Evolution of three *Pyrenophora* cereal pathogens: recent divergence, speciation and evolution of non-coding DNA

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Abstract

Three of the most important fungal pathogens of cereals are *P. tritici-repentis*, the cause of tan spot on wheat, and *P. teres* f. *teres* and *P. teres* f. *maculata*, the cause of spot form and net form of net blotch on barley, respectively. Orthologous intergenic regions were used to examine the genetic relationships and divergence times between these pathogens. Mean divergence times were calculated at 519 kya (±30) between *P. teres* f. *teres* and *P. teres* f. *maculata*, while *P. tritici-repentis* diverged from both *P. teres* forms 8.04 Mya (±138 ky). Individual intergenic regions showed a consistent pattern of co-divergence of the *P. teres* forms from *P. tritici-repentis*, with the pattern supported by phylogenetic analysis of conserved genes. Differences in calculated divergence times between individual intergenic regions suggested that they are not entirely under neutral selection, a phenomenon shared with higher Eukaryotes. *P. tritici-repentis* regions varied in divergence time approximately 5-12 Mya from the *P. teres* lineage, compared to the separation of wheat and barley some 12 Mya, while the *P. teres* f. *teres* and *P. teres* f. *maculata* intergenic region divergences correspond to the middle Pleistocene. The data suggest there is no correlation between the divergence of these pathogens the domestication of wheat and barley, and show *P. teres* f. *teres* and *P. teres* f. *maculata* are closely related but autonomous. The results are discussed in the context of speciation and the evolution of intergenic regions.

Introduction

Pyrenophora species are ascomycetes within the class Dothideomycetes. Several are important pathogens of cereals, notably *P. tritici-repentis* on wheat and *P. teres* f. *teres* and *P. teres* f. *maculata* on barley. *P. tritici-repentis* (PTR) is the cause of tan spot, while *P. teres* causes net blotch. These species are necrotrophs, causing cell death and feeding off the nutrients released, and have become recognised diseases only over the last century. The two barley forms closely resemble each other morphologically and are distinguished by their disease symptoms. *P. teres* f. *teres* (PTT) produces net form of net blotch (NFNB), while *P. teres* f. *maculata* (PTM) produces spot form of net blotch (SFNB). NFNB is typified by elongated lesions, where necrosis develops along leaf veins with occasional transverse striations. SFNB displays more ovoid lesions, often surrounded by a chlorotic zone.

The relationship of the two forms of *P. teres* to each other has remained unclear despite several DNA-based studies. These have suggested that PTT and PTM are closely related but autonomous and divergent genetic groups (Bakonyi and Justesen, 2007; Bogacki et al., 2010; Lehmensiek et al., 2010; Rau et al., 2007; Rau et al., 2003). However, PTT and PTM can be artificially hybridised to create progeny that are morphologically intermediate and are genetically stable (Campbell and Crous, 2003). Two studies have also suggested infrequent hybridisation may occur naturally (Campbell et al., 2002; Leišova et al., 2005). The uncertainty in these relationships has been due to the small amount of genomic DNA sequence information available and limitations in the marker types deployed. Long DNA sequences are often necessary to obtain a robust phylogenetic tree while some marker techniques, notably RAPDS, are anonymous in their DNA sequence content and context and are unreliable for inferring hybridisation events unless isolated and sequenced (e.g. Van De Zande and Bijlsma, 1995).

Wheat and barley are both believed to have been domesticated in the Fertile Crescent around 10,000 years ago (Badr et al., 2000; Heun et al., 1997; Özkan et al., 2002). Since then, widespread intensive cultivation and the development of genetically homogeneous crops has led to strong selective pressure on natural fungal populations or the emergence of entirely new pathogens (Brunner et al., 2007; Friesen et al., 2006; Ma et al., 2010; Stukenbrock et al., 2007). The history of the *Pyrenophora* diseases in this study and their relationship to their hosts is poorly understood, with the exception of PTR, which only recently became a serious pathogen on wheat through the horizontal transfer of the effector ToxA from *Stagonospora nodorum* (Friesen et al., 2006). Prior to this PTR was recorded as occasional and insignificant pathogen on wheat and other grasses (Nisikado, 1929). An obvious lack of fossil records for fungal pathogens means indirect dating techniques are needed to set molecular clocks and species diversification dates. The most reliable DNA sequences to base species divergence on are neutral regions where the nucleotide substitution rates are constant, as gene coding sequences, regulatory elements and introns may both be subject to selective constraints (Hare and Palumbi, 2003; Nielsen et al., 2004). In fungi, Oberhaensli *et al*. (2011) used intergenic regions and transposable elements to show a convergence between the evolution of powdery mildew and host speciation, while Martin *et al*. (2010) used inactive transposable elements to date the insertion age of truffle LTRs.

The advent of next generation sequencing technologies has made genome sequencing of fungal species rapid and affordable (Haridas et al., 2011; Nowrousian et al., 2010). Their relatively compact and simple genomes compared to most eukaryotes are suited to assembling the gene content using short sequencing reads. In this study we sequenced a representative of PTR and PTM, and, in combination with a pre-existing assembly for PTT (Ellwood *et al.*, 2010), we examined the genetic relationships, divergence, and host co-existence between these pathogens.

Material and methods

Genome sequencing

PTR isolate WAC11137 was acquired from the Department of Agriculture and Food, Western Australia (3, Baron Hay Court, South Perth, Western Australia 6151). PTM isolate SG1-1 was collected in Western Australia by S. Ellwood during 2009. One hundred base pair sequencing was performed on a Solexa GAII sequencing platform (Bentley *et al.*, 2008), using 300 bp paired-end libraries with a minimum 40 times genome coverage. Preparation of randomly sheared DNA, cluster formation, primer hybridization and DNA amplification reactions were according to the manufacturer's recommended protocol. Data was pre-filtered by Illumina's data pipeline. Removal of any contaminating DNA adaptors, tags, and trimming of bases with an Illumina quality score of less than 30 was performed using the FASTX-toolkit v 0.0.13.

PTR WAC11137 and PTM SG1-1 were assembled with Velvet v 1.1.02 (Zerbino and Birney, 2008). The optimal kmer (sequence overlap) length to construct the assemblies was determined by incrementally adjusting the kmer by 4 bp. The optimal kmer length was selected where N_{50} was minimal and L_{50} was maximal. L_{50} defined here is the length of the smallest N_{50} contig, where N_{50} is the minimum number of contigs required to represent 50 % of the genome. Protein coding sequences were identified with GeneMark-ES v.2 (Ter-Hovhannisyan *et al.*, 2008). GeneMark-ES uses a self-training algorithm optimized for features of fungal gene organization and incorporates an enhanced intron submodel to accommodate sequences with and without branch point sites. The Geneious (Drummond *et al.*, 2011) plugin Phobos (Mayer, 2010) was used to identify short tandem repeats or microsatellites. Larger interspersed repeats and transposon-like elements were predicted *denovo* using RepeatScout v 1.0.5 (Price *et al.*, 2005). These were combined with characterised transposable elements from a closely related Dothideomycete, *Stagonospora nodorum* (Hane and

Oliver, 2008), and their locations together with low complexity regions delineated with RepeatMasker 3.3.0 (Smit *et al.*, 1996-2011).

Calculation of divergence time

PTR, PTM, and PTT (Ellwood et al., 2010) genomic scaffolds with large intergenic regions were compared by BLASTN (Altschul *et al.*, 1997). Matching scaffolds delimited by orthologous genes were initially aligned in the Mauve genome viewer within Geneious, using the progressive alignment option. Intergenic regions were selected that were at 1.5 kbp from the nearest predicted flanking gene, which resided on separate scaffolds, and which lacked interspersed repeats. As a second precaution to ensure regions were not coding, they were queried against NCBI. Each region was aligned using MUSCLE (Edgar, 2004). The number of transitions and transversions between each pathogen were counted with a BioRuby script (Goto *et al.*, 2010). Indels were removed from the alignment length and hence the total number of informative sites. Evolutionary distance per site (K), divergence time, and standard errors were calculated using the Kimura 2-parameter model and the equations detailed in Kimura (1980), using an average substitution rate for fungi of 8.8×10^{-9} per site per year (Kasuga et al., 2002). FASTA alignments of intergenic regions used to calculate divergence times are presented in Supplementary file 1.

Multigene phylogeny

Phylogenetic relationships between PTR, PTM, PTT and *Cochliobolus heterostrophus* were inferred from 5 concatenated orthologous genes; actin (*act1*), β-tubulin (*tub2*), cytochrome P450 14α-demethylase (*cyp51A*), translation elongation factor-1α (*EF-1α*) and glyceraldehyde-3-phosphate dehydrogenase (*G3PD*). These regions were selected based on the number of informative sites among genes commonly used to infer phylogeny and correctly predicted between the different

assemblies. *C. heterostrophus* isolate C5 v 2 sequences were obtained from the DOE Joint Genome Institute (http://genome.jgi-psf.org/programs/fungi/index.jsf, Barbara Turgeon, pers. comm.). Concatenated sequences were aligned with MUSCLE and analysed by Metropolis coupled Markov chain Monte Carlo analyses with Mr Bayes (Huelsenbeck and Ronquist, 2001) within Geneious with *C. heterostrophus* selected as the outgroup. Unconstrained branch lengths were used with default parameters (gamma distribution approximated with four categories and a proportion of invariable sites, HKY substitution model, subsampling every 200th generation and a burn in length of 1,100,000). Branch bootstrap support values were obtained from a Neighbour Joining tree with 10,000 bootstrap replicates. MEGA v 5 (Tamura *et al.*, 2011) was used to portray trees. New gene sequences used in this study have been deposited in GenBank under accessions JQ314397-JQ314406. Aligned concatenated sequences are provided in Supplementary file 2.

Results and Discussion

Neutral substitution rates vary across gene loci, in part due to low numbers of scorable sites per gene, a particular issue in closely related taxa, and to differences in diversifying and purifying selection. We therefore selected intergenic regions on which to base divergence times between PTR, PTM, and PTT. As no fossil evidence for these species exists to calibrate a molecular clock, a mean substitution rate based on the third codon of protein coding genes in fungi was used, based on data from Kasuga *et al*. (2002), who showed the range of neutral substitution mutations were surprisingly similar across kingdoms.

A similar approach has previously been used to date divergence in truffles and powdery mildew (Martin et al., 2010; Oberhaensli et al., 2011) using transposable elements. However, in many fungi, transposable elements are subject to repeat-induced point (RIP) mutation, a fungal-specific genome defence mechanism that introduces G/C-to-A/T transition mutations into repeat copies during the sexual stage (Cambareri *et al.*, 1991). In the Dothideomycetes, the process has been identified in *Stagonospora* and *Leptosphaeria*, genera closely related to *Pyrenophora* (Hane et al., 2007; Rouxel et al., 2011), and more recently within *Pyrenophora* itself (James Hane, pers. comm.). In this study, therefore, aligned intergenic regions were identified on separate genomic scaffolds devoid of transposable elements. Transposable elements would not be expected to be prevalent in small DNA fragment short-read genome assemblies, as such repetitive DNA tends not to assemble (Ellwood et al., 2010). To ensure their absence, intergenic regions with interspersed repeats and transposable element-like sequences were identified and excluded from the analyses.

In total ten intergenic regions were identified that fitted the sampling criteria. The total alignment length was 35.5 kbp and the number of aligned bases per region ranged from 2.3 to 4.3 kbp (Table 1). The intergenic regions exhibited a range of divergence dates and the mean divergence dates for

the three fungi were calculated as just over 519 kya (±30) between PTM and PTT, and between PTR and both PTM and PTT an average of 8.04 Mya (±138 ky). The average ratio of transitions to transversions between PTM and PTT was 2:1 and between PTR compared to PTM and PTT was 1.53. These ratios are consistent with transitions being generated at a higher frequency than transversions and a bias or movement towards saturation of transitions at higher levels of genetic divergence (Yang and Yoder, 1999).

When divergence times in individual intergenic regions are examined, a consistent pattern of co-divergence of both forms of *P. teres* from PTR is evident but with varying dates between the regions. To validate these results, the phylogeny of five concatenated orthologous genes regions was compared using *C. heterostrophus* as an out group. The total alignment length was 8351 bp with 21 nucleotide changes between PTM and PTT. Tree topology was compared using Mr Bayes inference and neighbour joining. Each method produced trees entirely congruent with the divergence time model, with a relatively long branch separating PTR from the closely related *P. teres* forms (Figure 1).

In PTR, there are at least four groups of intergenic regions showing divergence at approximately 5, 7, 10 and 11 Mya from *P. teres*, while PTM and PTT show divergence dates from approximately 400 kya to 600 kya (Figure 2). The author proposes two theories to account for these different divergence times. The first is that despite the precautions described in materials and methods in selecting the intergenic regions, a proportion of these in *Pyrenophora* are functionally important and not under neutral selection. Andolfatto *et al*. (2005) showed in *Drosophila* that over 40% of intergenic nucleotides were evolutionarily constrained relative to synonymous sites, but also around 20% of nucleotides showed a greater than expected between-species divergence or positive selection. The majority of intergenic DNA has no known function but evolutionarily constrained intergenic regions may be due to several factors including the location of regulatory silencing

siRNA (Feng et al., 2009), large noncoding RNAs that associate with chromatin modifying complexes and affect gene expression (Khalil et al., 2009), and methylation sites thought to regulate cell context-specific alternative promoters (Maunakea et al., 2010). Positive selection may be explained by a recent study in sticklebacks, where 41% of loci associated with adaptive marine–freshwater evolution mapped entirely to non-coding regions of the genome (Jones et al., 2012). A second theory envisages hybridisation between recently diverging lineages still capable of interbreeding and may include lineages predating divergence and unknown intermediary lineages. Rare single hybridisation events, clonal (asexual) selection of progeny with favourable combinations of genes, and isolation restricting recombination would allow intergenic regions with different divergence dates to become fixed in a population. The mosaic of divergence times evident in PTR is a complex scenario suggesting several hybridisation events and might argue against this mechanism.

This study was partly conducted to determine whether the divergence times coincided with the divergence wheat and barley, the domestication of cereals or some other identifiable historical event. Wheat and barley diverged some 12 Mya (Chalupska *et al.*, 2008) and a lack of knowledge about host specificity before further host speciation and pathogen specialization cannot exclude a possible overlap of pathogen and host divergence. On the other hand, the divergence of PTM and PTT clearly predates the domestication of barley. Unlike in biotrophs, there is no studies to date indicating agriculturally important necrotrophs have co-evolved with their hosts, relying instead on horizontal transfer of genes conferring virulence or by strong selection pressure on local pathogen populations from wild relatives (Brunner et al., 2007; Friesen et al., 2006; Ma et al., 2010; Oliver and Solomon, 2008; Stukenbrock et al., 2007).

The divergence of PTM and PTT predating the domestication of barley confirms those studies suggesting the two forms of *P. teres* are genetically isolated (Bakonyi and Justesen, 2007; Bogacki

et al., 2010; Lehmensiek et al., 2010; Rau et al., 2007; Rau et al., 2003) and might be considered as different species. *P. graminea*, the cause of barley stripe, has yet to be sequenced. This species is considered as differentiated with *P. teres* as the magnitude between PTM and PTT (Rau et al., 2007). The mean and all individual intergenic divergence estimates fit within the middle Pleistocene of the Quaternary Period. This geological stage was punctuated by significant glaciation eras and long periods of isolation in glacial refuges may have provided conditions for speciation. More recently, isolation and specialisation may have continued in different centres of barley diversity and domestication in Asia and the Horn of Africa (Badr et al., 2000; Morrell and Clegg, 2007; Orabi et al., 2007). Literature describing two forms of *P. teres* dates back to the second half of the last century, with Smedegård-Petersen (1971) the first to report SFNB following detection in Denmark. The absence of previous information together with new reports and increases in severity of SFNB in all barley growing regions of the world (reviewed in Ficsor et al., 2010; Liu and Friesen, 2010; McLean et al., 2009) suggest this co-existence is recent, with different centres of barley diversity and domestication combined with modern travel providing a mechanism for the present co-existence. Alternatively, or as part of this process, wild hosts may have facilitated barley colonisation. PTT is reported to infect a range of gramineous species in the genera *Aegilops*, *Agropyron*, *Elymus*, *Hordeum*, *Hordelymus*, and *Stipa* (Brown *et al.*, 1993). Comparable data for PTM has not been collected, but barley colonisation by *P. teres* populations in new areas of cultivation, followed by adaption and specialisation, is also plausible.

The finding that intergenic regions in *Pyrenophora* are not entirely under neutral selection requires that genome-wide estimates of substitution rates, based on the number of mutations that have occurred in a known number of generations, are needed for more accurate estimates of rates within the regions. However, until such data is available, deploying a constant genome-wide substitution rate allows broad comparisons to be made, with the age of the common ancestor of PTR and P. teres being over fifteen times greater than that of the common ancestor PTM and PTT. The role of

Pyrenophora intergenic regions showing different divergence times can be resolved with more complete genome assemblies and by combining comparative genomic analyses with genetically diverse isolates and population-level data to unravel adaptive differences between populations and species.

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References

Altschul, S., et al., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research. 25**,** 3389-3402.

Andolfatto, P., 2005. Adaptive evolution of non-coding DNA in *Drosophila*. Nature. 437**,** 1149-1152.

Badr, A., et al., 2000. On the origin and domestication history of barley (*Hordeum vulgare*). Molecular Biology and Evolution. 17**,** 499-510.

Bakonyi, J., Justesen, A. F., 2007. Genetic relationship of *Pyrenophora graminea, P. teres* f. *maculata* and *P. teres* f. *teres* assessed by RAPD analysis. Journal of Phytopathology. 155**,** 76-83.

Bentley, D. R., et al., 2008. Accurate whole human genome sequencing using reversible terminator chemistry. Nature. 456**,** 53-59.

Bogacki, P., et al., 2010. Genetic structure of South Australian *Pyrenophora teres* populations as revealed by microsatellite analyses. Fungal Biology. 114**,** 834-841.

Brown, M. P., et al., 1993. Host range of *Pyrenophora teres* f. *teres* isolates from California. Plant Disease. 77**,** 942–947.

Brunner, P. C., et al., 2007. The origin and colonization history of the barley scald pathogen *Rhynchosporium secalis*. Journal of Evolutionary Biology. 20**,** 1311-1321.

Cambareri, E. B., et al., 1991. Recurrence of Repeat-Induced Point Mutation (RIP) in *Neurospora crassa*. Genetics. 127**,** 699-710.

Campbell, G. F., Crous, P. W., 2003. Genetic stability of net x spot hybrid progeny of the barley pathogen *Pyrenophora teres*. Australasian Plant Pathology. 32**,** 283-287.

Campbell, G. F., et al., 2002. Evidence of recombination between net- and spot-type populations of *Pyrenophora teres* as determined by RAPD analysis. Mycological Research. 106**,** 602-608.

Chalupska, D., et al., 2008. Acc homoeoloci and the evolution of wheat genomes. Proceedings of the National Academy of Sciences. 105**,** 9691-9696.

Drummond, A. J., et al., 2011. Geneious v 5.4, available from http://www.geneious.com/.

Edgar, R., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics. 5**,** 113.

Ellwood, S., et al., 2010. A first genome assembly of the barley fungal pathogen *Pyrenophora teres* f. *teres*. Genome Biology. 11**,** R109.

Feng, J., et al., 2009. Coding DNA repeated throughout intergenic regions of the *Arabidopsis thaliana* genome: evolutionary footprints of RNA silencing. Molecular BioSystems. 5**,** 1679-1687.

Ficsor, A., et al., 2010. First report of spot form of net blotch of barley caused by *Pyrenophora teres* f. *maculata* in Hungary. Plant Disease. 94**,** 1062-1062.

Friesen, T. L., et al., 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. Nat Genet. 38**,** 953-956.

Goto, N., et al., 2010. BioRuby: Bioinformatics software for the Ruby programming language. Bioinformatics. 1-3.

Hane, J. K., et al., 2007. Dothideomycete plant interactions illuminated by genome sequencing and EST analysis of the wheat pathogen *Stagonospora nodorum*. Plant Cell. 19**,** 3347-3368.

Hane, J. K., Oliver, R. P., 2008. RIPCAL: a tool for alignment-based analysis of repeat-induced point mutations in fungal genomic sequences. BMC Bioinformatics. 9**,** 478.

Hare, M. P., Palumbi, S. R., 2003. High intron sequence conservation across three mammalian orders suggests functional constraints. Molecular Biology and Evolution. 20**,** 969-978.

Haridas, S., et al., 2011. A biologist's guide to de novo genome assembly using next-generation sequence data: A test with fungal genomes. Journal of Microbiological Methods. 86**,** 368-375.

Heun, M., et al., 1997. Site of Einkorn wheat domestication identified by DNA fingerprinting. Science. 278**,** 1312-1314.

Huelsenbeck, J. P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 17**,** 754-755.
Jones, F. C., et al., 2012. The genomic basis of adaptive evolution in threespine sticklebacks. Nature. 484**,** 55-61.
Kasuga, T., et al., 2002. Estimation of nucleotide substitution rates in Eurotiomycete fungi. Molecular Biology and Evolution. 19**,** 2318-2324.
Khalil, A. M., et al., 2009. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proceedings of the National Academy of Sciences. 106**,** 11667-11672.
Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide substitutions. Journal of Molecular Evolution. 111-120.
Lehmensiek, A., et al., 2010. Population structure of South African and Australian *Pyrenophora teres* isolates. Plant Pathology. 59**,** 504-515.
Leišova, L., et al., 2005. Genetic diversity of *Pyrenophora teres* isolates as detected by AFLP analysis. Journal of Phytopathology. 153**,** 569-578.
Liu, Z. H., Friesen, T. L., 2010. Identification of *Pyrenophora teres* f. *maculata*, causal agent of spot type net blotch of barley in North Dakota. Plant Disease. 94**,** 480-480.
Ma, L.-J., et al., 2010. Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. Nature. 464**,** 367-373.
Martin, F., et al., 2010. Perigord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature. 464**,** 1033-1038.
Maunakea, A. K., et al., 2010. Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature. 466**,** 253-257.
Mayer, C., 2010. Phobos v 3.3.11, available from http://www.rub.de/spezzoo/cm/cm_phobos.htm.
McLean, M. S., et al., 2009. Epidemiology and control of spot form of net blotch (*Pyrenophora teres* f. *maculata*) of barley: a review. Crop and Pasture Science. 60**,** 303-315.
Morrell, P. L., Clegg, M. T., 2007. Genetic evidence for a second domestication of barley (*Hordeum vulgare*) east of the Fertile Crescent. Proceedings of the National Academy of Sciences. 104**,** 3289-3294.
Nielsen, C. B., et al., 2004. Patterns of intron gain and loss in fungi. PLoS Biology. 2**,** e422.
Nisikado, Y., 1929. Studies on the *Helminthosporium* diseases of Gramineae in Japan. Ber Ohara Inst Landw Forsch. 4**,** 111-126.
Nowrousian, M., et al., 2010. *De novo* assembly of a 40 Mb eukaryotic genome from short sequence reads: *Sordaria macrospora*, a model organism for fungal morphogenesis. PLoS Genetics. 6**,** e1000891.
Oberhaensli, S., et al., 2011. Comparative sequence analysis of wheat and barley powdery mildew fungi reveals gene colinearity, dates divergence and indicates host-pathogen co-evolution. Fungal Genetics and Biology. 48**,** 327-334.
Oliver, R. P., Solomon, P. S., 2008. Recent fungal diseases of crop plants: is lateral gene transfer a common theme? Molecular Plant-Microbe Interactions. 21**,** 287-293.
Orabi, J., et al., 2007. The Horn of Africa as a centre of barley diversification and a potential domestication site. Theoretical and Applied Genetics. 114**,** 1117-1127.
Özkan, H., et al., 2002. AFLP analysis of a collection of tetraploid wheats indicates the origin of Emmer and hard wheat domestication in southeast Turkey. Molecular Biology and Evolution. 19**,** 1797-1801.
Price, A. L., et al., 2005. De novo identification of repeat families in large genomes. Bioinformatics. 21**,** i351-358.
Rau, D., et al., 2007. Phylogeny and evolution of mating-type genes from *Pyrenophora teres*, the causal agent of barley 'net blotch' disease. Current Genetics. 51**,** 377-392.
Rau, D., et al., 2003. Population genetic structure of *Pyrenophora teres* Drechs. the causal agent of net blotch in Sardinian landraces of barley (*Hordeum vulgare* L.). Theoretical and Applied Genetics. 106**,** 947-959-959.

Rouxel, T., et al., 2011. Effector diversification within compartments of the *Leptosphaeria maculans* genome affected by Repeat-Induced Point mutations. Nat Commun. 2**,** 202.

Smedegård-Petersen, V., 1971. *Pyrenophora teres* f. *maculata* f. nov. and *Pyrenophora teres* f. *teres* on barley in Denmark. Aarsskrift Kongelige Veterinear of Landbohojskole. 124–144.

Smit, A. F. A., et al., 1996-2011. RepeatMasker Open-3.0, available from http://www.repeatmasker.org.

Stukenbrock, E. H., et al., 2007. Origin and domestication of the fungal wheat pathogen *Mycosphaerella graminicola* via sympatric speciation. Molecular Biology and Evolution. 24**,** 398-411.

Tamura, K., et al., 2011. MEGA5: Molecular evolutionary genetics analysis using Maximum Likelihood, evolutionary distance, and Maximum Parsimony methods. Molecular Biology and Evolution. doi:10.1093/molbev/msr121.

Ter-Hovhannisyan, V., et al., 2008. Gene prediction in novel fungal genomes using an *ab initio* algorithm with unsupervised training. Genome Research. 18**,** 1979-1990.

Van De Zande, L., Bijlsma, R., 1995. Limitations of the RAPD technique in phylogeny reconstruction in *Drosophila*. Journal of Evolutionary Biology. 8**,** 645-656.

Yang, Z., Yoder, A. D., 1999. Estimation of the transition/transversion rate bias and species sampling. Journal of Molecular Evolution. 48**,** 274-283.

Zerbino, D. R., Birney, E., 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Research. 18**,** 821-829.

Figure legends

Figure 1 Phylogram between PTM, PTT and PTR, with *C. heterostrophus* as an outgroup, based on an 8351 bp concatenated alignment of actin, β-tubulin, cyp51A, EF-1α and G3PD. The phylogram represents the consensus posterior output built by MrBayes. Neighbour Joining bootstrap support percentages are indicated at branching points. Scale bar represents substitutions per site.

Figure 2 Model of divergence between PTM, PTT and PTR based on mean intergenic divergence estimates. Grey bars represent the range of individual divergence time estimates for single intergenic regions for each comparison. Vertical scale bar is in million year intervals.

Table 1 Divergence time estimates of PTM, PTT and PTR.

Locus	Comparison	Aligned sites	Transitions	Transversions	Divergence time	Evolutionary distance per site (K)	Standard error (σK)
Locus 1							
	PTM/PTT	3,847	20	10	445,697	0.008	0.001
	PTT/PTR	3,427	222	104	5,839,474	0.103	0.006
	PTM/PTR	3,427	227	103	5,919,599	0.104	0.006
Locus 2							
	PTM/PTT	3,384	16	10	439,002	0.008	0.002
	PTT/PTR	2,933	202	148	7,434,579	0.131	0.007
	PTM/PTR	2,930	204	148	7,490,782	0.132	0.007
Locus 3							
	PTM/PTT	2835	28	7	708,753	0.012	0.002
	PTT/PTR	2406	263	137	10,875,296	0.191	0.010
	PTM/PTR	2428	264	136	10,763,935	0.189	0.010
Locus 4							
	PTM/PTT	3329	28	10	654,486	0.012	0.002
	PTT/PTR	3081	172	98	5,332,921	0.094	0.006
	PTM/PTR	3083	177	92	5,313,349	0.094	0.006
Locus 5							
	PTM/PTT	4357	29	18	617,772	0.011	0.002
	PTT/PTR	3580	316	253	10,231,709	0.180	0.008
	PTM/PTR	3592	315	258	10,270,356	0.181	0.008
Locus 6							
	PTM/PTT	3146	16	6	399,523	0.007	0.002
	PTT/PTR	2551	165	115	6,788,031	0.119	0.007
	PTM/PTR	2556	162	113	6,641,673	0.117	0.007
Locus 7							
	PTM/PTT	3255	12	10	385,855	0.007	0.001
	PTT/PTR	2913	194	144	7,207,790	0.127	0.007
	PTM/PTR	2906	192	140	7,087,926	0.125	0.007
Locus 8							
	PTM/PTT	2929	20	12	625,762	0.011	0.002
	PTT/PTR	2369	242	172	11,439,512	0.201	0.010
	PTM/PTR	2378	240	171	11,292,908	0.199	0.010
Locus 9							
	PTM/PTT	2932	21	8	566,371	0.010	0.002
	PTT/PTR	2414	279	158	11,988,192	0.211	0.011
	PTM/PTR	2393	283	160	12,309,594	0.217	0.011
Locus 10							
	PTM/PTT	3359	13	9	373,892	0.007	0.001
	PTT/PTR	2981	155	125	5,725,307	0.101	0.006
	PTM/PTR	2979	162	124	5,864,577	0.103	0.006
Total							
	PTM/PTT	33369	203	100	519,481	0.009	0.001
	PTT/PTR	28653	2209	1453	8,032,881	0.141	0.002
	PTM/PTR	28670	2225	1444	8,046,343	0.142	0.002