between vegetative growth and substrate decomposition

A negative correlation between growth and decay, indicative of an underlying trade-off between vegetative growth and substrate decomposition was observed. Although the data quality obtained for the decay experiment did not meet the requirements (non-independence) for revealing significance in local differences, the pattern observed may still provide insight to evaluate whether *F. pinicola* is trading-off between vegetative growth and substrate decomposition.

At the genetic level, functional genes regulating fungal decomposition (specifically breakdown of cellulose and lignin) is shown negatively associated with genes promoting stress tolerance (Treseder and Lennon 2015). This is further supported by comparing life history trade-offs between 34 saprotrophic fungi species across North America (Lustenhouwer et al. 2020). The authors found that decomposition rates strongly align with dominance-tolerance, supporting that life-history strategies of fungi fit Grimes C-S-R theory, originally developed for plants (Grime 1977). Simplified: R-selected fungi grow rapidly while exploiting available sugars, and as the supply of soluble carbohydrate declines, mycelial growth cease while initiating sporocarp production (Grime 1977). C-selected fungi are evolved to live extreme long lives by cover large areas (Grime 1977), such as *Armillaria mellea* that produces rhizomorphs that may spread from infected trees over to healthy ones (Rishbeth 1968). S-selected fungi are characterized by slow growth and decay rate, but stress-resistant evolved to remain over long-periods toward the terminal stages of fungal succession on decaying matter (Grime 1977). There can thus be a positive correlation between growth and decay in R-selected fungi, and a negative correlation between growth and decay, paid for by higher stress-tolerance, in S-selected fungi. Considering the R-selected traits demonstrated by *F. pinicola,* and a previous description of *F. pinicola* as a relatively rapid wood decomposer (Venugopal et al. 2016), one should expect *F. pinicola* to demonstrate a positive correlation between growth and decay. Yet, a negative correlation was observed. Hence, the negative correlation between growth and decay observed in *F. pinicola* contradict its general life history traits but confirm the hypothesis that: *F. pinicola* is experiencing a trade-off between vegetative growth and substrate decomposition (H3).

4.5 Local adaptation towards combative abilities

When comparing the combative abilities of *F. pinicola* from different locations towards four competing polypore species, no difference among locations were observed after 14 weeks of confrontation. Combative ability of nine white rot fungal species was recently shown to be affected by both substrate size and on the successional stage they naturally occur (Fukasawa et al. 2020). In addition, wood decay was slower in the stressful conditions close to the combative zone (Fukasawa et al. 2020). Hence, competition under other conditions, as confrontations on woody substrate or stressed by temperature or water potential, could have improved the evaluation of the competitive ability of *F. pinicola*. Thus, further research is needed before confirming or rejecting whether local climate in the study area affect the competitive ability of *F. pinicola* (H4).

4.6 Local adaptation or non-genetic effects?

Local adaptation is about genetic differentiation. Hence, differences observed in a common garden experiment should be interpreted with caution, as differences, may be due to non-genetic effects such as plasticity and maternal effects (Kawecki and Ebert 2004). Further, it should be noted that environmental heterogeneity often favors the evolution of adaptive phenotypic plasticity (Baythavong 2011). In absence of costs and constraints on plasticity, a genotype that in each habitat produces the locally optimal phenotype can become fixed (Baythavong 2011). A fixation of which, leads to adaptive phenotypic differentiation, without underlying genetic differentiation (Stinchcombe and Hoekstra 2008). Given the wide distribution of *F. pinicola*, across heterogeneous environmental conditions in Norway, adaptive phenotypic differentiation does not seem entirely unlikely. In several species, higher genetic variation has been observed among populations residing under stable climatic conditions, as compared to populations where the climate varies extensively (Pélabon et al. 2010, Stefansson et al. 2013, Hallsson and Björklund 2012). A logic next step would thus be to analyse genome-wide genetic variation across populations from different climate and link the observed phenotypic differences to underlying genetic polymorphisms. A comparison arguably justified, as the results presented in this study, strongly indicate climatic variability and habitat (substrate) requirements to affect evolution of local adaptation in *F. pinicola.*

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