University of South-Eastern Norway

Faculty of Technology, Natural Sciences and Maritime Sciences

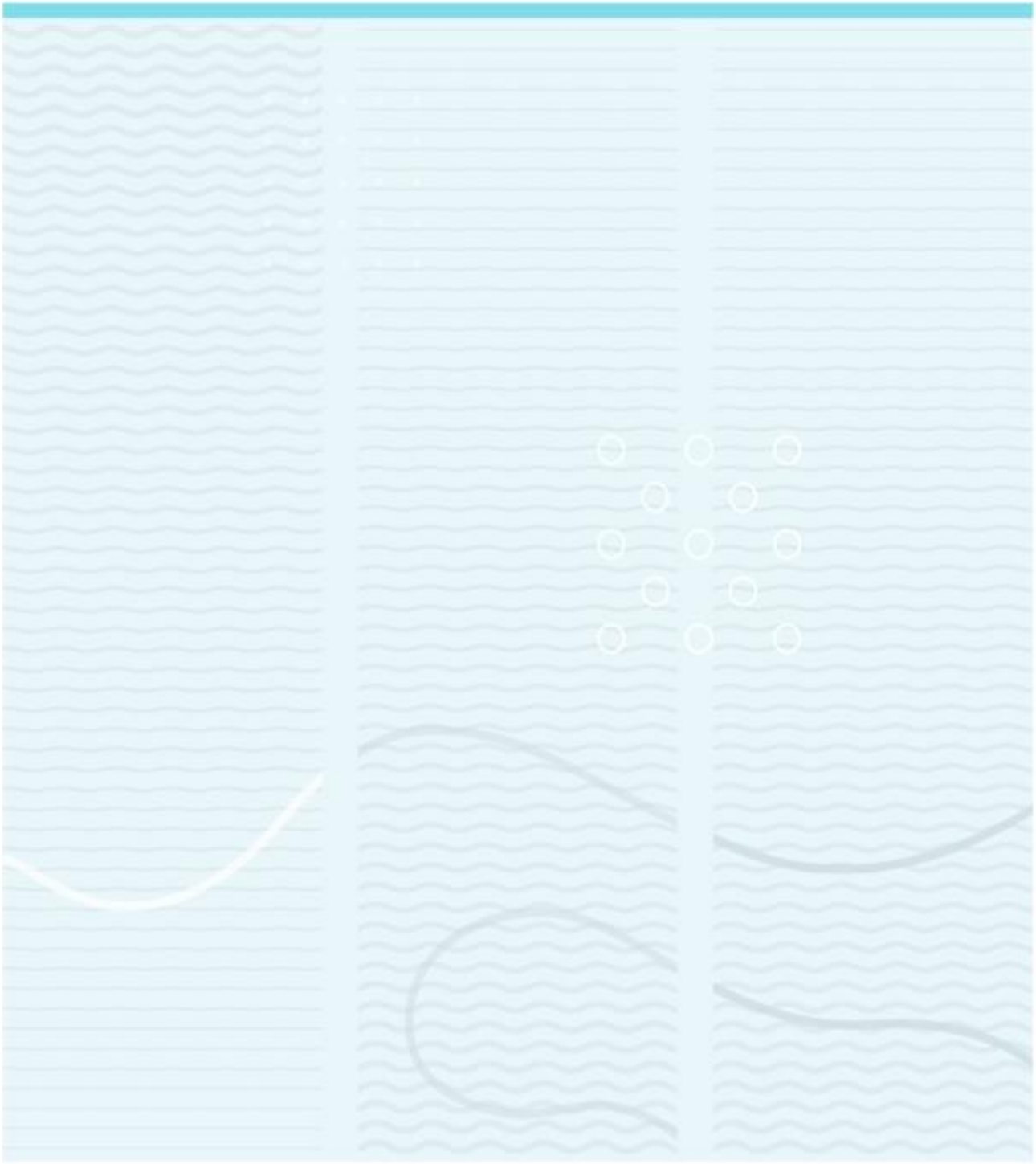
Master's Thesis

Study programme: Ecology and Environmental Management

Autumn 2022-Spring 2024

Rachel Carboni

Population Genomics of *Fomitopsis pinicola* in Norway



University of South-Eastern Norway
Faculty of Faculty of Technology, Natural Sciences and Maritime Sciences
Department of Natural Sciences and Environmental Health
PO Box 235
NO-3603 Kongsberg, Norway

http://www.usn.no

This thesis is worth 60 study points

Abstract

Understanding the genetic variability and population dynamics of fungal species is essential for unraveling their evolutionary history and adaptive potential. This study investigates the genomic structure of *Fomitopsis pinicola* populations across western and eastern Norway to determine the extent of reproductive isolation and gene flow, identify genomic regions potentially important for adaptation, as well as explore potential introduction pathways after the last glaciation period. Double Digest Restriction-Site Associated DNA sequencing (ddRADseq) and bioinformatics were used to identify genomic variability among the samples, including single nucleotide polymorphisms (SNPs), followed by comparative genomic analyses including fixation index matrix (F_{ST}) and principal component analysis (PCA). The results revealed an overall shallow population structure indicating high levels of gene flow consistent with previous studies of saprophytic fungi in Fennoscandia. However, subtle genetic differences among certain populations suggest areas of reduced gene flow and potential reproductive isolation. Ancestry analyses aligned with the post-glacial history of the main host species, *Picea abies*, highlighting its importance and influence on *F. pinicola* population structure. Redundancy analysis (RDA) revealed possible adaptations to local climates, especially temperature and precipitation. These findings corroborate the role of environmental factors in shaping fungal population genetics. Additionally, Gene Ontology (GO) analysis identified alleles potentially important for local adaptation. Further research utilizing whole-genome sequencing could potentially identify additional genes under selection, while investigations into specific environmental stressors may reveal targeted adaptations to extreme climatic variables. Overall, this study advances our understanding of fungal population genetics, especially in relation to climatic variables, and underscores the importance of considering genomic variation in conservation and population management strategies.

Key Words: *Fomitopsis pinicola*, local adaptation, population genomics, ddRADseq, adaptive divergence, gene flow, genotype-environment association, evolutionary history

Table of Contents

Forward

I would like to thank Håvard Kauserud and Sundy Maurice of the Oslo Mycology Group, as well as Cecilie Mathiesen at the University of Oslo for providing me with the cultured samples that were used in this study. I must also express my deepest gratitude to my advisor, Jørn Henrik Sønstebø, whose unwavering support and guidance have been invaluable throughout this thesis. Jørn Henrik's patience and kindness in answering my questions, no matter how simple or complex, created a nurturing environment that felt collaborative. His optimistic outlook in the lab was a constant source of encouragement and his calm demeanor and positive attitude were crucial for my overall sanity.

I would also like to acknowledge the contributions of the Molecular Ecology Research Group. The biweekly meetings created a sense of community and provided technical support and advice that was instrumental during the writing process. Thank you to Melanie Schreier for your help in the lab and for clearly explaining dilutions, and to Roland Pape for your help with fixing the projections in QGIS that caused me hours of trouble and you were able to fix in minutes. Lastly, I would like to thank the unconditional love and support provided by my friends and family, both near and far, as I embarked on this academic journey. I love you more than words can convey.

On a more practical note, this thesis assumes some knowledge of genomics and molecular ecology. Additional tables and figures are included in the Supplementary Information section, appended at the end of the thesis.

13.5.2024

Rachel Carboni

1 Introduction

In the realm of genomics, investigating the genetic basis of adaptation by studying population genomics and genotype-environment associations can provide indispensable insights into how organisms navigate the challenges posed by rapidly changing environmental landscapes and climate. Adaptive evolution, or adaptive divergence, refers to the development of new life forms from a common ancestor as a result of adaptations to environmental factors (Tusso et al., 2021). Adaptive divergence is a fundamental concept in evolutionary biology as it helps us understand how species evolve and adapt to different environments over time, contributing to biodiversity by generating new species and ecological niches. Deciphering how and why species evolve and diverge can inform conservation efforts and help protect and manage biodiversity more effectively. Including an evolutionary perspective in species distribution models can enhance projections by accounting for factors like dispersal, genetic adaptation, and population dynamics (Waldvogel et al., 2020). Fungi are optimal for studying adaptive evolution as they inhabit diverse habitats, have short generation times, and relatively small genomes. While many studies have been done on population genomics and allopatric isolation, more research focused on demographic history, responses to environmental factors, and adaptive evolution is needed (Zhang et al., 2022).

Local adaptation is the result of different environments exerting divergent selective pressures, resulting in variation in allele frequencies at genetic loci important for adaptation (Tigano & Friesen, 2016). Local adaptation is dependent on gene flow and local selection. Low gene flow can bolster local adaptation by allowing certain genotypes to be maintained, resulting in variation of allele frequencies. However, if there is high gene flow between populations, gene swamping can occur, which is when the increased number of introduced alleles dilute the adaptive genotypes. Genetic drift, a stochastic process wherein allele frequencies fluctuate randomly over generations, can diminish local adaptation by reducing genetic variation. Genetic drift, which is particularly strong in small populations, may inadvertently increase maladapted genetic variants due to its random nature (Blanquart et al., 2013). Understanding the interplay between selection, gene flow and genetic drift in influencing genetic diversity is crucial in population and conservation genetics with the assumption that this variation indicates adaptive potential (Cortázar-Chinarro et al., 2017; Waldvogel et al., 2020).

Large-scale genomic studies can enhance our comprehension of how species adapt and diversify. Comparing the genomes of populations within a species can identify any variation, broadening awareness of gene-specific and genome-wide effects of selection and shedding light on processes behind local adaptation (Gladieux et al., 2014). This process of isolating and identifying relevant genes related to selection or divergent adaptation between populations from next-generation sequencing has been termed "reverse-ecology" (Ellison et al., 2011) and has been used to improve predictions of how species will respond to global climate change and habitat fragmentation (Branco et al., 2017; Tusso et al., 2021). Understanding the basis of local adaptation is particularly important when planning restoration and reintroduction efforts because environmental stressors will affect individuals differently (Flanagan et al., 2018).

Fungi found across wide geographic regions often display significant genetic diversity. This diversity arises from factors such as geographic isolation, limited migration, and genetic drift among populations. This genetic variation may not always manifest visibly in observable phenotypic traits (Haight et al., 2016). *Fomitopsis pinicola* is a saprotrophic polypore found throughout the northern hemisphere. It obtains its nutrients by breaking down dead or decaying wood thereby aiding in the decomposition of fallen trees and woody debris in forest ecosystems. This decomposition process helps recycle nutrients back into the soil, making them available for other organisms and contributing to overall forest health (Abrego, 2022). *F. pinicola* has perennial fruiting bodies and a heterothallic sexual system, requiring different alleles for mating. Högberg et al.(1999) found *F. pinicola* to be highly diverse and adaptable, with little differentiation among populations. This genetic diversity results from the combination of genetic material from unrelated individuals, enhancing the adaptability and resilience of the population at both local and regional scales (Högberg et al., 1999).

Polypores, such as *F. pinicola*, propagate by releasing millions of airborne basidiospores, or unicellular haploid spores (Kauserud et al., 2008). The spores are often released at once and travel predominantly via the wind, but also by animals, water and even insects (Lunde et al., 2023). While it has been shown that some fungi are capable of long-distance spore dispersal, many are limited by barriers such as oceans and mountains (Peay et al., 2010). A study done by Peay et al. (2012), measuring ectomycorrhizal dispersal found that dispersal limitation reduces immigration rates. Norway is intersected by the Scandinavian Mountain range (Ehlers et al., 2011). Populations that are geographically isolated are often also reproductively isolated, encouraging divergent selection and specialization. Understanding how populations adapt to different ecological niches is important,

despite difficulties in determining the amount of gene flow and strength of selection (Tusso et al., 2021). Fragmentation can restrict gene flow and promote genetic drift, which can be observed in populations via changes in allele frequency. This could lead to lower genetic diversity and increased genetic differentiation between populations, as has been observed in certain fungi (Sønstebø et al., 2022).

Genotype-environment association studies are used to identify genetic variants that are associated with specific environmental factors or conditions. These studies analyze the relationship between genetic variations and environmental variables, uncovering how genetic factors contribute to an organism's ability to adapt to different environments (Branco et al., 2017). A study conducted by Branco et al. (2017), revealed two delineated populations of the species *Suillus brevipes*, one from a coastal environment and one an inland, mountainous environment. The populations had very low divergence and showed positive selection. The analysis revealed an adaptive gene to salt tolerance as a result from different abiotic environmental conditions. Another study conducted by Zhang et al. (2022), looked at single nucleotide polymorphisms (SNPs) of the species *Lentinula edodes* and found three distinct subgroups. Each subgroup had a different phenotypic and temperature response correlating to the geographical distribution. The question arises whether there is also genetic differentiation between populations of *F. pinicola* in other regions of the genome previously unstudied. If so, what are the genetic advantages resulting from the different environments?

Host species of *F. pinicola* include both hardwood and softwood species such as the genus *Pinus*, *Picea*, *Betula*, *Alnus*, *Populus*, *Pseudotsuga*, *Larix*, *Acer*, and *Tsuga*, with *Picea abies* being the most common host species in Norway (Haight et al., 2016; Ryvarden & Melo, 2017). Understanding species delimitation in the context of ecological and evolutionary processes emphasizes the role of shared evolutionary history and environmental factors in shaping species boundaries (Stengel et al., 2022). In general, to understand the evolutionary history and reconstruct possible introduction pathways after the last glaciation, this wide range of host species must be accounted for (Westergaard et al., 2019). To further complicate matters, the postglacial history of species migration in Norway is a debated topic that includes several different theories and timelines.

The complete disappearance of the Fenno-Scandinavian ice sheet occurred approximately 10.3 to 9.9 calibrated thousand years before the present (cal ka BP) (Alsos et al., 2022). The maximum extent of the Fenno-Scandinavian ice sheet was not reached simultaneously in Norway, and it is believed there have been several fluctuations of the glacier’s extent during that time (Ehlers et al., 2011; Hewitt, 2004). Pollen analyses and

sedimentary ancient DNA show sparse tundra vegetation around 14,600 years ago, with *Betula* emerging 12 to 10 cal ka BP. A significant botanical transition unfolded around 7.8 cal ka BP with the emergence of *Alnus incana*, marking the final arboreal species to establish in the region. Concurrently, pollen analyses indicate the expansion of *Pinus* and *Betula* forests during this period (Alsos et al., 2022).

Recurrent climatic cycles drove significant population movements in plant species, influencing genetic structures (Li et al., 2022). *Picia abies* has pronounced latitudinal patterns in both genetic and phenotypic variation which indicates a strong association between population structure and local adaptation. Adaptation aligns with environmental factors and historical recolonization routes along these environmental gradients (Tiret et al., 2022). It should be recognized that the present genetic diversity distribution in *P. abies* is the outcome of intricate interplay between historical and recent demographic events. This historical narrative encompasses ancient divergences, recurrent hybridization incidents, significant bottlenecks, and population migrations (Chen et al., 2019).

Theories include *P. abies* migrations from eastern (Sweden), northern (Russia) or southern (Baltics) populations (Li et al., 2022; Nota et al., 2022). Historical fossil pollen records indicate *P. abies* populations in northern Norway have a shallow population structure, possibly due to colonization from a glacial refugium. The colonization path appears to expand to the west and northwest, as the Fenno-Scandinavian ice sheet retreated (Tollefsrud et al., 2008, 2009, 2015). The existence of glacial refugia, small, ice-free areas, has been a much-debated topic in Norway for over 100 years (Ehlers et al., 2011). Westergaard postulated postglacial immigration of an alpine sedge species from North America (Westergaard et al., 2019). Parducci et al. (2012), theorized that there were ice corridors along the west coast of Norway during the Late Weichselian[1]. Haight et al. (2016) also hypothesized that the North American population of *F. pinicola* was older, as it had the greatest genetic diversity and largest divergence. A population genomic analysis will help understand the dispersal history of *F. pinicola* within Norway. Knowledge of historic isolation and migration is important for the prediction of future events and understanding biodiversity dynamics (Gillespie & Roderick, 2014).

The populations structure of *F. pinicola* has been studied for decades. A study done by Högberg et al. (1999), concluded that the gene pool of *F. pinicola* showed little or no differentiation, even over large

[1] Weichselian glaciation refers specifically to the area over Europe and involves the Fenno-Scandinavian ice sheet which once covered Norway (Ehlers et al., 2011).

geographical distances. The study sampled populations from Sweden, Russia, and Lithuania. The study postulated that it was unlikely that spore dispersal could reach hundreds of kilometers, therefore, the low genetic differentiation was due to a continuous linking of populations at both local and regional scales. It is currently believed that *F. pinicola* populations in Europe all belong to one intersterility group (Haight et al., 2016). Using a reduced-representation sequencing method to provide a more in-depth comparison of the genome could reveal any differences that were missed when only comparing a small selection of the genome. Double Digest Restriction-Site Associated DNA sequencing (ddRADseq), a cost-effective genomic analysis method, employs two restriction enzymes to target specific DNA regions by size. This technique generates sequence-specific libraries with barcoded adaptors for sample multiplexing, enabling uniform read coverage across genetic regions (Peterson et al., 2012). By reducing sequencing costs and improving efficiency, ddRADseq facilitates a more comprehensive exploration of the *F. pinicola* genome than the previous studies mentioned.

This study will explore differences in the genomic structure of *F. pinicola*, revealing if and to what extent the populations from western and eastern Norway are reproductively isolated. An understanding of genomic variability between populations will reveal more about the population structure and if there is gene flow between populations. Environmental association studies will allow genomic regions potentially important for adaptation to be identified. Lastly, the results of the analysis will shed light on introduction pathways after the last glaciation period and how this has influenced adaptation and demographic history. This research is important for conservation management efforts, as adaptive genetic variation can inform models of species' genetic evolutionary potential as it relates to climate change and other selective pressures (Rochat et al., 2021; Waldvogel et al., 2020).

2 Materials and Methods

2.1 Study area and Sampling Design

The samples used in this study were collected as part of a study done by Kauserud et al. (2024), in which *F. pinicola* samples were collected from nine geographic populations across Norway that represent different climatic conditions and substrates (Figure 1). At each 200 m^2 location, four sporocarps were collected from dead spruce (*Picea abies*) logs and four from dead alder (*Alnus incana*) logs. Samples were cultured by the University of Oslo and provided on petri dishes and stored at 20° C. Mycelium from each sample was extracted from the agar using a disposable scraping tool, placed in a 1.5ml Eppendorf tube and then stored at -20° C.

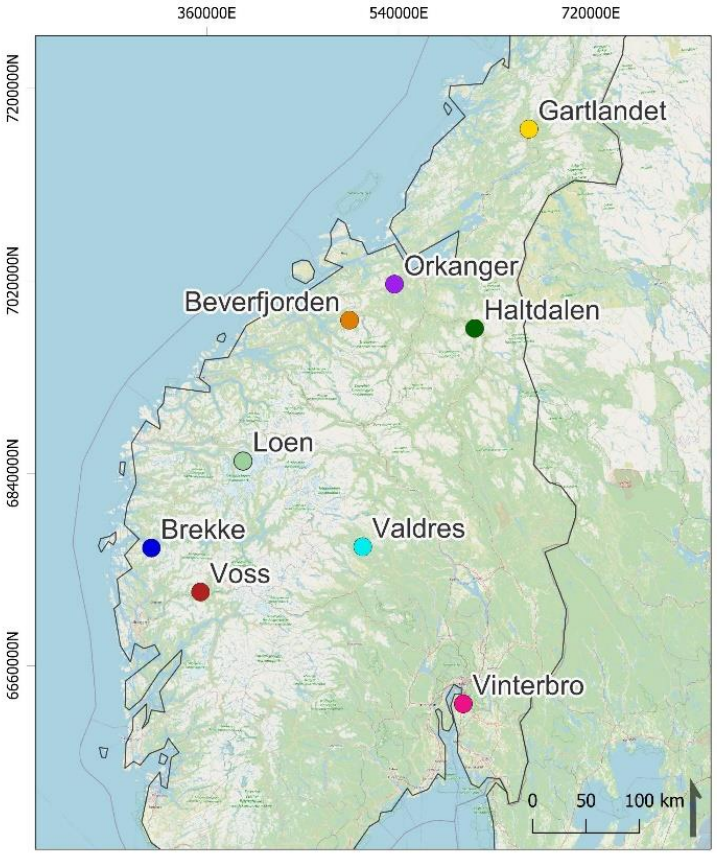


Figure 1 Sampling locations of the studied Fomitopsis pinicola populations collected in Norway. Eight samples were taken from each location, four from Picia abies logs and four from Alnus incana logs. Map created in QGIS v. 3.26.3 (QGIS Development Team, 2022. Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org)

2.2 DNA Extraction

Genomic DNA was extracted using the DNeasy Plant Pro Kit (Qiagen) as per the manufacturer's instructions with the following modifications: To better lyse the cells of the mycelium, the weighed samples were first manually cut using small laboratory scissors inside of the test tube. Small glass beads were used to mechanically disrupt the mycelium, as they are better suited to lyse the mycelium than the single large bead provided with the DNeasy Plant Pro Kit (Qiagen). After the CD1 lysing solution was added, the samples were heated at 65° C for 10 minutes at 500 RPM. Samples were then vortexed at the maximum speed of 2700 RPM for 10 minutes. Also, 100µl of Buffer EB was added in step 16. Samples were stored at -20° C.

2.3 Double Digest Restriction Associated DNA (ddRAD) Sequencing

Samples were sequenced using the ddRAD protocol developed by Vivian-Smith & Sønstebø (2017). Single stranded oligos were annealed into adaptors according to the protocol and working stocks were prepared. A NanoDrop One (Thermo Fisher Scientific, Waltham MA, USA) spectrophotometer was used to quantify genomic DNA and determine 260/280 nm ratios. Samples were prepared and diluted with quantities of 200 ng in 40 µl of nuclease free H_2O. Unidirectional digestion-ligation of the samples was performed according to the protocol with the following exceptions: The master mix used in the digestion-ligation was created using 0.5 µl of 100 U T4 DNA ligase (NEB) instead of 1 µl. The PstI endonuclease was used with the A-adaptor and the MspI endonuclease was used with the P1-adaptor. The P1-adaptor also contains a divergent Y-adaptor with short tags that allows for increased multiplexing. To create a unique barcode for each of the 72 samples, 24 of the forward PstI adapters were used along with three of the reverse MspI adapters. The unidirectional digestion-ligation step was performed with a digestion time of 2 hours.

Barcoded samples were pooled into three portions based on the three reverse adapters. Size selection was performed using the DNA electrophoresis protocol by Thermo Fisher Scientific and the E-Gel™ Power Snap electrophoresis device (Thermo Fisher Scientific). Three sequencing experiments were conducted, and the results of each run combined. For two of the sequence runs, a 400-base-read library size was selected with the target library peak of 480bp. The third sequencing run selected for a 200-base-read library size with the target library peak of 270bp. The size selected library was cleaned using the AMPure XP bead-based reagent

(Beckman Coulter, Inc). The library was amplified to produce enough genetic material and increase the number of fragments with adaptors. The amplification reactions were prepared for PCR using 25 µl DNA from the purified, size selected sample, 100 µl Platinum™ PCR SuperMix High Fidelity (Thermo Fisher Scientific), and 5 µl primer mix. The sample was then distributed between two 1.5ml Eppendorf tubes, each containing 65 µl. Amplification was done using 14-18 cycles, depending on the sequencing experiment.

The amplified ddRAD library was quantified using The Ion Library TaqMan™ Quantitation Kit (Thermo Fisher Scientific). Serial dilutions of the library were prepared according to the protocol using the recommended concentration of 50 pM. The ddRAD libraries were prepared for sequencing using the Ion Chef™ System (Thermo Fisher Scientific). Immediately following the run, the chip was placed in the Ion GeneStudio™ S5 Semiconductor Sequencer (Thermo Fisher Scientific) for sequencing.

2.4 Read Filtering and Variant Calling

The raw reads were demultiplexed using Cutadapt v.4.6 (Martin, 2011) with a maximum error rate of 1 error for every adapter. This process creates a separate file for each individual sample. The reads were then mapped to the NCBI reference genome (https://www.ncbi.nlm.nih.gov/nuccore/MPVS00000000.1) for *F. pinicola* using Bowtie2 v.2.5.3 (Langmead & Salzberg, 2012). An index file was created from the reference genome which allows Bowtie2 to efficiently and quickly search for matches between reads from the sequencing data and the reference genome (Langmead & Salzberg, 2012). Mapped reads were saved in sequence alignment map (SAM) format with corresponding log files. SAMtools v.1.20 (Danecek et al., 2021) was used to sort the files and save them as compressed binary alignment map (BAM) files. Variants were called using the genomic variant call format (GVCF) workflow in the Genome Analysis Tool Kit, GATK v.4.5.0.0 (Poplin et al., 2018). The GVCF records information for all sites, even if there is not a variant call located there. This allows for an accurate estimation of the confidence in whether the sites are homozygous, and this estimate is generated by the HaplotypeCaller's built-in reference model. Low quality variants were removed with GATK using the following command: QD<2.0||FS>60.0||MQ<40.0||MQRankSum< -12.5||ReadPosRankSum<-8.0" --filter-name "expression_fail". Further filtering of variants and individuals was performed with VCFtools v0.1.16 (Danecek et al., 2011) to remove individual samples with high level of missing data (>70%) and SNPs with a minimum

allele count greater than 2 using the "–mac" function. During this process, any genetic variation such as SNPs or insertions or deletions are identified in each individual sample.

2.5 Genetic Diversity and Divergence

Summary statistics, such as the expected and observed heterozygosity and fixation index matrix (F_{ST}), were calculated for each location using the R (R Core Team, 2022) package PopGenReport v.3.1 (Adamack & Gruber, 2014). Nucleotide diversity (π) is used to measure the degree of polymorphism within a population, with higher values suggesting greater genetic variability. The method introduced by Nei and Li was used (Nei & Li, 1979). Tajima's D compares the number of nucleotide differences between pairs of sequences and the number of segregating sites. The results indicate if the observed genetic variation in a population is more or less than what would be expected under a neutral model of evolution. A value of 0 indicates the observed variation the same as expected, with no evidence of selection. Positive values suggest a lack of rare genetic variants compared to what would be expected, possibly indicating balancing selection or a genetically stable population. Negative values suggest an excess of rare alleles and can indicate a recent selective sweep, population expansion after a recent bottleneck, or balancing selection in which one allele is favored thus reducing the genetic diversity (Tajima, 1989). Nucleotide diversity and Tajima's D were calculated using the R package PopGenome v.2.7.7 (Pfeifer et al., 2014). PopGenome was also used to plot the nucleotide diversity values for each population using a locally weighted scatterplot smoothing (LOESS) function to better show the relationship between populations. Tajima's D and nucleotide diversity were calculated within 10kb windows, with a step size of 7kb.

To assess population structure between the nine study locations, a principal component analysis (PCA) of nuclear genome variation was performed, as well as pairwise F_{ST} and an analysis of genetic admixture. The PCA was conducted using the R package SNPRelate v.1.36.1 (Zheng et al., 2012). The pairwise F_{ST} estimates the genetic differentiation between each location and was calculated using a custom script in R (Supplementary Figure S2) using the packages vcfR v.1.15.0 (Knaus & Grünwald, 2017), stringr v.1.5.1 (Wickham, 2023), and KRIS v.1.1.6 (Chaichoompu, 2018).

The F_{ST} was converted into a genetic distance matrix using ($F_{ST}/(1-F_{ST})$), and a heat map was visualized using the R package lattice v.0.22-5 (Sarkar, 2008). The ancestry for each sample was estimated using Admixture v.1.3.0 (Alexander & Lange, 2011). Cross validation error tests were calculated for the number of

ancestral populations (K) ranging from 1-10 (Supplementary Figure S4). Results from this analysis were visualized using R and QGIS v. 3.26.3 (QGIS Development Team, 2022. Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org).

2.6 Genotype-Environment Associations

A Redundancy Analysis (RDA) was performed to correlate the SNP dataset to environmental variables. The environmental metadata was downloaded from Worldclim v.2.1 (http://www.worldclim.org) (Fick & Hijmans, 2017) and includes 19 standard bioclimatic variables downloaded at a resolution of 30 seconds. The Point Sampling Tool in QGIS (QGIS Development Team, 2022) was used to extract the Worldclim data from each of the nine sampling locations. To investigate and reduce correlation among the climatic variables, the R package psych v.2.4.1 (Revelle, 2024) was used. All variables with a Spearman's correlation coefficient ($|\rho|$) >0.7 were excluded (Supplementary Figure S5), resulting in a selection of four climatic variables in the RDA analysis: annual mean temperature (BIO1), isothermality (BIO3), mean temperature of wettest quarter (BIO8), and annual precipitation (BIO12) (Supplementary Table S3). This method allows any outlier loci that exhibit a strong association to environmental conditions to be easily identified.

By constraining the analysis to environmental factors, RDA facilitates the detection of genetic outliers. These outliers are more likely to have undergone selection pressure, distinguishing them from loci primarily affected by other evolutionary mechanisms, like genetic drift and gene flow. This method has demonstrated a low incidence of false-positive signals of selection in populations with a shallow structure (Forester et al., 2018). The RDA was preformed using the R package Vegan R v.2.6-4 (Oksanen et al., 2022). An analysis of variance (ANOVA) on the fitted RDA model was done to test the significance of each environmental variable. Multicollinearity was also tested using the square root of the variance inflation factors (VIF). The resulting data frame of 126 candidate SNPs and their correlation with our four environmental predictors was downloaded to be used for further analysis. A correlation matrix was conducted using the R package Hmisc v.5.1-2 (Harrell Jr., 2023) to investigate the dependence between all 19 bioclimatic environmental variables. The result is a table containing the spearman correlation coefficients between each variable. The R package corrplot v.0.92 (Wei et al., 2021) was used to visualize the matrix (Supplementary Figure S8).

2.7 Annotation of Loci Under Selection

The SNPs of interest from the RDA analysis used the NCBI GenBank assembly, these contigs were matched to the JGI genome (https://mycocosm.jgi.doe.gov/Fompi3/Fompi3.home.html) using a custom BLAST program. The JGI scaffolds were then mapped to a list of known protein ID's and gene ontology (GO) terms downloaded from the JGI gene catalog using the Intersect tool from Bedtools v.2.31.0 (Quinlan & Hall, 2010). The R package topGO v.2.50.0 (Alexa & Rahnenführer, 2023) was used to perform a Gene Ontology (GO) analysis, a method used to interpret large sets of genes or proteins in terms of their associated biological processes, molecular functions, and cellular components (Alexa & Rahnenführer, 2023). A Fisher's exact test as well as a weighted algorithm was used to determine the significance of each GO term. The analysis reveals which biological processes or molecular functions are overrepresented or enriched compared to what would be expected by chance. The R package Rgraphviz v.4.3 (Hansen et al., 2023) was used to plot a subgraph of the most significant GO terms.

3 Results

3.1 Genetic Diversity

After read filtering and variant calling, 10,964 sites were identified as SNPs or as having genetic variation. The nucleotide diversity of each population ranged from 0.829 (Loen) to 1.219 (Orkanger). Mean expected heterozygosity (H_e) was 0.114 and ranged from 0.088 (Loen) to 0.132 (Voss). It should be noted that the sequencing reads from Loen were of low quality and could be the cause for low nucleotide diversity and H_e. The mean Tajima's D was 0.308 and ranged from -0.241(Vinterbro) to 0.597 (Valdres) (Supplementary Table S2). The nucleotide diversity values for each population, plotted using a locally weighted scatterplot smoothing (LOESS) function, reveal that the populations are relatively similar, although slight differences in nucleotide diversity indicate there may be factors contributing to genetic differentiation (Figure 2). Expected heterozygosity was lower than observed for all populations (Supplementary Figure S1).

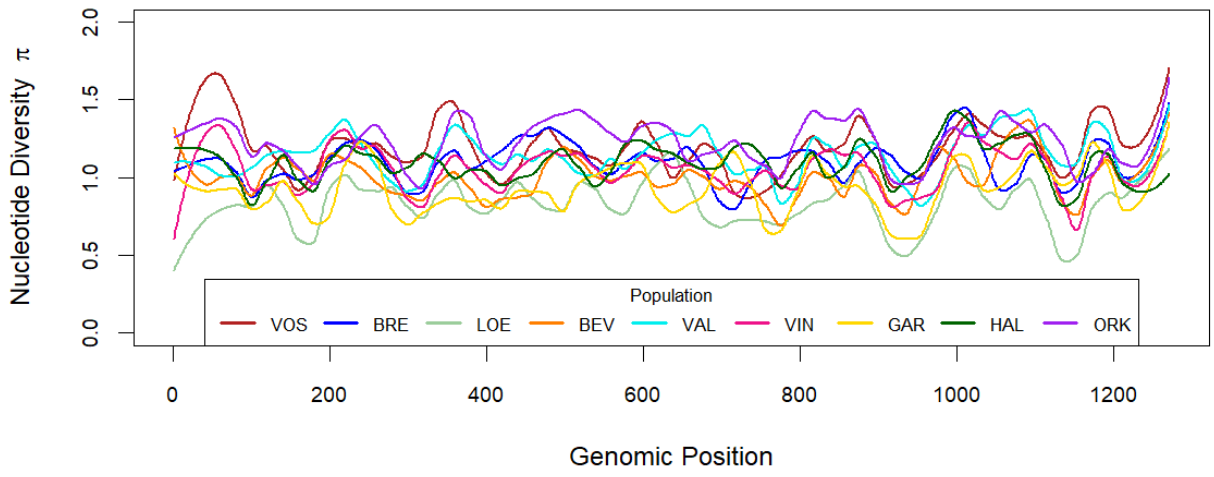


Figure 2 Nucleotide diversity of each of the populations using locally weighted scatterplot smoothing (LOESS) function across 10kb windows, with a step size of 7kb.

3.2 Genetic Divergence

The SNP-based PCA analysis calculated 32 principal components from the original 10,964 genetic variants. The first three axes explain 7.1%, 4.9% and 4.1% of the variance, respectively. The analysis clustered most samples together, while a few samples from Brekke, Voss, Haltdalen and Valdres populations are more distant (Figure 3). The PCA results were supported by the pairwise F_{ST} estimates with Brekke, Voss, Haltdalen

and Valdres populations showing moderately high values (Figure 4). Additionally, Loen had relatively high F_{ST} values which could be due to poor quality of the DNA, consequently resulting in a low number of reads.

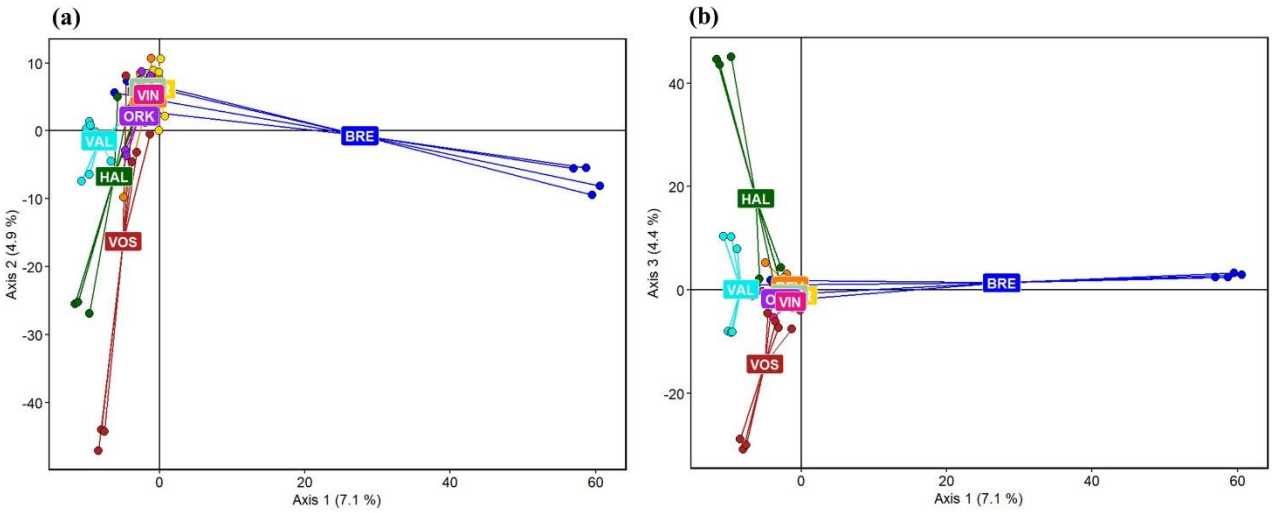


Figure 3 Principal component analysis (PCA) of the genomic variation of sampled Fomitopsis pinicola populations. The first two principal components (Axis 1 and 2) with percentage of variance explained (a), Axis 1 and 3 are shown along with percentage of variation explained (b).

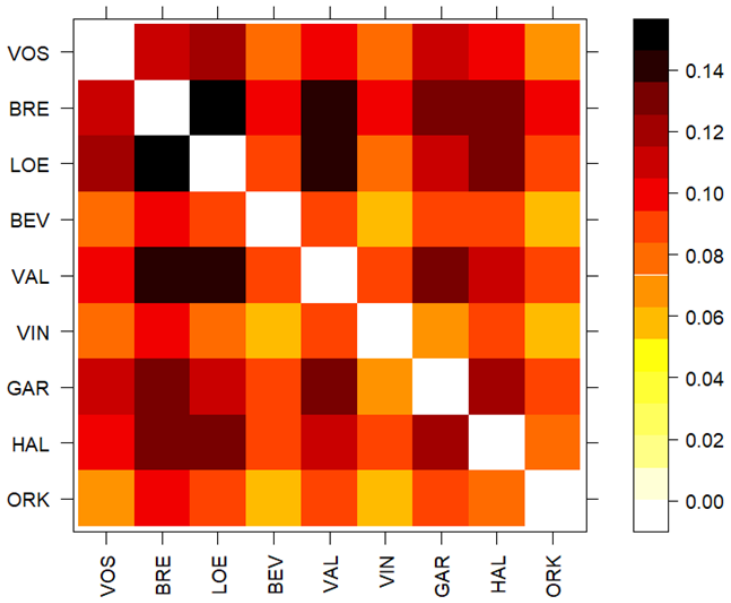


Figure 4 Heat map of pairwise $F_{ST}/(1-F_{ST})$ values between populations of Fomitopsis pinicola. Dark color with high F_{ST} values indicates a higher level of genetic differentiation.

An analysis of the population structure of *F. pinicola* using Admixture revealed the lowest cross-validation error was obtained for K = 1 (CV =0.447), suggesting a very shallow population structure. The cross-validation error rates for the other ancestry coefficients are as follows; K=2 (CV=0.462), K=3 (CV=0.4798), and K=4 (CV= 0.4872). For K=2, Voss and Valdres appear to be genetically similar with admixture of the Beverfjorden, Orkanger and Haltdalen populations. (Figure 5a). For K=3, Haltdalen and Valdres are modeled to show ancestral similarities. At K=4, Brekke, Voss and Valdres appear to have unique ancestries (Figure 5b).

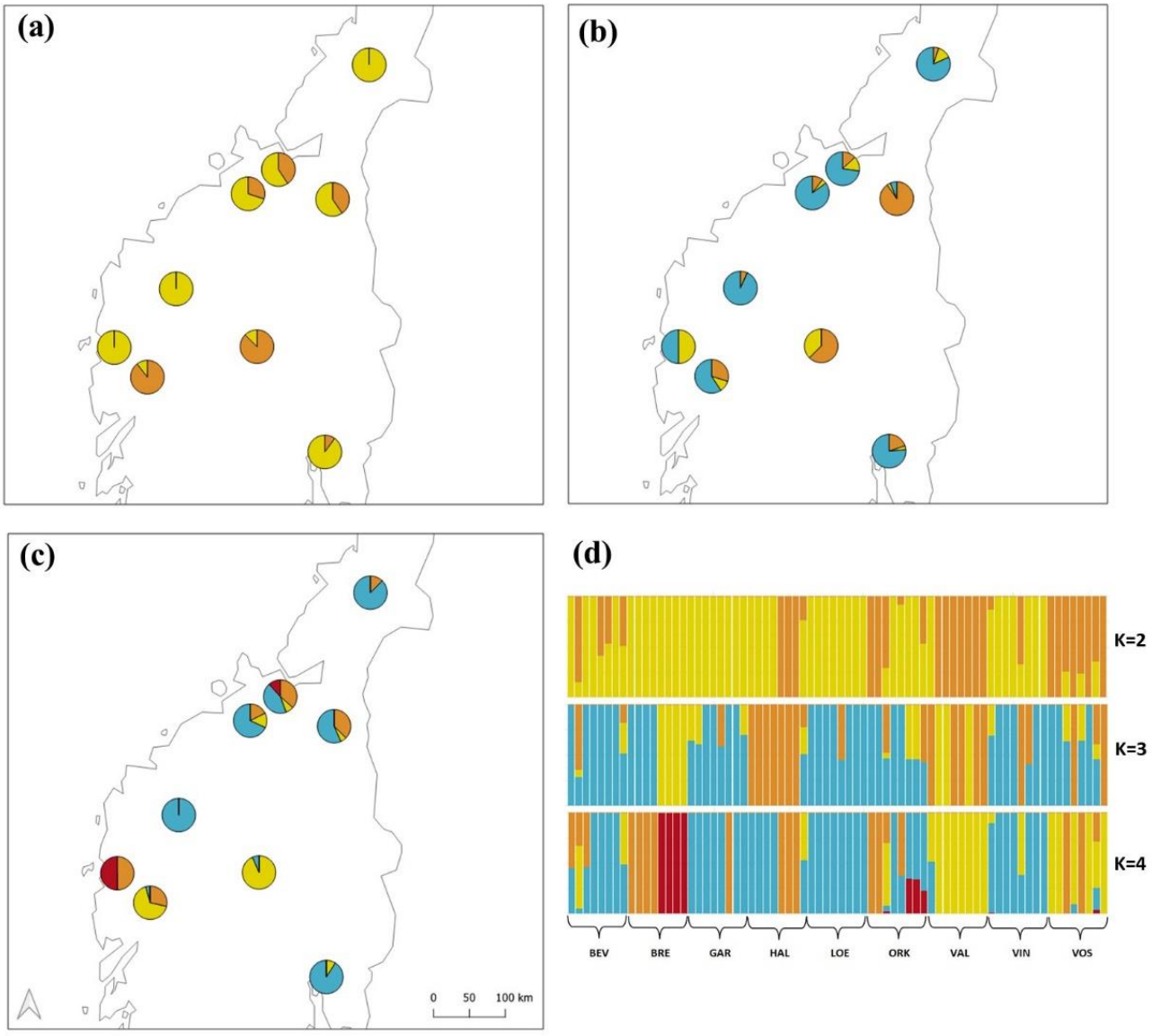


Figure 5 Admixture analysis of Fomitopsis pinicola. The mean individual membership ancestry coefficient per population is plotted on the map for K = 2 (a), K = 3 (b) and, K = 4 (c). Individual membership is plotted for K = 2–4 with populations also defined (d).

3.3 Genotype-Environment Associations

A redundancy analysis (RDA), constricting the SNP dataset to environmental variables revealed possible adaptations to local climates. The redundancy analysis revealed a significant relationship between the genetic variation captured by the SNP dataset and the environmental variables ($F=1.9021$, $p<0.001$). 10.2% of the total variance in the genetic data is explained by the environmental predictors included in the analysis ($R^2=0.102$). 126 candidate SNPs were identified as having a significant association with one of the four environmental predictors. Genetic variation from Brekke, Voss, Haltdalen and Valdres appear to have more variation explained from the environmental variables than the other populations, visualized in Figure 6. The redundancy analysis of axis 1 and axis 3 also reveals the Orkanger population as having a stronger relationship with the environmental variables. The ANOVA results indicate all four environmental variables are significant (Supplementary Table S5) and the results of the multicollinearity test, as indicated by the square root of the variance inflation factors (VIF), were consistently below the threshold value of 2, suggesting low levels of collinearity among the predictor variables (Supplementary Table S6). Figure 7 visually shows there are more SNPs associated with annual precipitation (47 SNPs) and the mean annual temperature (58 SNPs) then with isotherm (12 SNPs) or the mean temperature during the wettest quarter (9 SNPs).

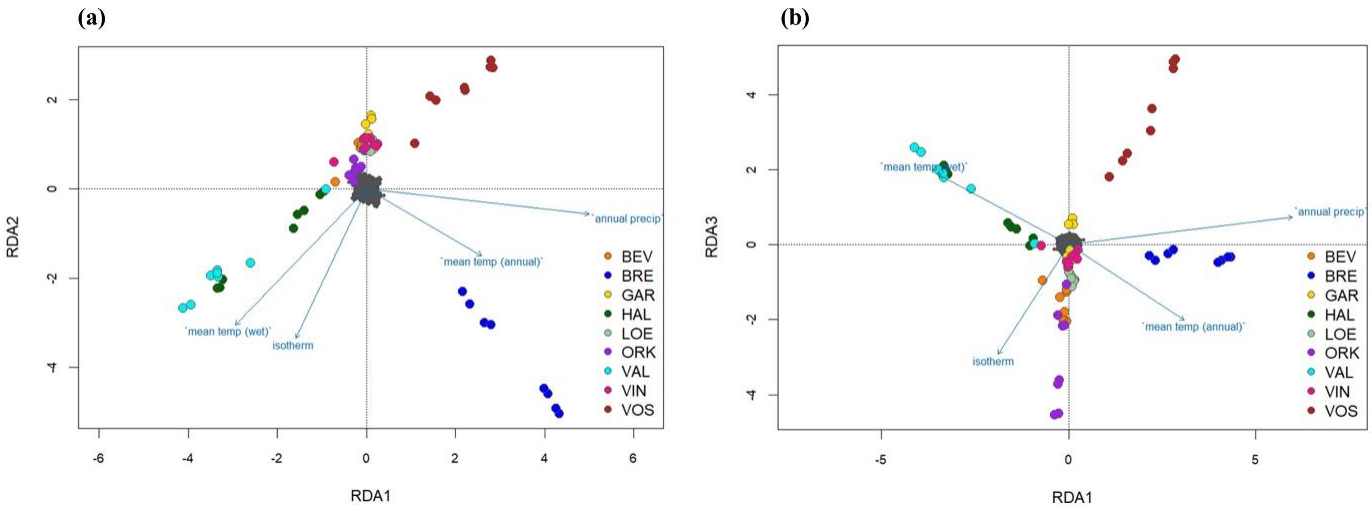


Figure 6 Redundancy analyses of the SNP variation explained by environmental variables. The points indicate the 22,191 alleles of F. Pinicola (colored by geographic region) inferred in the RDA analyses and projected along the first two axes RDA1 and RDA2, which explain 34.26% and 29.22% of the variation respectively (a). Redundancy analysis of the second two axes RDA1 and RDA 3, which explain 34.26% and 25.36% of the variation respectively (b).

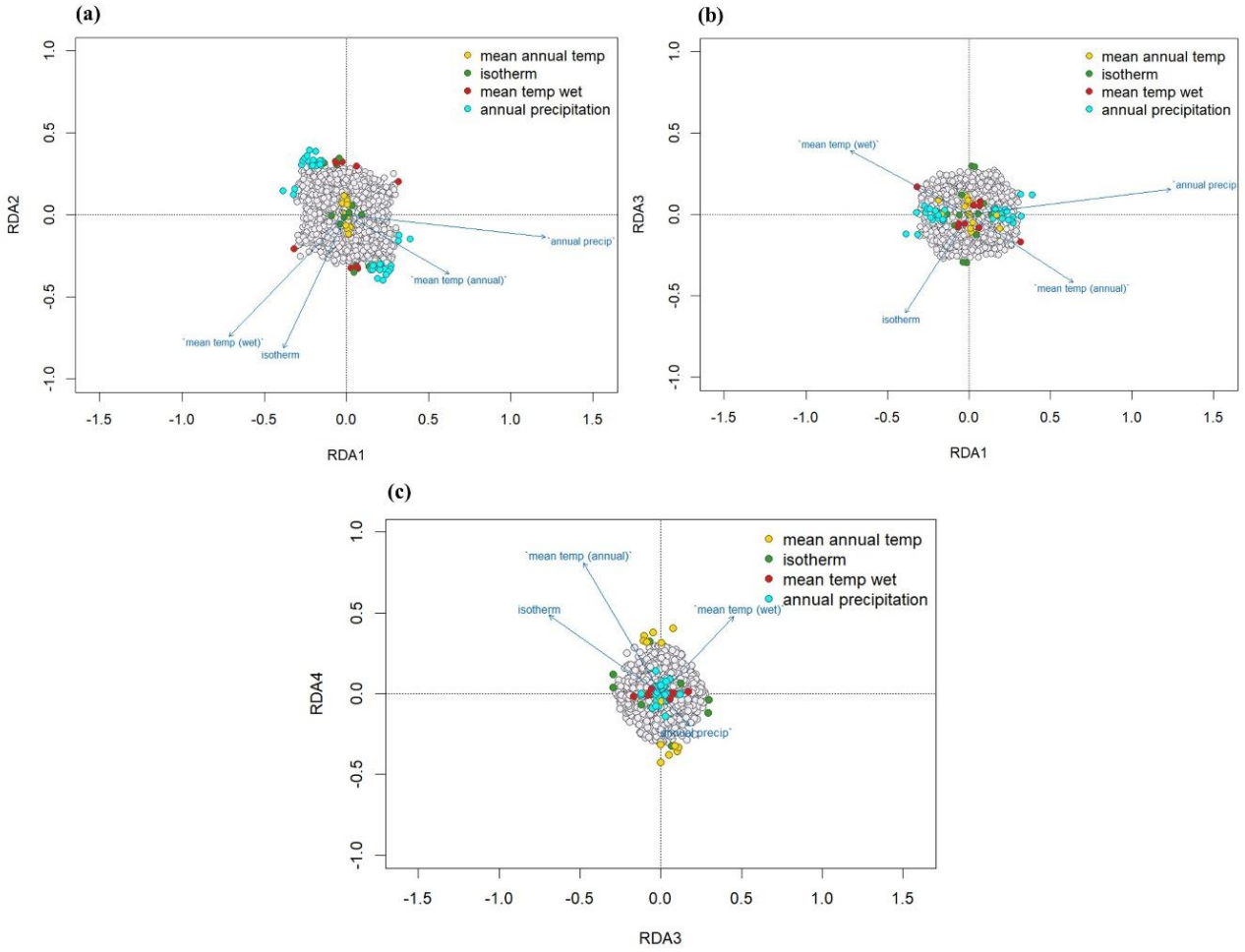


Figure 7 Redundancy analysis visualizing the association between candidate SNPs and environmental predictors. The color of each point corresponds to the predictor that the SNP is most strongly correlated with. The relationship between axis 1 and 2 (a), axis 1 and 3 (b), and axis 3 and 4 (c) are shown.

3.4 Annotation of Loci Under Selection

The results of the gene ontology (GO) analysis are summarized in Tables 1 and 2, and Figure 8. Tables 1 and 2 list the most significant GO terms based on the classic and weighted tests, respectively. The most significant terms are related to regulation of biological processes, nutrient uptake or cell signaling. Both the classic and weighted tests reveal the regulation of nitrogen utilization as the most significant term. Figure 8 is a visual representation of the classic GO results. The boxes indicate the 15 most significant terms with the box color representing the relative significance, ranging from dark red (most significant) to light yellow (least significant). The plot helps to better understand which of the significant GO terms are of interest, especially as they relate to the less significant terms.

Table 1 Significance of GO terms ranked from lowest P value from the classic Fisher's exact test. Included are the GO ID, the term (a brief annotation of the process or function), the number of genes annotated to each GO term, the number of significant genes for each term and the expected number of genes.

GO.ID	Term	Annotated	Significant	Expected	P value (classic)
0006808	regulation of nitrogen utilization	14	5	0.91	0.0013
0019740	nitrogen utilization	14	5	0.91	0.0013
0032101	regulation of response to external stimulus	14	5	0.91	0.0013
0032104	regulation of response to extracellular stimulus	14	5	0.91	0.0013
0032107	regulation of response to nutrient levels	14	5	0.91	0.0013
0009605	response to external stimulus	17	5	1.11	0.0035
0009991	response to extracellular stimulus	17	5	1.11	0.0035
0031667	response to nutrient levels	17	5	1.11	0.0035
0045454	cell redox homeostasis	12	4	0.78	0.0056
0048583	regulation of response to stimulus	29	6	1.89	0.0092
0019725	cellular homeostasis	14	4	0.91	0.0102
0042592	homeostatic process	18	4	1.17	0.0255
0065008	regulation of biological quality	20	4	1.3	0.0366

Table 2 Significance of GO terms ranked from lowest P value from the weighted algorithm. Included are the GO ID, the term (a brief annotation of the process or function), the number of genes annotated to each GO term, the number of significant genes for each term and the expected number of genes.

GO.ID	Term	Annotated	Significant	Expected	P value (weighted)
0006808	regulation of nitrogen utilization	14	5	0.91	0.0013
0045454	cell redox homeostasis	12	4	0.78	0.0056
0006289	nucleotide-excision repair	6	2	0.39	0.053
0009401	phosphoenolpyruvate-dependent sugar phosphotransferase system	1	1	0.07	0.0651
0042318	penicillin biosynthetic process	1	1	0.07	0.0651

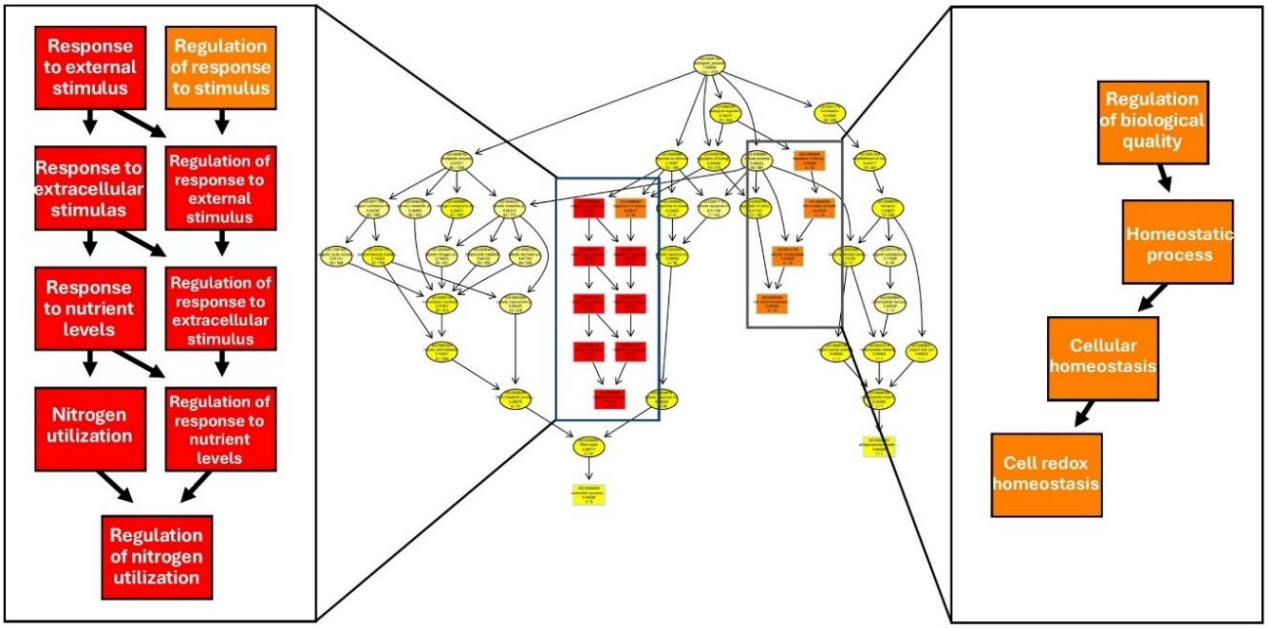


Figure 8 The subgraph induced by the top 15 GO terms identified by the classic algorithm for scoring GO terms for enrichment. Boxes indicate the 15 most significant terms. Box color represents the relative significance, ranging from dark red (most significant) to light yellow (least significant). The 13 most significant GO terms are enlarged for readability.

4 Discussion

4.1 Genetic Diversity

The presence of SNPs suggests the existence of genetic diversity, however the observed range of nucleotide diversity and expected heterozygosity across populations suggests variability in the extent of this diversity. This variability could be influenced by factors such as population size, historical demographic events, or selection pressures acting on different populations (Cortázar-Chinarro et al., 2017; Olson-Manning et al., 2012). The higher nucleotide diversity values suggest greater genetic variability, especially in the Orkanger, Voss, Valdres, Haltdalen and Vinterbro populations. Expected heterozygosity (H_e) was low for all populations which would suggest lower genetic diversity. In ddRAD sequencing, the genome is digested with restriction enzymes at specific recognition sites to generate fragments. If there are mutations present at these cutting sites, it can interfere with the efficiency of the digestion process, leading to incomplete digestion or altered fragment sizes. As a result, alleles present at mutated cutting sites may be underrepresented or missed entirely in the sequencing data, leading to an underestimation of heterozygosity (Magbanua et al., 2023).

Additionally, the Tajima's D results were slightly positive for six of the populations, which indicates a lack of rare genetic variants, possibly due to balancing selection or a stable population. The Vinterbro, Beverfjorden, and Orkanger populations had negative values of Tajima's D, indicating an excess of rare alleles or low frequency of polymorphism, which could be an indication of a recent expansion or bottleneck (Tajima, 1989). Tajima's D is sensitive to sample size (Larsson et al., 2013) and this should be considered when interpreting the results.

4.2 Genetic Divergence

The population genetic structure of *F. pinicola* was analyzed using methods such as principal component analysis, F_{ST}, and by using a model-based clustering algorithm in the program Admixture. In general, the results agree with earlier studies (Högberg et al., 1999; Kauserud & Schumacher, 2002; Sønstebø et al., 2022) in that the population genetic structure is generally shallow in wood decay fungi. Conversely, this study found that there is some polymorphism between certain populations, which could indicate limited gene flow and reproductive isolation. This aligns with the concept that genetic drift can drive genetic differentiation in fungal

populations, especially if the populations are small and isolated (Kauserud & Schumacher, 2002). These results diverge from the findings of previous studies that found *F. pinicola* to display a high amount of genetic diversity both within and between populations, which would indicate that populations are not reproductively isolated (Högberg et al., 1999). The results of the RDA suggest that genetic variation has a stronger association when constrained to climatic variables and that selection for these environmental factors is driving divergence.

While the results do not indicate two distinct populations of *Fomitopsis pinicola*, one in eastern Norway and one in western Norway, as hypothesized, they do indicate that some populations show genetic divergence. For example, the population in Brekke was divergent in the PCA and the F_{ST} analysis. The PCA also clearly separated the Voss and the Haltdalen and to some extent the Valdres populations. It should also be noted that Loen also exhibited higher F_{ST} values along with reduced genetic variation. This population is isolated along Lake Loenvatnet and encircled by very high mountains, suggesting that gene flow may be restricted (Sønstebø, personal communication, May 5, 2024). However, the DNA quality of the samples from this population was reduced, resulting in lower number of reads and reduced mapping which may have influenced the results. Investigating the geography of the other locations could reveal geographic or human induced factors that could cause restricted gene flow.

The results of the admixture analysis also suggest that there was no population structure, implying high levels of gene flow and genetic homogeneity. This could be an indication of ongoing connectivity between previously isolated populations although this cannot be determined by these results alone. It is worth noting that the results of K2-K4 do correspond to the results of the PCA and F_{ST} analysis in that the Brekke, Voss, Valdres and Haltdalen populations are genetically unique for each of these three ancestral coefficients. Also, the results of the K2-K4 analysis reveal that there is greater admixture in the northern populations, which aligns with the colonization path of *P. abies* after the Fenno-Scandinavian ice sheet retreated (Parducci et al., 2012; Tollefsrud et al., 2015). This could indicate that *F. pinicola* expanded to the north along with *P. abies*. It suggests that even though *F. pinicola* can grow on wood from many different trees it may be dependent on populations of *P. abies* for building enough inoculum to form a population (Sønstebø, personal communication, May 5, 2024). This may be the reason why the species is less abundant on the western part of Norway, where *P. abies* is also less prevalent than in eastern Norway, as observed in sightings reported by Artskart (Figure 9) (Artsdatabanken, 2022).

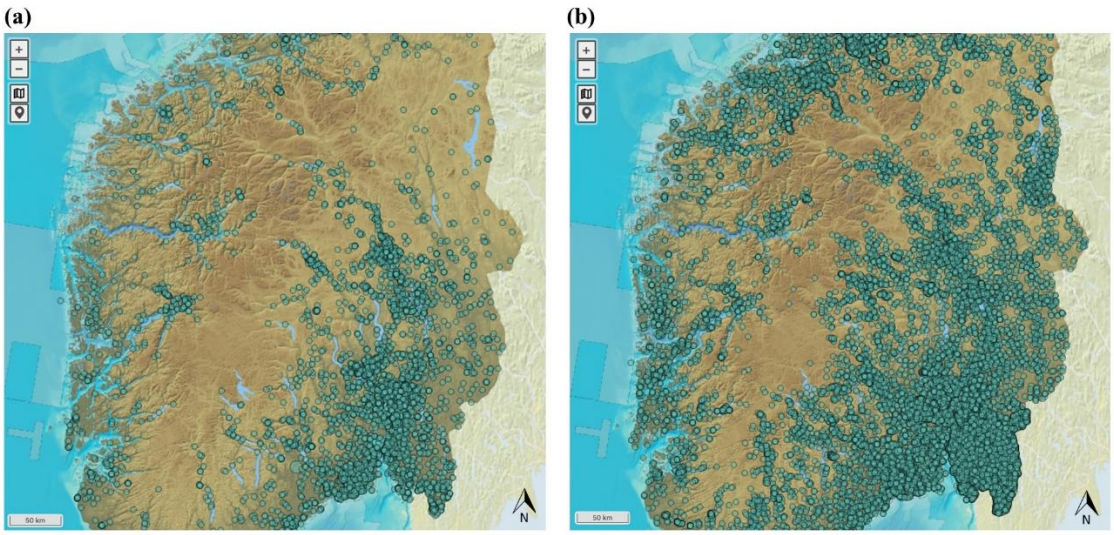


Figure 9 Maps showing where Fomitopsis pinicola (a) and Picia abies (b) have been observed and registered (blue points). Visually, there are less sightings on the west coast of Norway when compared to eastern Norway. Images from Artskart (Artsdatabanken, 2022).

4.3 Genotype-Environment Associations

This study also identified genomic regions significantly associated with four climatic variables. The results of the RDA indicate more structure than the PCA, suggesting genetic variation has a stronger association or response to these environmental factors. This echoes previous findings that environmental factors play a role in shaping fungal population genetic structure (Branco et al., 2017; Sønstebø et al., 2022). This type of ecological association helps to reveal environmental adaptation by detecting the alleles that hold statistical associations with these variables and can potentially indicate mechanisms for natural selection (Branco et al., 2017). The populations that had the most variation explained by the environmental variables were once again Brekke, Voss, Haltdalen, Valdres and Orkanger.

Both abiotic and biotic factors can eventually lead to adaptation and divergence in local populations, which influences genetic diversity, as fungi utilize complex signaling to sense and respond to external stressors (Bahn et al., 2007). The results of the RDA found SNPs associated with annual mean temperature, isothermality, mean temperature of wettest quarter, and annual precipitation. Adaptations to temperature are well documented in fungi (Branco et al., 2017; Mboup et al., 2012). Studies on *Neurospora crassa* have also highlighted the importance of temperature and latitude in driving local adaptation (Ellison et al., 2011; Gladieux et al., 2014), further underscoring the role of environmental variables in shaping fungal genetic diversity and distribution

patterns. It has also been found that temperature and moisture are important for fungal growth (Maurice et al., 2011) and moisture stress, such as drought or freezing, is a dominant regulator of competitive interactions among fungal species (Crowther et al., 2014).

Kauserud et al. (2024), which utilized the same samples of *F. pinicola* used in this study, conducted *in vitro* growth experiments to assess temperature and moisture dependent growth and decay rates. The results found a significant response to temperature dependent growth between populations grown at the highest (34°C) *in vitro* temperature, however they did not detect distinct variations between populations. Valdres and Haltdalen specifically showed large variation in growth as a response to temperature. These populations also exhibited genomic variation in this study. Also, the association between water potential and growth rate is not consistent across all populations and the significant results are dependent on which population is being considered. This study suggests that different populations may have adapted differently to water availability, leading to varying responses in growth rate under drought stress conditions or certain temperatures. Kauserud et al. (2024) also suggest that the variation could be due to random genetic changes over time. Overall, understanding the intricate interplay between temperature, precipitation, and other environmental factors is crucial for interpreting the mechanisms driving fungal adaptations and possibly explaining genetic divergence.

4.4 Loci Under Selection

Research has shown that genes responsible for regulating growth and fundamental metabolic processes exhibit consistent expression patterns across species, whereas genes involved in responding to stimuli, such as stress and effectors, are more likely to change (Gladieux et al., 2014). In line with these findings, the results of the gene ontology analysis revealed that the genes having to do with the regulation of response to nutrient levels, external stimulus, extracellular stimulus, and response to nutrient levels were significantly enriched among the genes in regions where signs of selection were identified. The GO analysis also revealed five significant genes that were associated with the regulation of nitrogen utilization for both the Fisher's exact test and the weighted analysis. Saprophytic fungi obtain nutrients from dead or decaying plant biomass, which is primarily rich in carbon, but often deficient in nitrogen. To overcome nitrogen limitations, fungi scavenge nitrogen from diverse sources, both inorganic and organic. This process involves activating various transporters and metabolic enzymes, which are regulated by the fungal cell to ensure their production matches nitrogen utilization needs

(Huberman et al., 2021). There could be other abiotic or biotic factors that explain the variation. Further research could be done on the association of other variables, such as nutrient availability or pH, on adaptive genes.

Some organisms have been shown to exhibit a broad, unspecific transcriptional response to environmental stress. This response involves the regulation of numerous genes, many of which may not have direct functional relevance to the specific stressor. In yeasts, specifically, this is known as a core stress response and can provide protection against various environmental challenges (López-Maury et al., 2008). Consequently, in the context of the gene ontology analysis, the significant enrichment of terms related to nutrient uptake and signaling pathways could reflect this general stress response strategy. These pathways may hold significance in coordinating cellular responses to environmental fluctuations, helping the organism adapt to and survive under challenging conditions. This study examined environmental factors such as mean temperature and annual precipitation. Future investigations into specific environmental stressors may reveal targeted adaptations to extreme climatic variables. Using ddRADseq allows for genome wide coverage, an improvement from past studies that compared only a few genes in comparative genomic studies, though it is still a reduced representation. Utilizing whole-genome sequencing would allow for comprehensive population genomic studies and identify more genes under selection (Yan et al., 2023).

5 Conclusion

This study investigated the genetic diversity, divergence, genotype-environment associations, and loci under selection in populations of *Fomitopsis pinicola* across eastern and western Norway. The application of ddRADseq has enabled a more extensive comparison of the *F. pinicola* genome than previous studies. Variability was observed in genetic diversity across populations, with certain regions displaying higher nucleotide diversity and expected heterozygosity, potentially influenced by factors such as population size, demographic history, and selection pressures. Additionally, Tajima's D analysis indicated potential signatures of balancing selection or recent population expansions, highlighting the dynamic nature of population genetics in *F. pinicola*.

The results reveal an overall shallow population structure and high levels of gene flow, yet genetic differences were found between some populations suggesting limited gene flow and localized reproductive isolation. Environmental association analyses identified genomic regions significantly associated with climatic variables, indicating the role of environmental factors in driving genetic divergence and adaptation. A gene ontology analysis revealed enrichment of genes related to nutrient uptake and signaling pathways, suggesting adaptive responses to environmental stressors. Further research utilizing whole-genome sequencing could identify additional genes under selection. Investigations into the effect of more specific environmental stressors, both using bioinformatics or *in vitro,* could reveal targeted adaptations to extreme climatic variables. Overall, the findings contribute to a deeper understanding of fungal population genetics and highlight the importance of considering both genetic and environmental factors in conservation and management strategies.

6 References

Abrego, N. (2022). Wood-inhabiting fungal communities: Opportunities for integration of empirical and theoretical community ecology. *Fungal Ecology*, *59*, 101112. https://doi.org/10.1016/j.funeco.2021.101112

Adamack, A. T., & Gruber, B. (2014). PopGenReport: Simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution*, *5*(4), 384–387. https://doi.org/10.1111/2041-210X.12158

Alexa, A., & Rahnenführer, J. (2023). *topGO: Enrichment Analysis for Gene Ontology. R package version 2.54.0*. https://bioconductor.org/packages/topGO

Alexander, D. H., & Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinformatics*, *12*(1), 246. https://doi.org/10.1186/1471-2105-12-246

Alsos, I. G., Rijal, D. P., Ehrich, D., Karger, D. N., Yoccoz, N. G., Heintzman, P. D., Brown, A. G., Lammers, Y., Pellissier, L., Alm, T., Bråthen, K. A., Coissac, E., Merkel, M. K. F., Alberti, A., Denoeud, F., Bakke, J., & Phylonorway Consortium. (2022). Postglacial species arrival and diversity buildup of northern ecosystems took millennia. *Science Advances*, *8*(39), eabo7434. https://doi.org/10.1126/sciadv.abo7434

Artsdatabanken 2022. Artskart. http://artskart.artsdatabanken.no/

Bahn, Y.-S., Xue, C., Idnurm, A., Rutherford, J. C., Heitman, J., & Cardenas, M. E. (2007). Sensing the environment: Lessons from fungi. *Nature Reviews Microbiology*, *5*(1), 57–69. https://doi.org/10.1038/nrmicro1578

Blanquart, F., Kaltz, O., Nuismer, S. L., & Gandon, S. (2013). A practical guide to measuring local adaptation. *Ecology Letters*, *16*(9), 1195–1205. https://doi.org/10.1111/ele.12150

Branco, S., Bi, K., Liao, H.-L., Gladieux, P., Badouin, H., Ellison, C. E., Nguyen, N. H., Vilgalys, R., Peay, K. G., Taylor, J. W., & Bruns, T. D. (2017). Continental-level population differentiation and environmental adaptation in the mushroom *Suillus brevipes*. *Molecular Ecology*, *26*(7), 2063–2076. https://doi.org/10.1111/mec.13892

Chen, J., Li, L., Milesi, P., Jansson, G., Berlin, M., Karlsson, B., Aleksic, J., Vendramin, G. G., & Lascoux, M. (2019). Genomic data provide new insights on the demographic history and the extent of recent material transfers in Norway spruce. *Evolutionary Applications*, *12*(8), 1539–1551. https://doi.org/10.1111/eva.12801

Chaichoompu, K., Abegaz, F., Tongsima, S., Shaw, P.J., Sakuntabhai, A., Pereira, L., & Van Steen, K. (2018). KRIS: Keen and Reliable Interface Subroutines for Bioinformatic Analysis. R package version 1.1.6. https://CRAN.R-project.org/package=KRIS

Cortázar-Chinarro, M., Lattenkamp, E. Z., Meyer-Lucht, Y., Luquet, E., Laurila, A., & Höglund, J. (2017). Drift, selection, or migration? Processes affecting genetic differentiation and variation along a latitudinal gradient in an amphibian. *BMC Evolutionary Biology*, *17*(1), 189. https://doi.org/10.1186/s12862-017-1022-z

Crowther, T. W., Maynard, D. S., Crowther, T. R., Peccia, J., Smith, J. R., & Bradford, M. A. (2014). Untangling the fungal niche: The trait-based approach. *Frontiers in Microbiology*, *5*, 579. https://doi.org/10.3389/fmicb.2014.00579

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., Durbin, R., & 1000 Genomes Project Analysis Group.

(2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T., McCarthy, S. A., Davies, R. M., & Li, H. (2021). Twelve years of SAMtools and BCFtools. *GigaScience*, *10*(2), giab008. https://doi.org/10.1093/gigascience/giab008

Ehlers, J., Gibbard, P. L., & Hughes, P. D. (2011). *Quaternary Glaciations—Extent and Chronology: A Closer Look*. Elsevier. http://ebookcentral.proquest.com/lib/ucsn-ebooks/detail.action?docID=742076

Ellison, C. E., Hall, C., Kowbel, D., Welch, J., Brem, R. B., Glass, N. L., & Taylor, J. W. (2011). Population genomics and local adaptation in wild isolates of a model microbial eukaryote. *Proceedings of the National Academy of Sciences*, *108*(7), 2831–2836. https://doi.org/10.1073/pnas.1014971108

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302–4315. https://doi.org/10.1002/joc.5086

Flanagan, S. P., Forester, B. R., Latch, E. K., Aitken, S. N., & Hoban, S. (2018). Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. *Evolutionary Applications*, *11*(7), 1035–1052. https://doi.org/10.1111/eva.12569

Forester, B. R., Lasky, J. R., Wagner, H. H., & Urban, D. L. (2018). Comparing methods for detecting multilocus adaptation with multivariate genotype–environment associations. *Molecular Ecology*, *27*(9), 2215–2233. https://doi.org/10.1111/mec.14584

Gillespie, R. G., & Roderick, G. K. (2014). Geology and climate drive diversification. Nature, 509(7500), Article 7500. https://doi.org/10.1038/509297a

Gladieux, P., Ropars, J., Badouin, H., Branca, A., Aguileta, G., de Vienne, D. M., Rodríguez de la Vega, R. C., Branco, S., & Giraud, T. (2014). Fungal evolutionary genomics provides insight into the mechanisms of adaptive divergence in eukaryotes. *Molecular Ecology*, *23*(4), 753–773. https://doi.org/10.1111/mec.12631

Haight, J.-E., Laursen, G. A., Glaeser, J. A., & Taylor, D. L. (2016). Phylogeny of *Fomitopsis pinicola*: A species complex. *Mycologia*, *108*(5), 925–938. https://doi.org/10.3852/14-225R1

Hansen, H., Gentry, J., Gentleman, R., Falcon, S., Hahne, F., & Sarkar, D. (2023). *Rgraphviz: Provides plotting capabilities for R graph objects. R package version 2.46.0.* https://doi.org/10.18129/B9.bioc.Rgraphviz

Harrell Jr., F. (2023). *Harrell Miscellaneous.* (5.1-2) [Computer software]. https://hbiostat.org/r/hmisc/

Hewitt, G. M. (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *359*(1442), 183–195. https://doi.org/10.1098/rstb.2003.1388

Högberg, N., Holdenrieder, O., & Stenlid, J. (1999). Population structure of the wood decay fungus *Fomitopsis pinicola*. *Heredity*, *83*(3), Article 3. https://doi.org/10.1038/sj.hdy.6885970

Huberman, L. B., Wu, V. W., Kowbel, D. J., Lee, J., Daum, C., Grigoriev, I. V., O'Malley, R. C., & Glass, N. L. (2021). DNA affinity purification sequencing and transcriptional profiling reveal new aspects of nitrogen regulation in a filamentous fungus. *Proceedings of the National Academy of Sciences*, *118*(13), e2009501118. https://doi.org/10.1073/pnas.2009501118

Kauserud, H., Colman, J. E., & Ryvarden, L. (2008). Relationship between basidiospore size, shape and life history characteristics: A comparison of polypores. *Fungal Ecology*, *1*(1), 19–23. https://doi.org/10.1016/j.funeco.2007.12.001

Kauserud, H., Justad, T. A., Vindenes, Y., Methlie, I.-S., Sønstebø, J. H., Skrede, I., & Maurice, S. (2024). Limited evidence of local adaptation of growth and decomposition rates in the widespread wood-decay fungus *Fomitopsis pinicola*. *Fungal Ecology*, *70*, 101353. https://doi.org/10.1016/j.funeco.2024.101353

Kauserud, H., & Schumacher, T. (2002). Population structure of the endangered wood decay fungus *Phellinus nigrolimitatus* (Basidiomycota). *Canadian Journal of Botany*, *80*(6), 597–606. https://doi.org/10.1139/b02-040

Knaus, B.J., Grünwald, N.J. (2017). "VCFR: a package to manipulate and visualize variant call format data in R." _Molecular Ecology Resources_, *17*(1), 44-53. ISSN 757, https://dx.doi.org/10.1111/1755-0998.12549

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

Larsson, H., Källman, T., Gyllenstrand, N., & Lascoux, M. (2013). Distribution of Long-Range Linkage Disequilibrium and Tajima's D Values in Scandinavian Populations of Norway Spruce (Picea abies). *G3: Genes|Genomes|Genetics*, *3*(5), 795–806. https://doi.org/10.1534/g3.112.005462

Li, L., Milesi, P., Tiret, M., Chen, J., Sendrowski, J., Baison, J., Chen, Z., Zhou, L., Karlsson, B., Berlin, M., Westin, J., Garcia-Gil, M. R., Wu, H. X., & Lascoux, M. (2022). Teasing apart the joint effect of demography and natural selection in the birth of a contact zone. *New Phytologist*, *236*(5), 1976–1987. https://doi.org/10.1111/nph.18480

Lunde, L. F., Boddy, L., Sverdrup-Thygeson, A., Jacobsen, R. M., Kauserud, H., & Birkemoe, T. (2023). Beetles provide directed dispersal of viable spores of a keystone wood decay fungus. *Fungal Ecology*, *63*, 101232. https://doi.org/10.1016/j.funeco.2023.101232

Magbanua, Z. V., Hsu, C.-Y., Pechanova, O., Arick, M., Grover, C. E., & Peterson, D. G. (2023). Innovations in double digest restriction-site associated DNA sequencing (ddRAD-Seq) method for more efficient SNP identification. *Analytical Biochemistry*, *662*, 115001. https://doi.org/10.1016/j.ab.2022.115001

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), Article 1. https://doi.org/10.14806/ej.17.1.200

Maurice, S., Coroller, L., Debaets, S., Vasseur, V., Le Floch, G., & Barbier, G. (2011). Modelling the effect of temperature, water activity and pH on the growth of *Serpula lacrymans*. *Journal of Applied Microbiology*, *111*(6), 1436–1446. https://doi.org/10.1111/j.1365-2672.2011.05161.x

Mboup, M., Bahri, B., Leconte, M., De Vallavieille-Pope, C., Kaltz, O., & Enjalbert, J. (2012). Genetic structure and local adaptation of European wheat yellow rust populations: The role of temperature-specific adaptation. *Evolutionary Applications*, *5*(4), 341–352. https://doi.org/10.1111/j.1752-4571.2011.00228.x

Nei, M., & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, *76*(10), 5269–5273.

Nota, K., Klaminder, J., Milesi, P., Bindler, R., Nobile, A., van Steijn, T., Bertilsson, S., Svensson, B., Hirota, S. K., Matsuo, A., Gunnarsson, U., Seppä, H., Väliranta, M. M., Wohlfarth, B., Suyama, Y., &

Parducci, L. (2022). Norway spruce postglacial recolonization of Fennoscandia. *Nature Communications*, *13*(1), Article 1. https://doi.org/10.1038/s41467-022-28976-4

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M. D., Durand, S., … Weedon, J. (2022). *vegan: Community Ecology Package* (2.6-4) [Computer software]. https://cran.r-project.org/web/packages/vegan/index.html

Olson-Manning, C. F., Wagner, M. R., & Mitchell-Olds, T. (2012). Adaptive evolution: Evaluating empirical support for theoretical predictions. *Nature Reviews. Genetics*, *13*(12), 867–877. https://doi.org/10.1038/nrg3322

Parducci, L., Jørgensen, T., Tollefsrud, M. M., Elverland, E., Alm, T., Fontana, S. L., Bennett, K. D., Haile, J., Matetovici, I., Suyama, Y., Edwards, M. E., Andersen, K., Rasmussen, M., Boessenkool, S., Coissac, E., Brochmann, C., Taberlet, P., Houmark-Nielsen, M., Larsen, N. K., … Willerslev, E. (2012). Glacial Survival of Boreal Trees in Northern Scandinavia. *Science*, *335*(6072), 1083–1086. https://doi.org/10.1126/science.1216043

Peay, K. G., Bidartondo, M. I., & Elizabeth Arnold, A. (2010). Not every fungus is everywhere: Scaling to the biogeography of fungal–plant interactions across roots, shoots and ecosystems. *New Phytologist*, *185*(4), 878–882. https://doi.org/10.1111/j.1469-8137.2009.03158.x

Peay, K. G., Schubert, M. G., Nguyen, N. H., & Bruns, T. D. (2012). Measuring ectomycorrhizal fungal dispersal: Macroecological patterns driven by microscopic propagules. *Molecular Ecology*, *21*(16), 4122–4136. https://doi.org/10.1111/j.1365-294X.2012.05666.x

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. *PLOS ONE*, *7*(5), e37135. https://doi.org/10.1371/journal.pone.0037135

Pfeifer, B., Wittelsbürger, U., Ramos-Onsins, S. E., & Lercher, M. J. (2014). PopGenome: An Efficient Swiss Army Knife for Population Genomic Analyses in R. *Molecular Biology and Evolution*, *31*(7), 1929–1936. https://doi.org/10.1093/molbev/msu136

Poplin, R., Ruano-Rubio, V., DePristo, M. A., Fennell, T. J., Carneiro, M. O., Auwera, G. A. V. der, Kling, D. E., Gauthier, L. D., Levy-Moonshine, A., Roazen, D., Shakir, K., Thibault, J., Chandran, S., Whelan, C., Lek, M., Gabriel, S., Daly, M. J., Neale, B., MacArthur, D. G., & Banks, E. (2018). *Scaling accurate genetic variant discovery to tens of thousands of samples* (p. 201178). bioRxiv. https://doi.org/10.1101/201178

QGIS Development Team (2022). QGIS Geographic Information System Developers Manual. Open Source Geospatial Foundation Project

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: A flexible suite of utilities for comparing genomic features. Bioinformatics, 26(6), 841–842. https://doi.org/10.1093/bioinformatics/btq033

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Revelle, W. (2024). psych: Procedures for Psychological, Psychometric, and Personality Research. Northwestern University, Evanston, Illinois. R package version 2.4.1, https://CRAN.R-project.org/package=psych.

Rochat, E., Selmoni, O., & Joost, S. (2021). Spatial areas of genotype probability: Predicting the spatial distribution of adaptive genetic variants under future climatic conditions. Diversity and Distributions, 27(6), 1076–1090. https://doi.org/10.1111/ddi.13256

Ryvarden, L., & Melo, I. (2017). *Poroid fungi of Europe* (2nd rev. ed., Vol. 37). Fungiflora.

Sarkar, D. (2008). *Lattice: Multivariate Data Visualization with R*. Springer. https://doi.org/10.1007/978-0-387-75969-2

Sønstebø, J. H., Trucchi, E., Nordén, J., Skrede, I., Miettinen, O., Haridas, S., Pangilinan, J., Grigoriev, I. V., Martin, F., Kauserud, H., & Maurice, S. (2022). Population genomics of a forest fungus reveals high gene flow and climate adaptation signatures. *Molecular Ecology*, *31*(7), 1963–1979. https://doi.org/10.1111/mec.16369

Stengel, A., Stanke, K. M., Quattrone, A. C., & Herr, J. R. (2022). Improving Taxonomic Delimitation of Fungal Species in the Age of Genomics and Phenomics. *Frontiers in Microbiology*, *13*. https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2022.847067

Tajima, F. (1989). Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism. *Genetics*, *123*(3), 585–595.

Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation with gene flow. *Molecular Ecology*, *25*(10), 2144–2164. https://doi.org/10.1111/mec.13606

Tiret, M., Olsson, L., Grahn, T., Karlsson, B., Milesi, P., Lascoux, M., Lundqvist, S., & García-Gil, M. R. (2022). Divergent selection predating the Last Glacial Maximum mainly acted on macro-phenotypes in Norway spruce. *Evolutionary Applications*, *16*(1), 163–172. https://doi.org/10.1111/eva.13519

Tollefsrud, M. M., Kissling, R., Gugerli, F., Johnsen, Ø., Skrøppa, T., Cheddadi, R., Van Der Knaap, W. O., Latałowa, M., Terhürne-Berson, R., Litt, T., Geburek, T., Brochmann, C., & Sperisen, C. (2008). Genetic consequences of glacial survival and postglacial colonization in Norway spruce: Combined analysis of mitochondrial DNA and fossil pollen. *Molecular Ecology*, *17*(18), 4134–4150. https://doi.org/10.1111/j.1365-294X.2008.03893.x

Tollefsrud, M. M., Latałowa, M., van der Knaap, W. O., Brochmann, C., & Sperisen, C. (2015). Late Quaternary history of North Eurasian Norway spruce (*Picea abies*) and Siberian spruce (*Picea obovata*) inferred from macrofossils, pollen and cytoplasmic DNA variation. *Journal of Biogeography*, *42*(8), 1431–1442. https://doi.org/10.1111/jbi.12484

Tollefsrud, M. M., Sønstebø, J. H., Brochmann, C., Johnsen, Ø., Skrøppa, T., & Vendramin, G. G. (2009). Combined analysis of nuclear and mitochondrial markers provide new insight into the genetic structure of North European *Picea abies*. *Heredity*, *102*(6), 549–562. https://doi.org/10.1038/hdy.2009.16

Tusso, S., Nieuwenhuis, B. P. S., Weissensteiner, B., Immler, S., & Wolf, J. B. W. (2021). Experimental evolution of adaptive divergence under varying degrees of gene flow. *Nature Ecology & Evolution*, *5*(3), Article 3. https://doi.org/10.1038/s41559-020-01363-2

Vivian-Smith, A., & Sønstebø, J. H. (2017). *A streamlined ddRAD tag protocol for use with the Ion Torrent sequencer, as a versatile probe for populatio...* https://www.protocols.io/view/a-streamlined-ddrad-tag-protocol-for-use-with-the-khuct6w

Waldvogel, A., Feldmeyer, B., Rolshausen, G., Exposito-Alonso, M., Rellstab, C., Kofler, R., Mock, T., Schmid, K., Schmitt, I., Bataillon, T., Savolainen, O., Bergland, A., Flatt, T., Guillaume, F., &

Pfenninger, M. (2020). Evolutionary genomics can improve prediction of species' responses to climate change. *Evolution Letters*, *4*(1), 4–18. https://doi.org/10.1002/evl3.154

Wei, T., Simko, V., Levy, M., Xie, Y., Jin, Y., Zemla, J., Freidank, M., Cai, J., & Protivinsky, T. (2021). *corrplot: Visualization of a Correlation Matrix* (0.92) [Computer software]. https://cran.r-project.org/web/packages/corrplot/index.html

Westergaard, K. B., Zemp, N., Bruederle, L. P., Stenøien, H. K., Widmer, A., & Fior, S. (2019). Population genomic evidence for plant glacial survival in Scandinavia. *Molecular Ecology*, *28*(4), 818–832. https://doi.org/10.1111/mec.14994

Wickham, H. (2023). stringr: Simple, Consistent Wrappers for Common String Operations. R package version 1.5.1, https://CRAN.R-project.org/package=stringr.

Yan, D., Liu, J., Fan, Y., Lian, Z., Dang, Z., & Niu, J. (2023). Genomic insights into genetic diversity and local adaptation of a dominant desert steppe feather grass, *Stipa breviflora* Griseb. *Frontiers in Plant Science*, *14*, 1170075. https://doi.org/10.3389/fpls.2023.1170075

Zhang, J., Shen, N., Li, C., Xiang, X., Liu, G., Gui, Y., Patev, S., Hibbett, D. S., Barry, K., Andreopoulos, W., Lipzen, A., Riley, R., He, G., Yan, M., Grigoriev, I. V., Shan Kwan, H., Kit Cheung, M., Bian, Y., & Xiao, Y. (2022). Population genomics provides insights into the genetic basis of adaptive evolution in the mushroom-forming fungus *Lentinula edodes*. *Journal of Advanced Research*, *38*, 91–106. https://doi.org/10.1016/j.jare.2021.09.008

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, *28*(24), 3326–3328. https://doi.org/10.1093/bioinformatics/bts606

7 List of Figures and Tables

Tables		
Number	Page	Description
1	22	Significance of GO terms ranked from lowest P value from the classic Fisher's exact test. Included are the GO ID, the term (a brief annotation of the process or function), the number of genes annotated to each GO term, the number of significant genes for each term and the expected number of genes.
2	22	Significance of GO terms ranked from lowest P value from the weighted algorithm. Included are the GO ID, the term (a brief annotation of the process or function), the number of genes annotated to each GO term, the number of significant genes for each term and the expected number of genes.

8 Supplementary Information

Table S1 Summary of DNA purity and concentration of each of the 72 samples. A indicates samples taken from Alnus logs and P indicates samples taken from Pinus logs.

Sample	a260/a280	ng/µL
GAR_A1	1.79	6.1
GAR_A2	1.18	7.7
GAR_A3	1.82	31.9
GAR_A4	1.78	6.9
GAR_P1	2.01	5
GAR_P2	1.66	4.8
GAR_P3	2.05	7.9
GAR_P4	1.93	11.7
LOE_A1	1.66	4.8
LOE_A2	1.62	6.3
LOE_A3	1.67	5.6
LOE_A4	1.79	6
LOE_P1	1.78	16.6
LOE_P2	1.95	10.5
LOE_P3	1.74	7.7
LOE_P4	1.49	1.8
VIN_A1	1.77	12.1
VIN_A2	1.8	25.4
VIN_A3	1.88	8.3
VIN_A4	1.86	25
VIN_P1	1.64	3.6
VIN_P2	1.92	12.2
VIN_P3	1.82	23.6
VIN_P4	1.78	58.1
BRE_A1	1.53	4.8
BRE_A2	1.75	15.5
BRE_A3	1.82	30.3
BRE_A4	1.8	20.5
BRE_P1	1.68	9.4
BRE_P2	1.74	7.6
BRE_P3	1.73	11.6
BRE_P4	1.81	45.9
ORK_A1	1.88	6.3
ORK_A2	1.8	11.2
ORK_A3	1.83	22.1
ORK_A4	1.99	6.4

Sample	a260/a280	ng/µL
ORK_P1	2.15	1.1
ORK_P2	1.84	21.9
ORK_P3	1.96	12
ORK_P4	1.89	23.5
VOS_A1	1.79	8.3
VOS_A2	1.99	14.6
VOS_A3	1.78	20.6
VOS_A4	1.73	4.7
VOS_P1	1.68	6.4
VOS_P2	2.2	7.2
VOS_P3	1.79	22.4
VOS_P4	1.81	33.7
BEV_A1	1.73	15.6
BEV_A2	1.77	13.6
BEV_A3	1.81	17.1
BEV_A4	1.82	17.4
BEV_P1	2.32	7.7
BEV_P2	1.82	33.2
BEV_P3	1.79	20.5
BEV_P4	1.81	33.3
VAL_A1	1.82	43.2
VAL_A2	1.91	7.7
VAL_A3	1.81	24.5
VAL_A4	1.8	12.7
VAL_P1	1.82	21.6
VAL_P2	1.93	16.8
VAL_P3	1.84	35.8
VAL_P4	1.79	20.1
HAL_A1	1.77	6
HAL_A2	1.81	25
HAL_A3	1.77	19.4
HAL_A4	1.81	22.6
HAL_P1	1.69	3.2
HAL_P2	1.78	8.7
HAL_P3	1.9	9.1
HAL_P4	1.7	7.2

Table S2 Summary of mean nucleotide diversity, Tajima's D, and the observed and expected heterozygosity results for each population.

Location	Mean Nucleotide Diversity	Tajima's D	H_o	H_e
VOS	1.195	0.308	0.110	0.132
BRE	1.098	0.414	0.063	0.117
LOE	0.829	0.265	0.020	0.088
BEV	0.999	-0.118	0.057	0.107
VAL	1.138	0.597	0.104	0.125
VIN	1.048	-0.241	0.051	0.108
GAR	0.922	0.186	0.042	0.097
HAL	1.087	0.478	0.078	0.119
ORK	1.219	-0.107	0.099	0.131

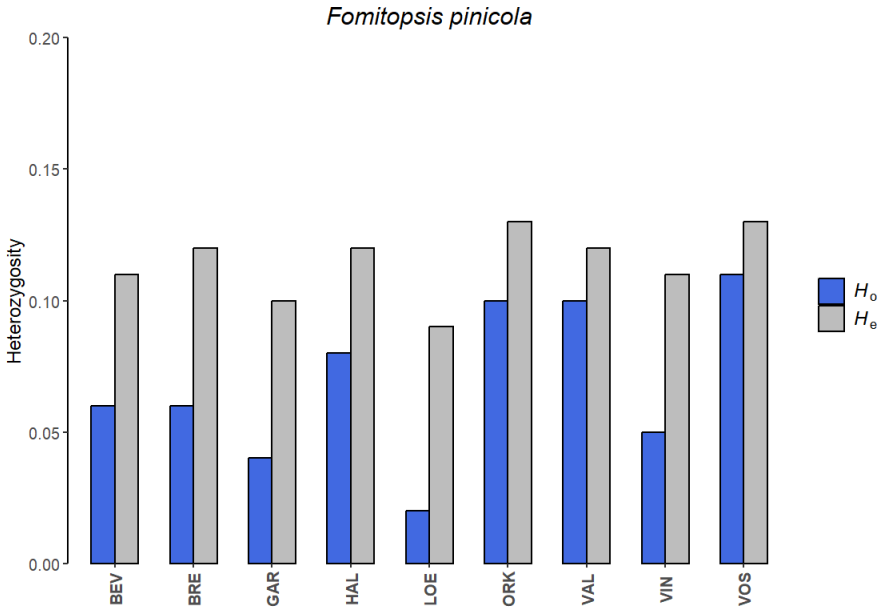


Figure S1 Observed and expected heterozygosity for each population. Observed heterozygosity is lower than expected for each population, especially for Loen.

```
#library(vcfR)
#library(stringr)
#library(KRIS)

obj.vcfR <- read.vcfR("filename.vcf")

# Character matrix containing the genotypes
geno <- extract.gt(obj.vcfR)
# Positions in bp
position <- getPOS(obj.vcfR)
# Chromosome information
chromosome <- getCHROM(obj.vcfR)

G <- matrix(NA, nrow = nrow(geno), ncol = ncol(geno))
G[geno %in% c("0/0", "0|0")] <- 0
G[geno  %in% c("0/1", "1/0", "1|0", "0|1")] <- 1
G[geno %in% c("1/1", "1|1")] <- 2

ind <- colnames(obj.vcfR@gt)[-1]

pop <- as.data.frame(str_split_fixed(ind, "_", 2))
colnames(pop)<- c("pop", "ind")

pop_order <- c("VOS", "BRE", "LOE", "BEV", "VAL", "VIN", "GAR", "HAL", "ORK"
)

#fst.hudson: Calculate the average fixation index (Fst) between two groups of
individuals from Single-nucleotide polymorphism (SNP)
fst<- matrix(0, nrow = length(pop_order), ncol = length(pop_order))
for(j in 1:(length(pop_order))){
  for(i in 1:(length(pop_order))){

    if(j>i){fst[i,j]<-fst.hudson(t(G), idx.p1 = which(pop$pop==pop_order[j]),
idx.p2 = which(pop$pop==pop_order[i]))}else{fst[i,j]<-0}
  }
}

colnames(fst)<- pop_order
rownames(fst)<- pop_order

dgen <- as.dist(t(fst/(1-fst)))
```

Figure S2 Custom code used in R to estimate the pairwise F_{ST} by estimating the genetic differentiation between each location as well as convert F_{ST} into a genetic distance matrix. Code provided by (Sønstebø, personal communication, February 7, 2024).

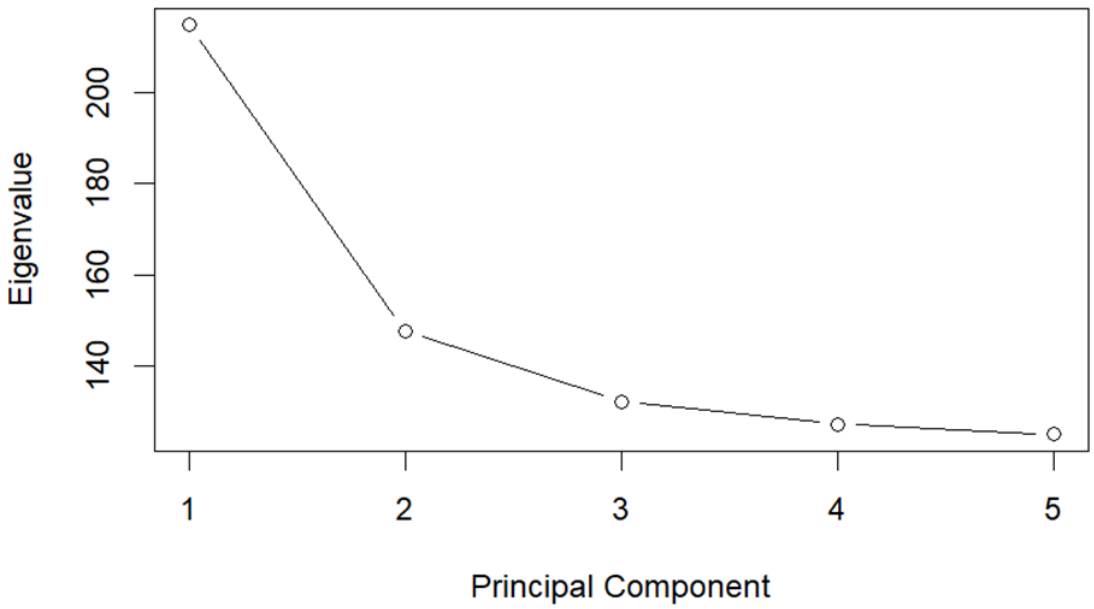


Figure S3 Plot used to determine the number of axes that captures the most meaningful variation in PCA analysis. The nmber of axes chosen for the PCA was three.

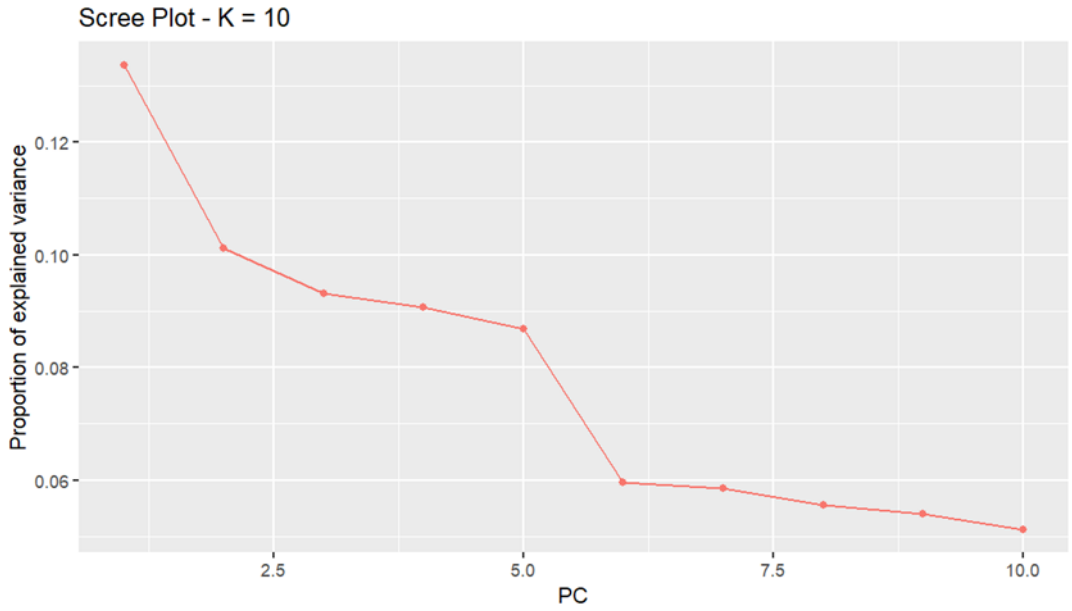


Figure S4 Cross validation error tests were calculated for the number of ancestral populations (K) ranging from 1-10.

Table S3 Overview of the bioclimatic variables used in the RDA analysis (http://www.worldclim.org).

Code	Variable	Description	Unit
BIO1	mean annual air temperature	Mean annual daily mean air temperatures averaged over 1 year	°C
BIO3	isothermality	Ratio of diurnal variation to annual variation in temperatures	°C
BIO8	mean daily mean air temperatures of the wettest quarter	The wettest quarter of the year is determined (to the nearest month)	°C
BIO12	annual precipitation amount	Accumulated precipitation amount over 1 year	mm

Table S4 Summary of the bioclimatic variables taken from each of the nine locations that were used in the RDA analysis.

Location	Annual mean temperature (°C) (BIO1)	Isothermality (°C) (BIO3)	Mean temperature of wettest quarter (°C) (BIO8)	Annual precipitation (mm) (BIO12)
BEV	5.04	28.76	1.68	1197
BRE	7.25	29.51	4.90	2813
GAR	4.18	26.30	4.48	1286
HAL	2.51	29.03	10.75	743
LOE	5.95	28.51	2.82	1392
ORK	4.42	29.24	0.93	1180
VAL	2.08	29.23	12.22	569
VIN	5.93	26.61	6.00	734
VOS	3.18	23.37	0.12	2270

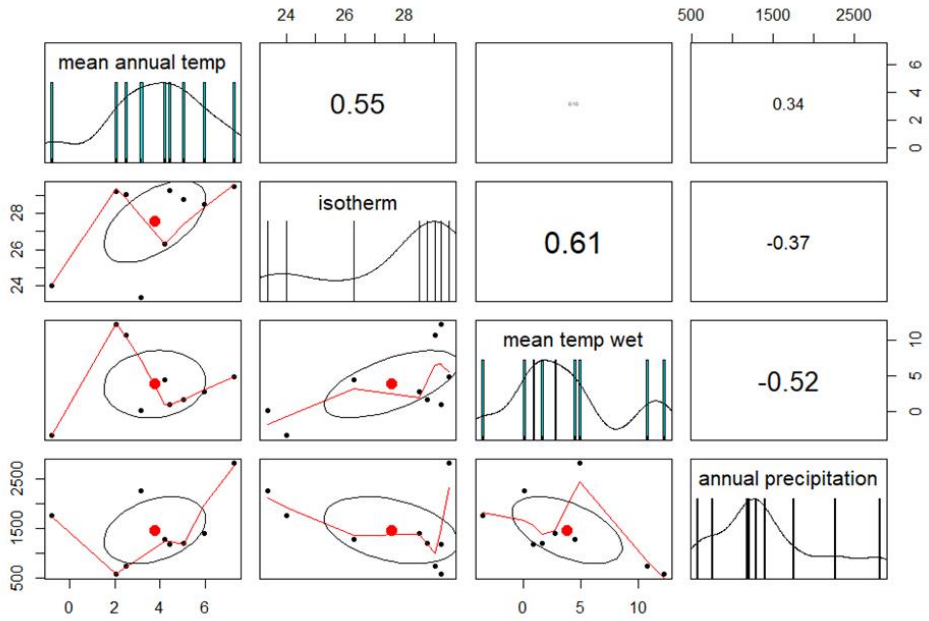


Figure S5 Visualization of pairwise relationships between the four bioclimatic variables (1,3,8, and 12) chosen for the RDA analysis.The desired correlation coefficient was less then 0.7.

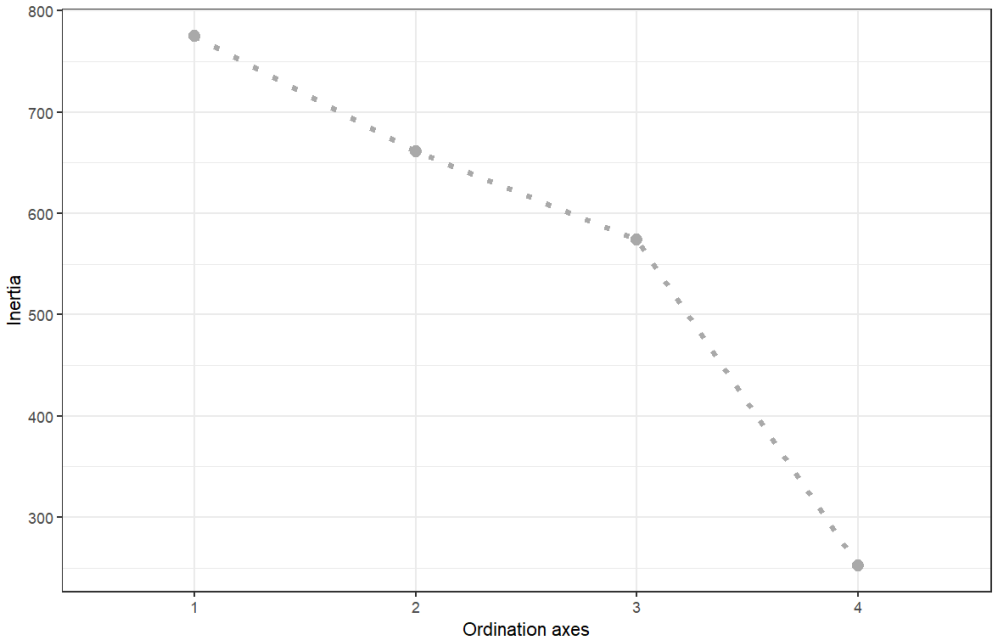


Figure S6 Plot used to determine the number of axes that captures the most meaningful variation in RDA analysis. The nmber of axes chosen for the RDA was four. Three is ideal.

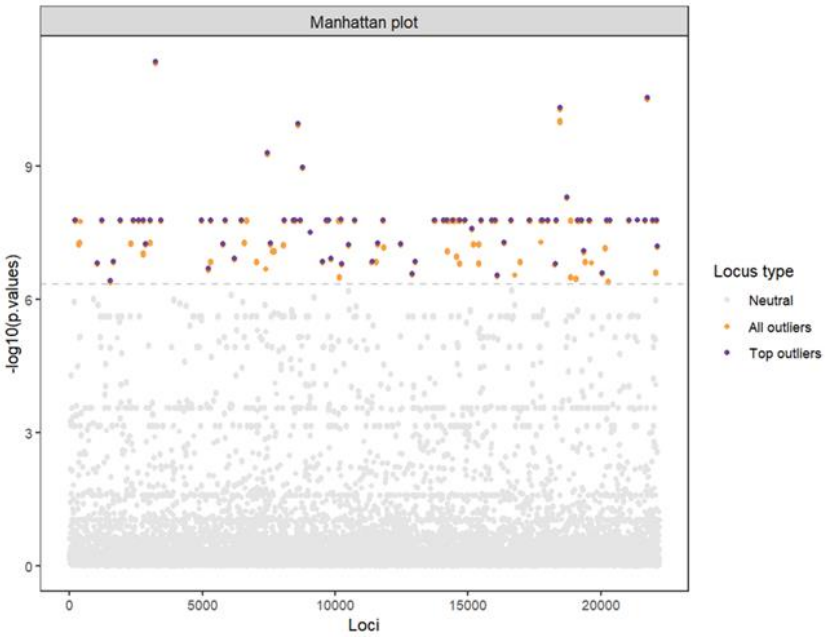


Figure S7 Manhattan plot showing the SNPs from the RDA analysis. Grey are neutral, orange are all outliers and purple are the top outliers. Outliers are SNPs that had a significant association with one of the four bioclimatic environmental variables..

Table S5 The ANOVA results of the RDA indicate all four environmental variable are significant.

	Df	**Variance**	**F**	**Pr(>F)**
Mean temp (annual)	1	460.1	1.5470	0.004
Isotherm	1	703.3	2.3646	0.001
Mean temp (wet)	1	524.0	1.7619	0.001
Annual precip	1	575.5	1.9350	0.001
Residual	67	19928.0		

Table S6 The results of the multicollinearity test for the RDA, as indicated by the square root of the Variance Inflation Factors (VIF), were consistently below the threshold value of 2.

	VIF
Mean temp (annual)	1.68
Isotherm	1.88
Mean temp (wet)	1.39
Annual precip	1.54

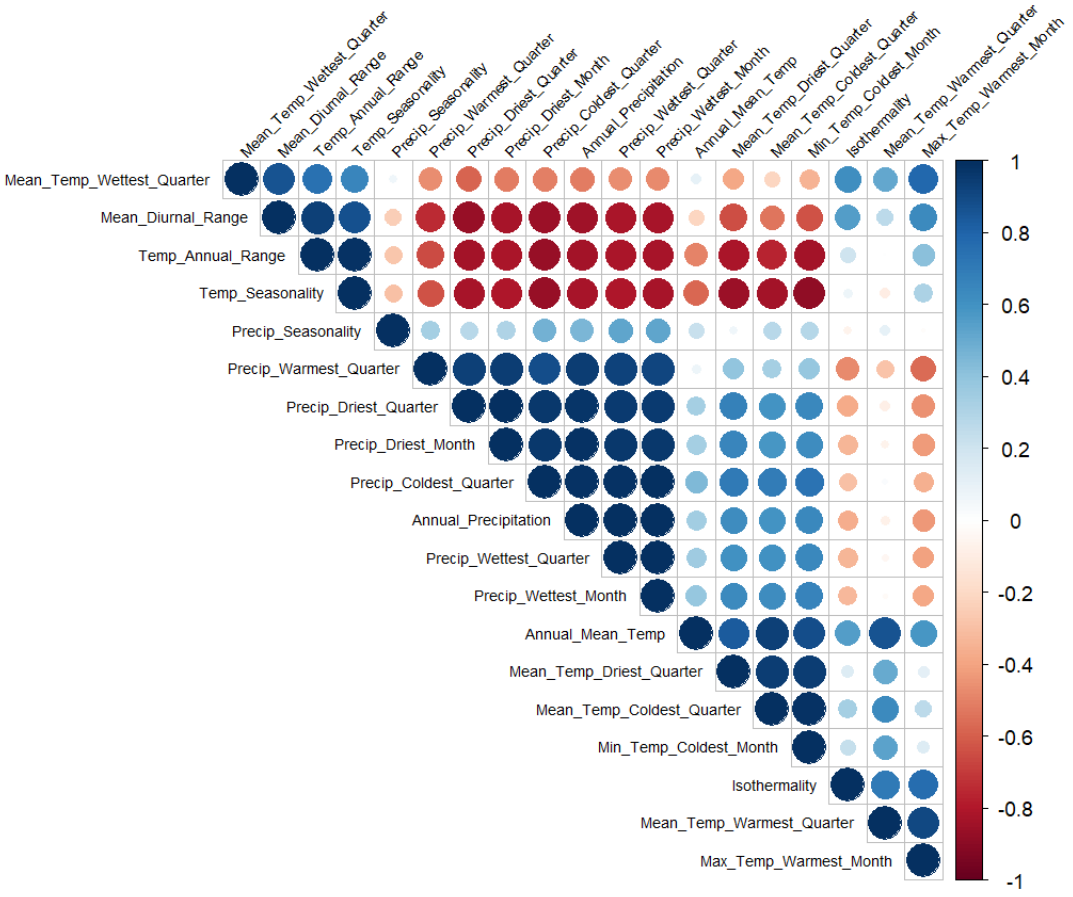


Figure S8 Correlation matrix between environmental variables assessed by calculating Spearman´s ρ. There is a high correlation between the precipitation variables and temperature.

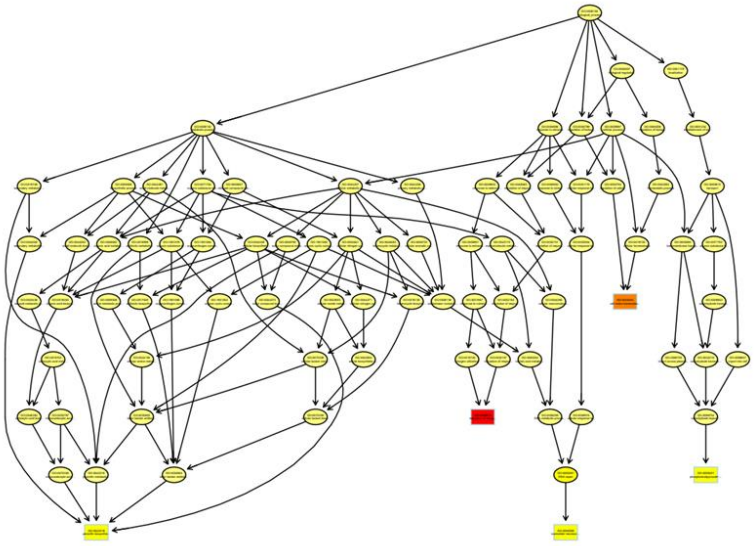


Figure S9 The subgraph induced by the top 5 GO terms identified by the weighted algorithm for scoring GO terms for enrichment. Boxes indicate the 15 most significant terms. Box color represents the relative significance, ranging from dark red (most significant).