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Deep population structure linked to host vernalization requirement in the barley net blotch fungal pathogen

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Abstract

Invasive fungal pathogens pose a substantial threat to widely cultivated crop species, owing to their capacity to adapt to new hosts and new environmental conditions. Gaining insights into the demographic history of these pathogens and unravelling the mechanisms driving coevolutionary processes are crucial for developing durably effective disease management programmes. *Pyrenophora teres* is a significant fungal pathogen of barley, consisting of two lineages, Ptt and Ptm, with global distributions and demographic histories reflecting barley domestication and spread. However, the factors influencing the population structure of *P. teres* remain poorly understood, despite the varietal and environmental heterogeneity of barley agrosystems. Here, we report on the population genomic structure of *P. teres* in France and globally. We used genotyping-by-sequencing to show that Ptt and Ptm can coexist in the same area in France, with Ptt predominating. Furthermore, we showed that differences in the vernalization requirement of barley varieties were associated with population differentiation within Ptt in France and at a global scale, with one population cluster found on spring barley and another population cluster found on winter barley. Our results demonstrate how cultivation conditions, possibly associated with genetic differences between host populations, can be associated with the maintenance of divergent invasive pathogen populations coexisting over large geographic areas. This study not only advances our understanding of the coevolutionary dynamics of the Pt-barley pathosystem but also prompts further research on the relative contributions of adaptation to the host versus adaptation to abiotic conditions in shaping Ptt populations.

Impact Statement

Many invasive fungal pathogens have successfully followed major crop species throughout their intercontinental range, but continue to represent dynamic biotic threats. During their geographic expansion, invasive fungal populations were subjected to heterogeneous environmental conditions, or different populations of hosts, which could result in adaptation processes. Understanding this history of colonization can allow us to better prevent the emergence of crop diseases, and to better control them.

One such fungus, *Pyrenophora teres*, negatively impacts barley production globally by causing net blotch disease. In this study, we characterized the genetic makeup of *P. teres* in France and how it compares with what can be sampled in other regions of the world. We found that both the net and spot forms of *Pyrenophora teres* can be in the same area in France, but the spot form is more common. We also discovered that the net form populations associated with winter and spring barley are different, which was not known until now. This study opens up numerous experimental perspectives aimed at evaluating whether the two populations of net form are adapted to their hosts or to the conditions of cultivation of their hosts, with the goal of implementing measures that force the pathogen to maladaptation.

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Abbreviations: AMOVA, analysis of molecular variance; DAPC, discriminant analysis of principal components; GBS, genotyping-by-sequencing; PCA, principal component analysis; Pt, *Pyrenophora teres*; Ptm, *Pyrenophora teres* form *maculata*; Ptt, *Pyrenophora* teresform *teres*.

Raw genotyping-by-sequencing data are available under BioProject PRJEB66440; raw sequencing reads for reference assembly are available under BioProject PRJEB70711; single-nucleotide polymorphisms and reference genome assembly for isolate FRA0042 are available under doi 10.5281/zenodo.8424841.

Data statement: All supporting data, code and protocols have been provided within the article or through supplementary data files.Five supplementary figures and twelve supplementary tables are available with the online version of this article.

DATA SUMMARY

Raw genotyping-by-sequencing data are available under BioProject PRJEB66440; raw sequencing reads for reference assembly are available under BioProject PRJEB70711; single-nucleotide polymorphisms and reference genome assembly for isolate FRA0042 under doi https://zenodo.org/records/10021844.

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INTRODUCTION

Numerous prominent fungal pathogens that affect major crops exhibit extensive geographic ranges [1–3]. These introduced fungal pathogens, which now thrive on physiologically- and phenologically-homogenous hosts planted over vast areas, have reached their current distribution following the colonization of a variety of environments, with demographic histories strongly constrained by the actions of their human vectors [4, 5]. As fungal pathogens established in new territories, they encountered new environmental conditions, new wild or domesticated host species, or new host varieties, potentially leading to local adaptation [6–9]. In ascomycetes, adaptation to new hosts (i.e. specialization) is often associated with the differentiation of new lineages or species, which is expected for pathogens that use senescent host tissues as a substrate for reproduction, with no or little migration of gametes between infection and mating [10–12]. The relative weakness of non-ecological (i.e. intrinsic) prezygotic reproductive barriers in plant pathogenic ascomycetes [13] may also facilitate the emergence of local adaptation through hybridization or the introgression of adaptive variants [14–17]. Molecular population genetic analyses of whole-genome polymorphism data can reveal the demographic history of invasive fungal pathogens, provide insight into the mechanisms underlying coevolutionary processes [4, 18–22], and thereby help the implementation of durably efficient disease management programmes [23–25]. Understanding how crop diseases spread, adapted, and co-evolved with their hosts requires a comprehensive picture of the evolutionary trajectory of fungal pathogens in conjunction with the (often complex) history of breeding, cultivation, and trade of their hosts [7, 26–29].

Pyrenophora teres is a major fungal pathogen of barley, present in all areas of barley cultivation [30–33]. *Pyrenophora teres* exists as two distinct lineages (called forms) that cause different symptoms. *Pyrenophora teres* form *teres* (Ptt) produces net-like lesions while *P. teres* f. *maculata* (Ptm) causes brown spots surrounded by chlorosis, thus referred to as 'net form net blotch' and 'spot form net blotch', respectively. Ptt and Ptm cross easily in lab conditions [34], their artificial hybrids are fertile [35], and they can produce clonal hybrid lineages in the field [36]. However, population genomic studies revealed neither pervasive admixture nor rampant gene flow [37–40], which suggests that ecologically-based reproductive barriers contribute to the maintenance of Ptt and Ptm in sympatry on the same host [41]. Ptt and Ptm diverged well before the onset of plant domestication and agriculture, likely in different regions and/or on different hosts, with relatively recent secondary contact on barley [42, 43]. Although Ptt and Ptm both have global distributions, their relative abundance changes over time and space, with one form generally predominant in a given region [44–46]. The genetic structure of Ptm has been elucidated regionally, but its global structure remains unknown [37, 47–49]. Analyses of whole-genome sequencing data revealed that Ptt is structured into different populations, some tending to be geographically restricted to different regions, while others are distributed across multiple continents [29, 50]. Demographic modelling showed that the population structure of Ptt was shaped by the history of domestication and worldwide spread of barley. However, besides the contingencies linked to the history of the host and their impact on population demography, the nature of the host features responsible for the structure of Ptt remains poorly known despite the significant spatiotemporal heterogeneity of the barley agrosystem. Barley is an ancient crop that adapted to a broad spectrum of agricultural environments during a process of widespread range extension [51]. Domesticated barley is genetically structured into different populations, and the observed associations between agronomically-relevant phenotypes and population structure suggest that selection has played a role in the origin and/or maintenance of population differentiation [52, 53]. In particular, domesticated barley populations can vary in terms of cold requirement, with winter barley that requires lower temperatures for vernalization, and can therefore be sown in winter or late fall, and spring barley that does not require low temperatures and can be sown in spring. Domesticated barley populations can also vary in terms of grain characteristics, which is reflected in the morphology of ears, with six-row barley more often used as an animal or human feed, and two-row barley favoured for malting and brewing. Some regions, such as France, have varied environmental characteristics, which allow the cultivation of different types of barley over large areas, providing ideal conditions to study the interplay between barley host diversity and the population structure of the pathogen. Here, we describe the population genetic structure of *P. teres* in France by using genotyping-by-sequencing (GBS) data. These data were used (1) to assess what are the forms of *P. teres* associated with barley crops in continental France and what is their distribution, (2) to investigate the contribution of various invasive populations from other continents to the genetic makeup of *P. teres* in France, and (3) to identify the host features associated with population differentiation.

METHODS

Sampling, isolation, DNA extraction, and genotyping-by-sequencing

Random sampling of symptomatic barley leaves was performed from 2018 to 2021 in barley nurseries and experimental fields. A total of 16 localities in 12 administrative divisions (i.e. *départements*) in France were included, and samples from several

varieties and different barley types in terms of vernalization and row number were obtained. After sampling, the symptomatic tissue was either processed immediately for single-conidium isolation, or preserved at 4°C until processing. Monosporic cultures were obtained by three steps: (i) a colony was obtained by the inoculation of a PDA Petri dish with one conidium obtained from the symptomatic tissue after incubation in a humid chamber, (ii) a quick DNA extraction was made using Chelex 100 by Bio-Rad, and the DNA was used to conduct a PCR using the primers and conditions developed by Leisova *et al*. [54] to screen for the presence of *Pyrenophora teres*, then, (iii) for the colonies in which the presence of the pathogen was confirmed, a single conidium was taken to produce new colonies that were used in the rest of the study. Alternatively, because some of the strains did not produce any more conidia after being obtained from the leaf, isolation was performed by cutting and transferring the tip of a hyphae.

DNA extraction was performed following the methodology proposed by Carlsen *et al*. [33] with some modifications. For each isolate obtained, five plugs were inoculated into a 250 ml Erlenmeyer containing 60 ml of modified Fries medium [33], which was incubated at 27°C and 120 r.p.m. for 7 days. The content of the Erlenmeyer was blended and mixed with an additional 60 ml of Fries medium and incubated for two more days under the same conditions. After incubation, the mycelium was rinsed with sterile distilled water and recovered using a piece of veil fabric as the filter (diameter of pores <0.3 mm). The liquid was removed by pressing the mycelium contained in the fabric with the help of paper towels. The mycelium was frozen at −20°C and lyophilized during 24–36 h, depending on the amount of humidity. The lyophilized tissue was placed in a 2 ml microcentrifuge tube and ground using liquid nitrogen and a drill with plastic blue pellet pestles (Sigma-Aldrich). Just the sufficient amount to reach the line of 200 µl of the tube was used for the extraction, following the protocol described by Carlsen *et al*. [33] but adding an extra RNAse treatment. The quality of the DNA was assessed using Nanodrop and electrophoresis on agarose gel, and the quantity was measured using the fluorometry method of the Qubit dsDNA High Sensitivity assay kit. The DNA samples were stored at −20°C until used.

Genotyping-by-sequencing (GBS) libraries were constructed using 100 ng of DNA for each isolate. DNA was digested with the ApeKI enzyme for 2 h at 75°C. A specific barcode was added to each sample using a ligation reaction (T4 DNA ligase; 30 min at 22°C). Barcoded samples (5 µl of each) were pooled, and DNA was amplified. Purification was performed to remove adaptors and primer dimers using the Wizard Genomic DNA Kit (Promega), and quality was verified with a bioanalyser by measuring the quantity and length (150–300 bp) of the fragments. Libraries were sequenced using NovaSeq 6000 Illumina technology. Sequencing reads (150 bp) were demultiplexed using GBSx 1.3 [55], and trimmed for barcodes and adaptors using Trimmomatic 0.39 [56]. Reads were inspected with Fastqc 0.11.9 and Multiqc 1.11 [57, 58].

Assignment to the two forms of *Pyrenophora teres* and estimation of nucleotide divergence

For taxonomic affiliation, GBS reads were mapped to a reference genome of each of the possible forms of *P. teres* [59, 60] using Bowtie 2.5 [61] with default parameters. SNP calling and filtering were performed using Bcftools 1.16 [62]. After plotting for each individual, using histograms, the distribution of mapping quality (MQ) and sequencing depth, filtering cutoffs were set at MQ ≥40, and sequencing depth by individual ≥3. Sites with more than 50% missing data were removed using Vcftools 0.1.16 [63]. The Variant Calling Files (VCFs; one file with Ptt as the reference, and one file with Ptm) were converted into FASTA pseudo-alignment files. Neighbour-net phylogenetic networks were constructed using Splitstree 4.17.2 [64] with FASTA pseudo-alignment files as input. The networks were built based on the p-distance (also called Hamming distance, which is the number of positions at which two vectors are different), as calculated by Splitstree, and isolates were assigned to the reference genome to which they were the closest according to this metric.

Divergence between the two forms was quantified by computing net divergence as $D_a = d_{XY} - ((\pi_{Ptt} + \pi_{Ptm})/2))$, with d_{XY} representing nucleotide divergence and π representing nucleotide diversity. The mapping of short reads from Ptt on a Ptm reference, and vice versa, resulted in a significant underestimate of nucleotide diversity in both forms (not shown); it was therefore not possible to estimate the net divergence between the two forms using SNP calling data. We thus used public genomic data for six isolates of Ptt and five of Ptm (Table S1, available in the online version of this article) to estimate D_a from the analysis of single-copy sequences identified with Busco 5.5.0 [65] after masking the genomes using RepeatMasker v4 [66] with repeat families obtained with RepeatModeler 2.0.3 [67]. The sequences of the 5562 single-copy genes identified in all eleven genomes were individually aligned using mafft 7 [68], and d_{XY} and π were computed using the EggLib 3.3.0 [69] Python package.

Population subdivision in Ptt

Given the low prevalence of Ptm, population subdivision was only analysed in Ptt. To minimize the divergence between the reference genome and the genotyped isolates, as none of the assembled genomes available before this work were closely related to the French Ptt population, we sequenced the whole genome of one of the French isolates and used the assembly as the reference genome for calling SNPs in aligned GBS reads.

The DNA of isolate FRA0042 was used to build a library with an insert size of 550 bp, using the TruSeq Nano DNA Library Preparation Kit (Illumina). The library was sequenced on an Illumina NovaSeq 6000 machine. Sequencing reads were inspected and trimmed using the same approach as with the GBS data, and assembled using different K-mer sizes using ABySS [70]. The chosen K-mer size was the one that maximized N50, L50, and assembly size (Table S2). The quality of the resulting assembly was assessed using BUSCO after masking the repeats using RepeatModeler and RepeatMasker. GBS reads were mapped onto the reference genome using Bowtie 2.5 [61] with default parameters and were first filtered to obtain good-quality variants using Bcftools 1.16 [62]. Quality filtering cutoffs were set at MQ ≥50, and sequencing depth by individual ≥3, after examination of the distribution of these statistics for each individual. Indels were filtered out (i.e. genotypes identified as indels were converted to missing data).

Population subdivision was inferred in R 4.2.1 using approaches that make no assumption of linkage or Hardy-Weinberg equilibrium: the clustering algorithm sNMF (sparse non-negative matrix factorization) implemented in the package LEA [71], and DAPC (Discriminant Analysis of Principal Components) implemented in the package poppr [72]. Only biallelic single-nucleotide polymorphisms (SNPs) that passed filtering cutoffs and with less than 50% missing genotypes were used in analyses of population subdivision. The host features and other factors associated with population differentiation were investigated using Principal Component Analysis (PCA) and Analysis of Molecular Variance (AMOVA) using the packages poppr and adegenet in R. The factors considered were the barley type, year of sampling, barley variety, site of origin, and the clusters identified with clustering algorithms.

Summary statistics of nucleotide variation

The dataset used to compute summary statistics included all genotypes passing quality cutoffs and indel filtering (i.e. the dataset combined single nucleotide polymorphisms, sites with missing data and monomorphic sites). Nucleotide diversity π, and nucleotide divergence d_{XY} were estimated in 100Kbp windows using the program pixy 1.27b [73], which provides unbiased estimates of diversity and divergence in the presence of missing data. Tajima's D was calculated in non-overlapping 100kbp windows using the Scikit-allel [74] Python package. Linkage disequilibrium decay (LDD) was assessed using the package PopLDdecay [75] by generating a pseudo-diploidized VCF.

Global population structure of Ptt

The evolutionary relationships between the French isolates of Ptt and populations from other countries were determined by combining our GBS data with previously released whole-genome sequencing data (*n*=103) [29], as well as with new GBS data we generated for six isolates from Canada [76] and nine isolates from the USA (Table S3). The fifteen isolates were treated with the same methodology as the French isolates for growth, DNA extraction, and genotyping.

All genomic and GBS reads were mapped onto the Ptt reference genome [59]. This reference genome was used because it was previously used for the dataset we combined our data with [29], and because it is one of the most complete Ptt genomes publicly available. The two quality-filtered VCFs (one obtained with GBS sequencing reads, the other with whole genome sequencing reads) were combined using Bcftools merge. The resulting VCF was further filtered by removing indels, and keeping only biallelic variants with less than 5% missing genotypes. The final file comprised a total of 322 isolates from various countries, including the USA (*n*=29), Canada (*n*=6), Morocco (*n*=27), Iran (*n*=9), Azerbaijan (*n*=21), Denmark (*n*=5), and more samples from France (*n*=204 with GBS data [this study] and *n*=21 with whole-genome data [29]) (Table S3). Population subdivision was analysed by PCA with the package poppr in R, and using the clustering algorithm sNMF as described above for the French population.

RESULTS AND DISCUSSION

Ptt outpaces Ptm in frequency as a barley pathogen in France

A total of 207 isolates of *Pyrenophora teres* were obtained from four collection campaigns in 2018 (*n*=39), 2019 (*n*=11), 2020 (*n*=94), and 2021 (*n*=63). Isolates represented 16 localities distributed in 12 administrative divisions of France (i.e. *départements*), and three to 48 isolates were obtained per locality depending on symptomatic tissue availability. Isolates were obtained from more than 15 barley varieties representing two-row spring barley (*n*=26) and winter barley (*n*=181). Information about the type of barley in terms of vernalization requirements (winter vs. spring) was available for all 207 isolates, while the name and row number of the barley variety were available for 204 isolates. Sampling information is provided in Table S4.

The GBS reads covered 30 and 32%, respectively, of the reference genomes of Ptt and Ptm [59, 60] (Table S5). SNP calling and filtering identified 1.1e5 SNPs for the dataset with Ptt as the reference genome, and 8.4e4 SNPs for the dataset with Ptm as the reference genome. Neighbour-net networks built from these datasets showed that most of the isolates (*n*=204) were closely related to the reference genome of Ptt (Fig. 1a), with a p-distance from the Ptt reference genome of 0.08 differences/polymorphic site on average. Only three isolates were closely related to the reference genome of Ptm (Fig. 1b), with a

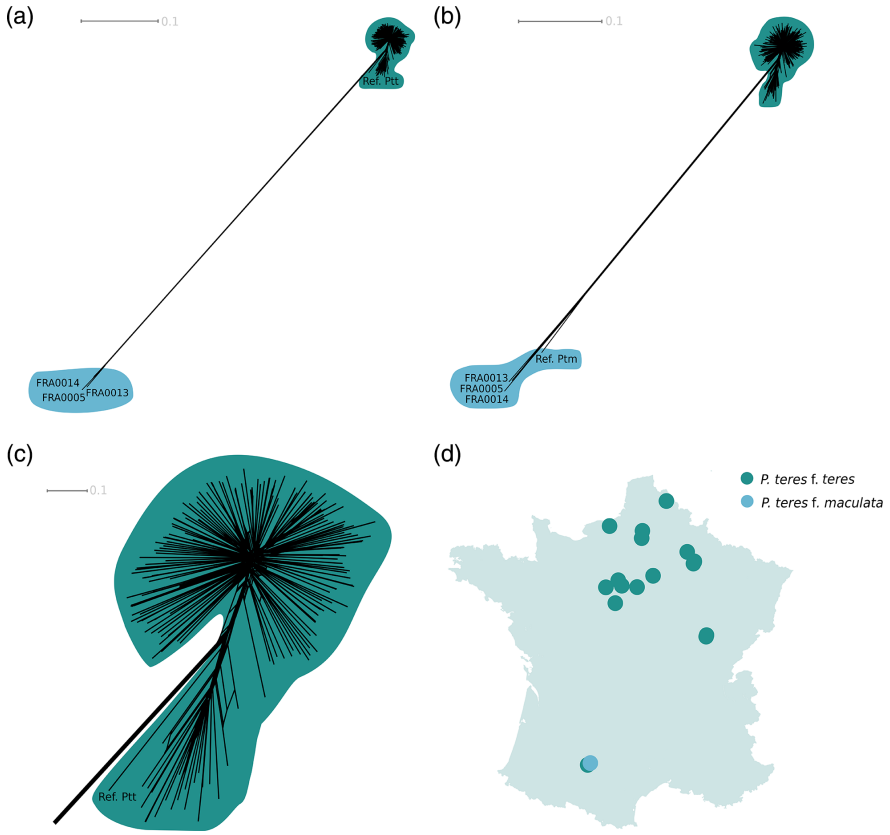

Fig. 1. Identification of the two different forms of *Pyrenophora teres* present among isolates from France and their geographic distribution inferred by the analysis of neighbour-net networks generated from genotyping-by-sequencing (GBS) data. Two main groups are highlighted, the green group showing isolates identified as Ptt, and the light blue group showing isolates identified as Ptm. Isolate names are not shown for the Ptt group. (a.) Network constructed from the dataset obtained by mapping GBS reads onto the reference genome of *Pyrenophora teres* f. *teres* (Ptt), which is referred to as 'Ref. Ptt'. (b.) Network constructed from the dataset obtained by mapping GBS reads onto the reference genome of *Pyrenophora teres* f. *maculata*, which is referred to as 'Ref Ptm'. (c.) Closer view of the group formed by isolates that were the most closely related to the Ptt reference genome. (d.) Map of geographic distribution of Ptt and Ptm in France. Dots correspond to sampling sites, and their colour to the different taxa found at each site. The two dots at the bottom left of the map correspond to the same geographical site; one of the two dots was moved slightly to avoid complete overlap. The p-distance scale bar (in number of differences per polymorphic site) is displayed at the top of panels a, b, and c.

p-distance from the Ptm reference genome of 0.2 differences/polymorphic site on average (Tables S6 and S7). Net divergence (D_a) between the two forms, as estimated from alignments of single-copy orthologs identified in publicly available genomes, was 0.006 /bp (s.d. 0.016) (Table S8), which can be used to estimate that the two forms diverged 0.128 M years ago, assuming a substitution rate of 2.2e-8 per base pair [77, 78]. The estimated level of net divergence between the two forms of *P. teres* places them in the grey zone of speciation (0.5–2% net synonymous divergence), in which taxonomy is often controversial, and hybridization possible [79]. Given the low frequency at which Ptm was found, more extensive sampling would be needed to confidently dismiss the possibility of hybridization between the two forms. However, the absence of reticulations along the branches connecting Ptt and Ptm indicated the absence of hybrids in our dataset (Fig. 1a, b). This suggests that intrinsic postzygotic barriers (i.e. incompatibilities [41]), as well as possible extrinsic prezygotic barriers that remain to be identified (e.g. adaptation to the host [6, 80]), may contribute efficiently to the maintenance of the two forms on the same crop species without pervasive hybridization.

We showed that Ptt is largely dominant over Ptm in France, with only 1.4% of isolates assigned to Ptm, which suggests there was a swap during the past three decades since the most prevalent form of *P. teres* in France 30 years ago was Ptm [81]. The study of this pathogen in other regions showed that the prevalence of the forms can rapidly change depending on control strategies like the presence of resistant plant material [82, 83]. Our findings suggest that the prevalence of the two forms in France could change again in the future – as fast as a couple of decades, according to the contrasting results of a previous assessment [60] – highlighting the need for regular monitoring of *P. teres* populations.

Ptt was present in all of the sampled areas in France, whereas Ptm was only found in the southwest of the country, where it coexisted with Ptt in the same field. This pattern is similar to what has been reported in other regions of Eastern and Northern Europe, North America, Italy, the Maghreb, and Egypt, where the two forms are present, but Ptt clearly predominates [37, 46, 64, 80, 83–92]. Our results, however, contrast with what was observed in East Africa, South Africa, and Southeastern Australia, where Ptm is more prevalent [44, 82, 93]. The differences in prevalence could be explained by environmental conditions, or by the heterogeneity in the distribution of varieties with differences in levels of resistance to the two net blotch pathogens [80]. Based on our sampling, we cannot favour one hypothesis over the other due to the low prevalence of Ptm. Our findings do indicate, however, that it is possible for both forms to coexist on different hosts in sympatry, which opens up opportunities for future experiments to test the hypothesis that the two forms of net blotch pathogens are adapted to different populations of barley.

Pyrenophora teres f. *teres* is subdivided into two main clusters in France

Due to the low occurrence of the Ptm form among the isolates, exploring its population structure was not feasible. We focused our investigations on the *n*=204 Ptt isolates, for which we analysed population structure by mapping GBS reads onto the assembled genome of the French isolate FRA0042. The genome assembly of FRA0042 consisted of 6.8e4 contigs larger than 1 Mb, and was characterized by an N50 of 328 Kb, an L50 of 30, and a Busco score of 94.4%. On average across isolates, 43% of the 32 Mb reference genome was covered by GBS reads (Table S5). The final filtered dataset included 6.3e4 SNPs.

Analyses with the sNMF algorithm and Discriminant Analysis of Principal Components (DAPC) identified two well-differentiated clusters within Ptt. In analyses with sNMF, cross-entropy decreased monotonically with increasing of the K value, which corresponds to the number subdivisions (i.e. genetic clusters) in the dataset (Fig. S1 and Table S9). However, only the model with K=2 clusters showed two well-separated groups (Fig. 2a). Increasing K did not identify clear new clusters, and this simply resulted in a breakup of ancestry proportions between different clusters. In the DAPC, the minimum value of the Bayesian information criterion (BIC) was observed at K=3 (Fig. S1), but only K=2 presented a clear pattern of subdivision (Fig. 2). Clusters identified in models with K≥3 exhibited significant overlap along the second discriminant function (Figs 2e and S1). Analysis of molecular variance (AMOVA) revealed that differentiation was relatively high between clusters (Table S10), with 45% of the total variation being distributed between clusters. Net divergence, which scales linearly with time under a strict isolation model [78], was D_a=0.0014 between the two clusters, which represents 20% of the estimated net divergence between Ptt and Ptm (Table S11). Despite the recent divergence between clusters, admixture appeared limited, with only 0.5% of isolates presenting ancestry proportions between 0.2 and 0.8 in analyses with sNMF (Table S4).

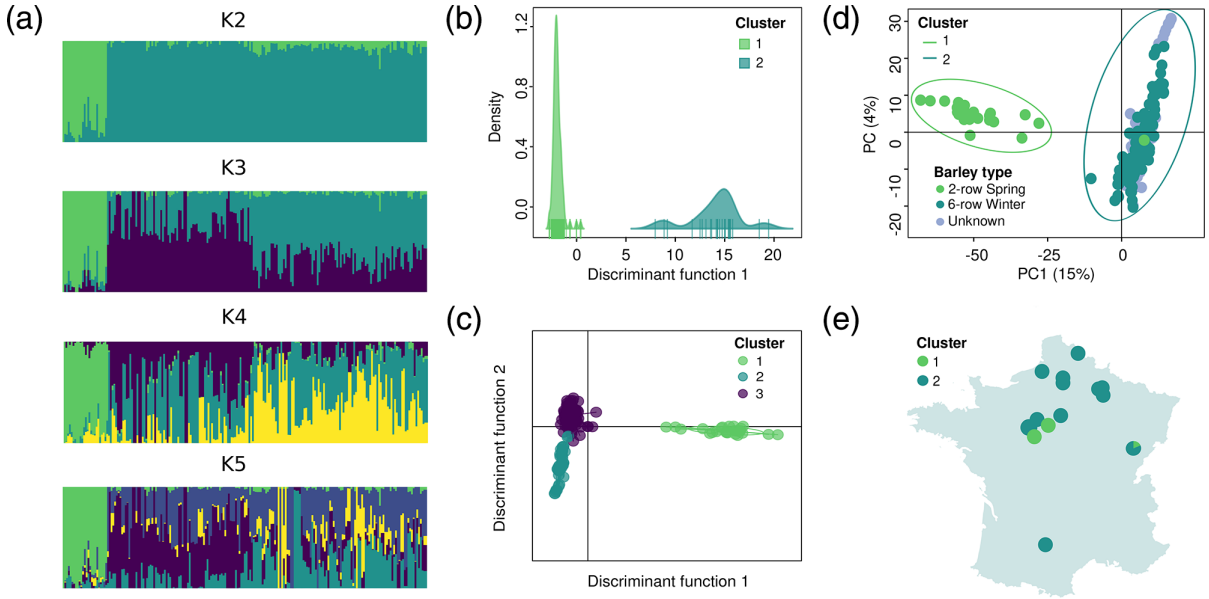

Fig. 2. Population subdivision in *Pyrenophora teres* f. *teres* from France, estimated from genotyping-by-sequencing data for 204 isolates from 16 sampling sites. (a.) Ancestry proportions in K clusters estimated with sNMF, with each isolated represented by a segment subdivided in K intervals. (b.) Discriminant Analysis of Principal Components (DAPC) with two clusters, based on 20 principal components. (c.) DAPC with three clusters, based on 25 principal components. (d.) Principal Component Analysis of genotype data, with each isolate represented by a dot coloured based on its type of barley of origin, and ellipses showing clusters of isolates identified using DAPC and sNMF analysis. (e.) Geographical distribution of the two main *Pyrenophora teres* f. *teres* clusters in France, with pie charts representing the proportion of isolates from each cluster at each sampling site.

The two main clusters identified in Ptt displayed similar genetic structures. Summary statistics were calculated based on 2.5e6 sites passing quality filters. The two clusters displayed close estimates of nucleotide diversity (cluster 1: π=0.00434; cluster 2: π=0.00433), despite differences in Tajima's D suggesting contrasted demographic histories (cluster 1: D=1.47; cluster 2: D=−0.72; Table S11). Patterns of linkage disequilibrium decay were consistent with a history of recombination, and thus sexual reproduction, both at the scale of Ptt and clusters within Ptt, with linkage disequilibrium reaching half of its maximum value within less than 5e3 bp (Fig. S2). Analyses of molecular variances revealed limited sub-structure within clusters, with more than 90% of variation distributed within locations or varieties (Table S12), which is consistent with the lack of clear subdivision observed in models with K≥2 clusters with the sNMF algorithm and the DAPC. Together, these analyses indicate a history of recent and widespread gene flow within clusters, and therefore the lack of strong barriers to dispersal and the absence of lineages strongly differentiated by the effect of local adaptation to host varieties.

The two major clusters identified within French Ptt were associated with different types of barley. We relied on PCAs to visualize the relationship between population structure and the isolates' metadata, i.e. barley variety, barley type, year of sampling, and site of origin. We found that the type of barley was the factor most clearly associated with population subdivision, with the first cluster being formed almost entirely by isolates from two-row spring barley, except isolate FRA0049, and the second cluster grouping isolates from six-row winter barley (Figs 2d and S3, Tables S4 and S9). The two clusters were thus distributed according to the location of the types of barley sampled, as shown in Fig. 2e. In our sampling, only one type of barley was generally sampled per location, and consequently, only one cluster was detected. Interestingly, there was only one area (*Côte d'Or*, in Burgundy, *n*=25) in which both clusters were present (cluster 1: *n*=6; cluster 2: *n*=18) and no significant difference in the membership proportions estimated by sNMF between the isolates from this area and other regions (*n*=179) was found, neither when assigned to cluster one nor to cluster two (Wilcoxon's test p-value=0.562 and 0.067 respectively, see Fig. S4). This indicates that the two populations can coexist without any detectable increase in admixture levels, and adaptation to the host may play a role in maintaining these populations. However, grain type and vernalization requirement were associated in the barley varieties we sampled (i.e. all two-row varieties were spring barleys, and all six-row varieties were winter barleys, the only two samples from two-row winter barley obtained were classified as Ptm), and we, therefore, cannot predict which of these two traits would be more likely to be associated with the differentiation between the two clusters in a more diverse barley panel.

The global structure of Ptt is associated with the vernalization requirements of barley varieties

To determine where the French isolates branch into the global structure of Ptt, but also to further evaluate the association between barley type and population structure, we combined our GBS dataset with publicly available whole-genome data [29] and new GBS data we generated by sequencing extra samples from North America. The combined dataset, including 133609 SNPs without missing data, was first submitted to PCA. Principal components (PCs) 1, 2, and 3, represented 18, 8, and 5% of total variation, respectively. While PC2 did not reveal any evident association between differentiation and metadata (host or region of origin; Fig. S5), PC1 and PC3 subdivided the dataset into several clusters that mirrored relatively well geographical origins (Fig. 3a), consistent with previous findings [29]. PC1 identified three clusters: a first cluster mostly comprised of isolates collected in Azerbaijan and isolates collected in France from six-row winter varieties, a second cluster mostly comprised of isolates from Iran, and a third cluster grouping the remaining isolates. PC3 split the third cluster identified along PC1 into one cluster mostly comprised of isolates from North America, one cluster mostly comprised of isolates from North Africa, and one last cluster mostly comprised of isolates collected from two-row spring varieties in France. Our results thus demonstrate that what has previously been called the 'Caucasus cluster' [29] is also present at a relatively high frequency in Europe.

Although some geographical component to population structure was observed, another factor associated with differentiation within global populations of Ptt was the type of barley, and more precisely, the vernalization requirement. We were able to collect metadata for 46% of isolates from the entire genome dataset, which, when combined with the GBS isolates with known barley type, formed a new dataset where the type of grain and vernalization requirement were no longer strictly associated. This allowed us to independently assess the association of population differentiation with each factor using PCA. Although neither of the two principal components, PC1 and PC3 could distinguish between isolates from two-row or six-row barley (Fig. 3b), PC1 was able to separate isolates from winter and spring barley in an almost mutually exclusive manner (Fig. 3c).

Further characterization of the global population of Ptt was performed using the sNMF clustering algorithm (Fig. 3d). With K=2 clusters, the results of the PCA and sNMF clustering were similar, with ancestry proportions defining two main groups that corresponded to the same two groups identified with PC1: one first group formed by isolates from spring barley, and a second group formed almost exclusively by isolates from winter barley. With increasing K values, the cluster of winter barley was always maintained; however, the group of spring barley could be further subdivided into three well-differentiated groups, the first group corresponded to almost entirely North African isolates, some isolates from the Middle East, and one from the USA, the second group consisted of isolates from Europe, Caucasus, the Middle East and some from North Africa, and the third group was comprised of isolates from North America only.

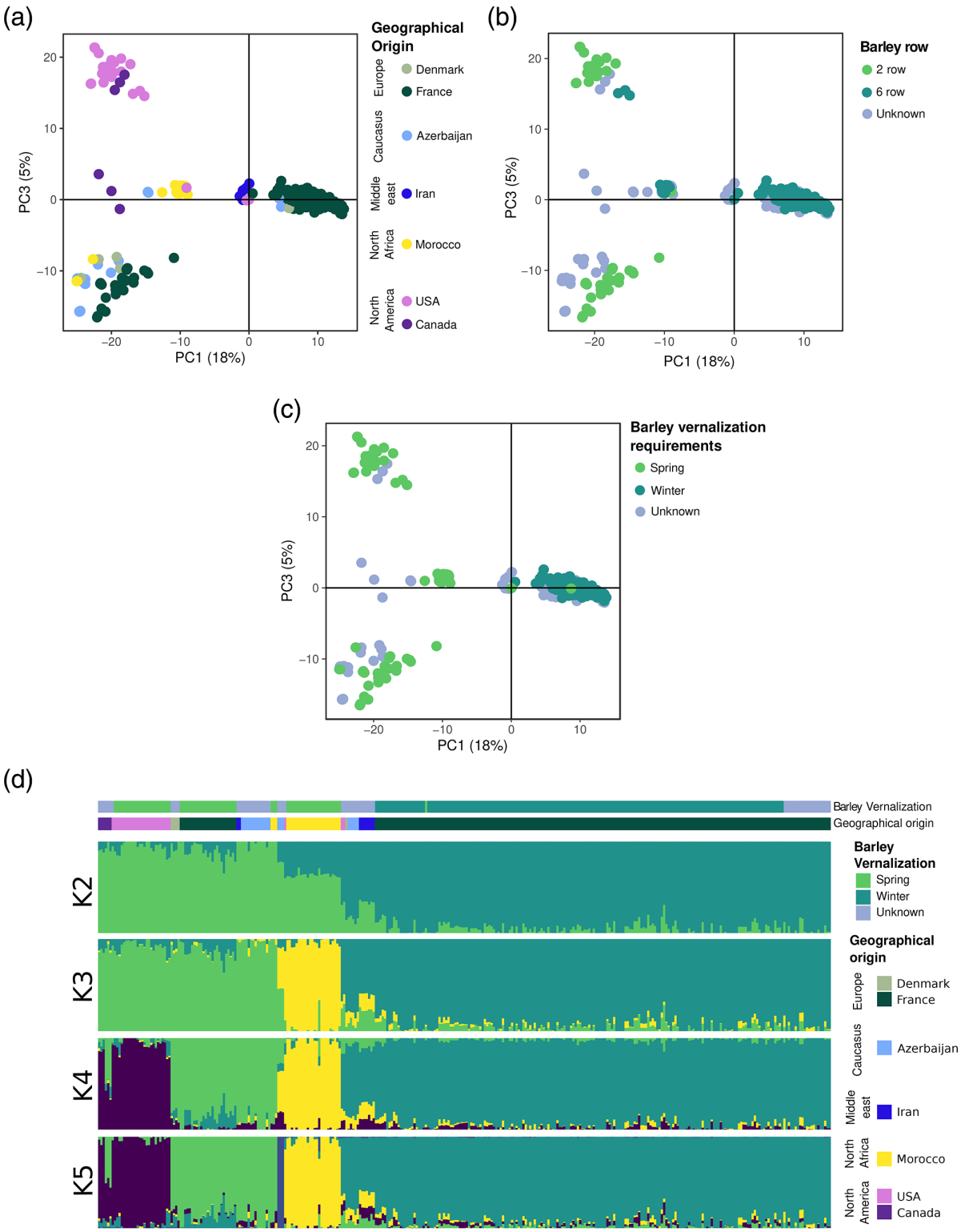

Fig. 3. Association between population structure in *Pyrenophora teres* f. *teres* isolates collected globally, and the vernalization requirement of their barley host, as visualized using principal components analysis of single-nucleotide polymorphisms. Each dot represents an isolate. (a.) Isolates coloured by country of origin. (b.) Isolates coloured according to the number of rows of the barley variety from which they were obtained. (c.) Isolates coloured according to the vernalization requirement of the barley variety from which they were obtained. (d.) Ancestry proportions in K clusters estimated with sNMF, with each isolated represented by a segment subdivided in K intervals, barley type from where they were obtained along with the geographical origin are indicated on the top.

Our analysis thus suggests that the previously described Caucasian cluster [29], from which the spring barley population of North Africa originated, and from which the spring barley populations of North America and Europe diverged relatively long ago, is actually a population associated with winter barley and not specific to the Caucasus. These findings do not contradict previous conclusions [29] but rather provide additional insights into the evolutionary history of Ptt by suggesting that adaptation to hosts, or their growing conditions, would have played a crucial role in the emergence of Ptt.

CONCLUDING REMARKS

A growing body of evidence suggests that adaptation plays a major role in the successful emergence and dissemination of pathogens across heterogeneous environments in time and space [4, 7, 8, 12, 16, 94, 95]. In our study, we used a molecular ecology approach to show that there are genetic differences between populations of Ptt that are associated with winter and spring barley in France, as well as on a broader geographical scale. The existence of such a pattern suggests that the populations of the pathogen are differentially adapted to the two types of barley, and/or to the conditions in which they are cultivated. This work opens up numerous research perspectives to determine what are the barriers, particularly prezygotic [10, 11], that contribute to the maintenance of the two populations, and in particular, what is the relative contribution of adaptation to the host or adaptation to abiotic conditions. Our results also highlight the importance of testing new resistant barley materials with both spring and winter barley isolates of Ptt, especially in areas where both types of barley are used.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Bebber DP, Gurr SJ. Crop-destroying fungal and oomycete pathogens challenge food security. *Fungal Genet Biol* 2015;74:62–64.
2. Palm ME. Systematics and the impact of invasive fungi on agriculture in the United States. *BioScience* 2001;51:141.
3. Palm ME, Rossman AY. *Invasion Pathways of Terrestrial Plant-Inhabiting Fungi. Bioinvasions: Pathways, Vectors, and Management Strategies*. New York, USA: Island Press, New York; 2003, pp. 31–43.
4. Gladieux P, Feurtey A, Hood ME, Snirc A, Clavel J, *et al*. The population biology of fungal invasions. *Mol Ecol* 2015;24:1969–1986.
5. Bebber DP, Holmes T, Gurr SJ. The global spread of crop pests and pathogens. *Glob Ecol Biogeogr* 2014;23:1398–1407.
6. Gladieux P, Guérin F, Giraud T, Caffier V, Lemaire C, *et al*. Emergence of novel fungal pathogens by ecological speciation: importance of the reduced viability of immigrants. *Mol Ecol* 2011;20:4521–4532.
7. Feurtey A, Lorrain C, McDonald MC, Milgate A, Solomon PS, *et al*. A thousand-genome panel retraces the global spread and adaptation of a major fungal crop pathogen. *Nat Commun* 2023;14:1059.
8. Thierry M, Charriat F, Milazzo J, Adreit H, Ravel S, *et al*. Maintenance of divergent lineages of the rice blast fungus *Pyricularia oryzae* through niche separation, loss of sex and post-mating genetic incompatibilities. *PLoS Pathog* 2022;18:e1010687.
9. McMullan M, Percival-Alwyn L, Sawford K, Kaithakottil G, Grey M, *et al*. Analysis of wild plant pathogen populations reveals a signal of adaptation in genes evolving for survival in agriculture in the beet rust pathogen (*Uromyces beticola*). *Evol Biol* 2021.
10. Servedio MR, Van Doorn GS, Kopp M, Frame AM, Nosil P. Magic traits in speciation: 'magic' but not rare? *Trends Ecol Evol* 2011;26:389–397.
11. Giraud T, Villaréal L, Austerlitz F, Le Gac M, Lavigne C. Importance of the life cycle in sympatric host race formation and speciation of pathogens. *Phytopathology* 2006;96:280–287.
12. Giraud T, Gladieux P, Gavrilets S. Linking the emergence of fungal plant diseases with ecological speciation. *Trends Ecol Evol* 2010;25:387–395.
13. Le Gac M, Giraud T. Existence of a pattern of reproductive character displacement in *Homobasidiomycota* but not in *Ascomycota*. *J Evol Biol* 2008;21:761–772.
14. Menardo F, Praz CR, Wyder S, Ben-David R, Bourras S, *et al*. Hybridization of powdery mildew strains gives rise to pathogens on novel agricultural crop species. *Nat Genet* 2016;48:201–205.
15. McMullan M, Rafiqi M, Kaithakottil G, Clavijo BJ, Bilham L, *et al*. The ash dieback invasion of Europe was founded by two genetically divergent individuals. *Nat Ecol Evol* 2018;2:1000–1008.
16. Hessenauer P, Fijarczyk A, Martin H, Prunier J, Charron G, *et al*. Hybridization and introgression drive genome evolution of Dutch elm disease pathogens. *Nat Ecol Evol* 2020;4:626–638.
17. Rogério F, Van Oosterhout C, Ciampi-Guillardi M, Correr FH, Hosaka GK, *et al*. Means, motive and opportunity for biological invasions: genetic introgression in a fungal pathogen. *Mol Ecol* 2023;32:2428–2442.
18. Ebert D, Fields PD. Host-parasite co-evolution and its genomic signature. *Nat Rev Genet* 2020;21:754–768.
19. Gladieux P, Ropars J, Badouin H, Branca A, Aguileta G, *et al*. Fungal evolutionary genomics provides insight into the mechanisms of adaptive divergence in eukaryotes. *Mol Ecol* 2014;23:753–773.
20. Stukenbrock EH, Bataillon T. A population genomics perspective on the emergence and adaptation of new plant pathogens in agro-ecosystems. *PLoS Pathog* 2012;8:e1002893.

21. Barrett LG, Thrall PH, Burdon JJ, Linde CC. Life history determines genetic structure and evolutionary potential of host-parasite interactions. *Trends Ecol Evol* 2008;23:678–685.
22. Stukenbrock EH, McDonald BA. The origins of plant pathogens in agro-ecosystems. *Annu Rev Phytopathol* 2008;46:75–100.
23. Thrall PH, Oakeshott JG, Fitt G, Southerton S, Burdon JJ, *et al*. Evolution in agriculture: the application of evolutionary approaches to the management of biotic interactions in agro-ecosystems. *Evol Appl* 2011;4:200–215.
24. Zhan J, Thrall PH, Burdon JJ. Achieving sustainable plant disease management through evolutionary principles. *Trends Plant Sci* 2014;19:570–575.
25. Williams PD. Darwinian interventions: taming pathogens through evolutionary ecology. *Trends Parasitol* 2010;26:83–92.
26. LeBlanc N, Cubeta MA, Crouch JA. Population genomics trace clonal diversification and intercontinental migration of an emerging fungal pathogen of boxwood. *Phytopathology* 2021;111:184–193.
27. Dutech C, Barrès B, Bridier J, Robin C, Milgroom MG, *et al*. The chestnut blight fungus world tour: successive introduction events from diverse origins in an invasive plant fungal pathogen. *Mol Ecol* 2012;21:3931–3946.
28. Sotiropoulos AG, Arango-Isaza E, Ban T, Barbieri C, Bourras S, *et al*. Global genomic analyses of wheat powdery mildew reveal association of pathogen spread with historical human migration and trade. *Nat Commun* 2022;13:4315.
29. Taliadoros D, Feurtey A, Wyatt N, Barrès B, Gladieux P, *et al*. Emergence and spread of the barley net blotch pathogen coincided with crop domestication and cultivation history. *PLoS Genet* 2024;20:e1010884.
30. Liu Z, Ellwood SR, Oliver RP, Friesen TL. Pyrenophora teres: profile of an increasingly damaging barley pathogen. *Mol Plant Pathol* 2011;12:1–19.
31. Jayasena KW, Van Burgel A, Tanaka K, Majewski J, Loughman R. Yield reduction in barley in relation to spot-type net blotch. *Austral Plant Pathol* 2007;36:429.
32. Galano T, Bultosa G, Fininsa C. Malt quality of 4 barley (*Hordeum vulgare L.*) grain varieties grown under low severity of net blotch at Holetta, West Shewa, Ethiopia. *Afri J Biotechnol* 2011;10:797–806.
33. Carlsen SA, Neupane A, Richards JK, Faris JD, *et al*. Characterizing the *Pyrenophora teres f. maculata*–barley interaction using pathogen genetics. *G3: Genes, Genomes, Genetics* 2017;7:2615–2626.
34. Smedegård-Petersen V. Inheritance of genetic factors for symptoms and pathogenicity in hybrids of *Pyrenophora teres* and *Pyrenophora graminea*. *J Phytopathol* 1977;89:193–202.
35. Dahanayaka BA, Vaghefi N, Snyman L, Martin A. Investigating in vitro mating preference between or within the two forms of *Pyrenophora teres* and its hybrids. *Phytopathology* 2021;111:2278–2286.
36. Turo C, Mair W, Martin A, Ellwood S, Oliver R, *et al*. Species hybridisation and clonal expansion as a new fungicide resistance evolutionary mechanism in *Pyrenophora teres* spp. *Evol Biol* 2021.
37. Akhavan A, Turkington TK, Kebede B, Xi K, Kumar K, *et al*. Genetic structure of *Pyrenophora teres* f. teres and *P. teres* f. *maculata* populations from western Canada. *Eur J Plant Pathol* 2016;146:325–335.
38. Çelik AO, Ölmez F, Karakaya A. Mating type idiomorphs of *Pyrenophora teres* in Turkey. *Zemdirbyste-Agriculture* 2018.
39. Poudel B, McLean MS, Platz GJ, McIlroy JA, Sutherland MW, *et al*. Investigating hybridisation between the forms of *Pyrenophora teres* based on Australian barley field experiments and cultural collections. *Eur J Plant Pathol* 2019;153:465–473.
40. Leišova L, Minarı˘ková V, Kučera L, Ovesná J. Genetic diversity of *Pyrenophora teres* isolates as detected by AFLP analysis. *J Phytopathol* 2005;153:569–578.
41. Yuzon JD, Wyatt NA, Vasighzadeh A, Clare S, Navratil E, *et al*. Hybrid inferiority and genetic incompatibilities drive divergence of fungal pathogens infecting the same host. *Genetics* 2023;224:iyad037.
42. Rau D, Attene G, Brown AHD, Nanni L, Maier FJ, *et al*. Phylogeny and evolution of mating-type genes from Pyrenophora teres, the causal agent of barley "net blotch" disease. *Curr Genet* 2007;51:377–392.
43. Ellwood SR, Syme RA, Moffat CS, Oliver RP. Evolution of three *Pyrenophora* cereal pathogens: recent divergence, speciation and evolution of non-coding DNA. *Fungal Genet Biol* 2012;49:825–829.
44. Louw J, Holz G. Relative importance of the barley net blotch pathogens *Pyrenophora teres* f. *teres* (net-type) and *P. teres* f. *maculata* (spot-type) in South Africa. *Afr Plant Prot* 1996;2:89–95.
45. McLean MS, Howlett BJ, Hollaway GJ. Epidemiology and control of spot form of net blotch (*Pyrenophora teres* f. *maculata*) of barley: a review. *Crop Pasture Sci* 2009;60:303.
46. Liu ZH, Friesen TL. Identification of *Pyrenophora teres* f. *maculata*, causal agent of spot type net blotch of barley in North Dakota. *Plant Dis* 2010;94:480.
47. Liu ZH, Zhong S, Stasko AK, Edwards MC, Friesen TL. Virulence profile and genetic structure of a North Dakota population of *Pyrenophora teres* f. *teres*, the causal agent of net form net blotch of barley. *Phytopathology* 2012;102:539–546.
48. Serenius M, Manninen O, Wallwork H, Williams K. Genetic differentiation in *Pyrenophora teres* populations measured with AFLP markers. *Mycol Res* 2007;111:213–223.
49. Vasighzadeh A, Sharifnabi B, Javan-Nikkhah M, Seifollahi E, Landermann-Habetha D, *et al*. Population genetic structure of four regional populations of the barley pathogen *Pyrenophora teres* f. *maculata* in Iran is characterized by high genetic diversity and sexual recombination. *Plant Pathol* 2021;70:735–744.
50. Dahanayaka BA, Vaghefi N, Knight NL, Bakonyi J, Prins R, *et al*. Population structure of *Pyrenophora teres* f. *teres* barley pathogens from different continents. *Phytopathology* 2021;111:2118–2129.
51. Zohary D, Hopf M. *Domestication of Plants in the Old World*. Oxford University Press, 2000.
52. Jones H, Civáň P, Cockram J, Leigh FJ, Smith LM, *et al*. Evolutionary history of barley cultivation in Europe revealed by genetic analysis of extant landraces. *BMC Evol Biol* 2011;11:1–12.
53. Russell J, Mascher M, Dawson IK, Kyriakidis S, Calixto C, *et al*. Exome sequencing of geographically diverse barley landraces and wild relatives gives insights into environmental adaptation. *Nat Genet* 2016;48:1024–1030.
54. Leisova L, Minarikova V, Kucera L, Ovesna J. Quantification of *Pyrenophora teres* in infected barley leaves using real-time PCR. *J Microbiol Methods* 2006;67:446–455.
55. Herten K, Hestand MS, Vermeesch JR, Van Houdt JKJ. GBSX: a toolkit for experimental design and demultiplexing genotyping by sequencing experiments. *BMC Bioinformatics* 2015;16:73.
56. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–2120.
57. Andrews S. *FastQC: A Quality Control Tool for High Throughput Sequence Data*. Cambridge, United Kingdom: Babraham Bioinformatics, Babraham Institute; 2010.
58. Ewels P, Magnusson M, Lundin S, Käller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* 2016;32:3047–3048.
59. Wyatt NA, Richards JK, Brueggeman RS, Friesen TL. Reference assembly and annotation of the *Pyrenophora teres* f. *teres* isolate 0-1. *G3: genes, Genomes, Genetics* 2018;8:1–8.
60. Wyatt NA, Friesen TL. Four reference quality genome assemblies of *Pyrenophora teres* f. *maculata*: a resource for studying the barley spot form net blotch interaction. *Mol Plant Microbe Interact* 2021;34:135–139.
61. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012;9:357–359.
62. Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* 2011;27:2987–2993.
63. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, *et al*. Twelve years of SAMtools and BCFtools. *Gigascience* 2021;10:giab008.

64. Douiyssi A, Rasmusson DC, Roelfs AP. Responses of barley cultivars and lines to isolates of *Pyrenophora teres*. *Plant Dis* 1998;82:316–321.
65. Manni M, Berkeley MR, Seppey M, Zdobnov EM. BUSCO: assessing genomic data quality and beyond. *Curr Protoc* 2021;1:e323.
66. Smit AFA, Hubley R, Green P. Repeatmasker Open-4.0. 2013–2015. 2015.
67. Smit AFA, Hubley R. 2015 Repeatmodeler Open-1.0. repeat Masker; 2008. http://www repeatmasker org
68. Katoh K, Toh H. Recent developments in the MAFFT multiple sequence alignment program. *Brief Bioinform* 2008;9:286–298.
69. Siol M, Coudoux T, Ravel S, De Mita S. EggLib 3: a python package for population genetics and genomics. *Mol Ecol Resour* 2022;22:3176–3187.
70. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJM, *et al*. ABySS: a parallel assembler for short read sequence data. *Genome Res* 2009;19:1117–1123.
71. Frichot E, Mathieu F, Trouillon T, Bouchard G, François O. Fast and efficient estimation of individual ancestry coefficients. *Genetics* 2014;196:973–983.
72. Kamvar ZN, Tabima JF, Grünwald NJ. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2014;2:e281.
73. Korunes KL, Samuk K. pixy: unbiased estimation of nucleotide diversity and divergence in the presence of missing data. *Mol Ecol Resour* 2021;21:1359–1368.
74. Miles A, Io BP, Murillo R, Ralph P, Harding N, *et al*. Cggh/Scikit-Allel: V1. 3.3 (version V1. 3.3). *Zenodo* 2021;3:3.
75. Zhang C, Dong S-S, Xu J-Y, He W-M, Yang T-L. PopLDdecay: a fast and effective tool for linkage disequilibrium decay analysis based on variant call format files. *Bioinformatics* 2019;35:1786–1788.
76. Akhavan A, Turkington TK, Askarian H, Tekauz A, Xi K, *et al*. Virulence of *Pyrenophora teres* populations in western Canada. *Can J Plant Pathol* 2016;38:183–196.
77. Latorre SM, Reyes-Avila CS, Malmgren A, Win J, Kamoun S, *et al*. Differential loss of effector genes in three recently expanded pandemic clonal lineages of the rice blast fungus. *BMC Biol* 2020;18:88.
78. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci U S A* 1979;76:5269–5273.
79. Roux C, Fraïsse C, Romiguier J, Anciaux Y, Galtier N, *et al*. Shedding light on the grey zone of speciation along a continuum of genomic divergence. *PLoS Biol* 2016;14:e2000234.
80. Serenius M, Mironenko N, Manninen O. Genetic variation, occurrence of mating types and different forms of *Pyrenophora teres* causing net blotch of barley in Finland. *Mycol Res* 2005;109:809–817.
81. Arabi MI, Barrault G, Sarrafi A, Albertini L. Variation in the resistance of barley cultivars and in the pathogenicity of *Drechslera teres* f. sp. *maculata* and D. *teres* f. sp. *teres* isolates from France. *Plant Pathol* 1992;41:180–186.
82. McLean MS, Howlett BJ, Hollaway GJ. Spot form of net blotch, caused by *Pyrenophora teres* f. *maculata*, is the most prevalent foliar disease of barley in Victoria, Australia. *Austral Plant Pathol* 2010;39:46.
83. Marshall JM, Kinzer K, Brueggeman RS. First report of *Pyrenophora teres* f. maculata the cause of spot form net blotch of barley in Idaho. *Plant Dis* 2015;99:1860.
84. Lammari H-I, Rehfus A, Stammler G, Fellahi ZEA, Benbelkacem A, *et al*. Occurrence and frequency of spot form and net form of net blotch disease of barley in Algeria. *J Plant Dis Prot* 2020;127:35–42.
85. Cherif M, Harrabi M, Morjane H. Distribution and importance of wheat and barley diseases in Tunisia 1989 to 1991. *Rachis* 1994;13:25–34.
86. Rau D, Brown AHD, Brubaker CL, Attene G, Balmas V, *et al*. Population genetic structure of *Pyrenophora teres* Drechs. the causal agent of net blotch in Sardinian landraces of barley (*Hordeum vulgare L.*). *Theor Appl Genet* 2003;106:947–959.
87. Leisova L, Kucera L, Minarikova VOvesnaJAFLP-based PCR markers that differentiate spot and net forms of *Pyrenophora teres*. *Plant Pathol* 2005;54:66–73.
88. Statkevičiūtė G, Brazauskas G, Semaškienė R, Leistrumaitė A, Dabkevičius Z. *Pyrenophora teres* genetic diversity as detected by ISSR analysis. *Agriculture* 2010;97:91–98.
89. Damgaci E. Arpa Ağbenek (*Pyrenophora teres* Drechs.) hastalığının yayılış durumu, neden olduğu verim kaybı ve verim bileşenlerine etkisi üzerinde araştırmalar. *Plant Protection Bulletin/Bitki Koruma Bülteni* 2014;54.
90. Lartey RT, Caesar-TonThat TC, Caesar AJ, Sainju UM, Evans RG. First report of spot form net blotch caused by *Pyrenophora teres* f. *maculata* on barley in the Mon-Dak area of the United States. *Plant Dis* 2013;97:143.
91. Haggag WM. First report of the spot form of net blotch of barley caused by *Pyrenophora teres* f. sp. m*aculata* in Egypt. *J Plant Pathol* 2010;92:S118.
92. Ficsor A, Bakonyi J, Tóth B, Tomcsányi A, Palágyi A, *et al*. First report of spot form of net blotch of barley caused by *Pyrenophora teres* f. *maculata* in Hungary. *Plant Dis* 2010;94:1062.
93. Teferi TA, Wubshet ML, Aregawi TB. Occurrence and intensity of net and spot blotch of barley in South Tigray, Ethiopia. *Glob J Pests Dis Crop Protect* 2015;3:113–123.
94. Hessenauer P, Feau N, Gill U, Schwessinger B, Brar GS, *et al*. Evolution and adaptation of forest and crop pathogens in the Anthropocene. *Phytopathology* 2021;111:49–67.
95. Stauber L, Badet T, Feurtey A, Prospero S, Croll D. Emergence and diversification of a highly invasive chestnut pathogen lineage across southeastern Europe. *elife* 2021;10:e56279.