ORIGINAL ARTICLE

QTL mapping of temperature sensitivity reveals candidate genes for thermal adaptation and growth morphology in the plant pathogenic fungus *Zymoseptoria tritici*

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Different thermal environments impose strong, differential selection on populations, leading to local adaptation, but the genetic basis of thermal adaptation is poorly understood. We used quantitative trait locus (QTL) mapping in the fungal wheat pathogen *Zymoseptoria tritici* to study the genetic architecture of thermal adaptation and identify candidate genes. Four wild-type strains originating from the same thermal environment were crossed to generate two mapping populations with 263 (cross 1) and 261 (cross 2) progeny. Restriction site-associated DNA sequencing was used to genotype 9745 (cross 1) and 7333 (cross 2) single-nucleotide polymorphism markers segregating within the mapping population. Temperature sensitivity was assessed using digital image analysis of colonies growing at two different temperatures. We identified four QTLs for temperature sensitivity, with unique QTLs found in each cross. One QTL had a logarithm of odds score > 11 and contained only six candidate genes, including *PBS2*, encoding a mitogen-activated protein kinase kinase associated with low temperature tolerance in *Saccharomyces cerevisiae*. This and other QTLs showed evidence for pleiotropy among growth rate, melanization and growth morphology, suggesting that many traits can be correlated with thermal adaptation in fungi. Higher temperatures were highly correlated with a shift to filamentous growth among the progeny in both crosses. We show that thermal adaptation has a complex genetic architecture, with natural populations of *Z. tritici* harboring significant genetic variation for this trait. We conclude that *Z. tritici* populations have the potential to adapt rapidly to climate change and expand into new climatic zones.

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INTRODUCTION

Temperature has a strong effect on many life history traits, including growth, development and reproduction (Angilletta *et al.*, 2006; de Jong and van der Have, 2009). This is especially true for ectotherms, such as fungi, whose internal temperature directly reflects the surrounding environment (Angilletta *et al.*, 2002). Two general strategies for adapting to different thermal environments are phenotypic plasticity and genetic differentiation (Dybdahl and Kane, 2005; Knies *et al.*, 2006; Yamori *et al.*, 2010). Phenotypic plasticity is an individual-based response that allows the same genotype to produce different phenotypes in different environments (de Jong and van der Have, 2009). Genetic differentiation is a population-based response where individuals carrying different alleles are selected according to the local environment. Over generations, selection pressure for different temperature optima can lead to changes in the genetic architecture of temperature adaptation. For example, nonsynonymous substitutions can generate protein isoforms that differ for thermal stability and/or optimized function at different temperatures (Schoville *et al.*, 2012). In a population, genetic diversity for thermal adaptation can be maintained by antagonistic pleiotropy (genetic trade-offs), in which an allele that has optimal function at one temperature exhibits maladaptation at a different temperature (Fields, 2001). A better understanding of the genetic basis of thermal adaptation is likely to

provide insight into the ability of species to adapt to global climate change (Austin, 2007).

Thermal adaptation is important for many plant pathogenic fungi. Before 2000, the wheat stripe rust pathogen *Puccinia striiformis* f. sp. *tritici* caused epidemics only in cooler wheat-growing regions. After 2000, two new clonal lineages of the pathogen able to cause epidemics in warmer climates emerged, allowing the pathogen to expand into previously unfavorable environments (Milus *et al.*, 2009; Mboup *et al.*, 2012). In the globally distributed barley pathogen *Rhynchosporium commune*, there was evidence for local adaptation to grow at higher temperatures (22 °C), a key adaptation that may have enabled the global emergence of a pathogen that originated in the cold climate of Scandinavia (Zaffarano *et al.*, 2006; Linde *et al.*, 2009; Stefansson *et al.*, 2013).

In some cases, pathogenic fungi grow saprophytically as filamentous hyphae outside their host, and then undergo a morphological transformation to yeast-like growth after infecting their host. These morphological switches enhance pathogenicity by facilitating dispersal within the host and enabling evasion of host immune responses (Nadal *et al.*, 2008; Gauthier, 2015). In other cases, the switch is in the opposite direction, which is mainly the case for plant pathogens (e.g. *Ustilago maydis*), where yeast-like growth is observed outside the host and filamentous growth is observed within the host.

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For some fungal pathogens (e.g. *Talaromyces marneffei*; Boyce and Andrianopoulos, 2013), a change in temperature triggers a switch in growth morphology (Gauthier, 2015).

Fungi use several mechanisms to cope with changing temperatures, including the induction or upregulation of proteins related to thermal stress (e.g. heat-shock proteins (HSPs); Feder and Hofmann, 1999), changes in cellular composition (e.g. activation of the high-osmolarity glycerol (HOG) pathway resulting in glycerol accumulation in cells; Panadero *et al.*, 2006) and adjustments in membrane fluidity (e.g. lipid saturation in cell membranes; Leach and Cowen, 2014). Little is known regarding the genetic basis of how fungi tolerate different temperatures. The majority of research on this topic has been conducted in yeasts and is oriented around the regulation of HSPs (Feder and Hofmann, 1999; Leach and Cowen, 2014). HSPs are regulated through the heat-shock transcription factor 1, which has been extensively studied in *Saccharomyces cerevisiae* (Sorger and Pelham, 1988; Nicholls *et al.*, 2009). Quantitative trait locus (QTL) mapping of high-temperature growth in the progeny of a cross between a heat-tolerant clinical strain of *S. cerevisiae* and a heat-sensitive strain isolated from a rotting fig identified four linked genes involved in high-temperature growth, but none of these genes had a function known to affect thermal adaptation (Steinmetz *et al.*, 2002; Sinha *et al.*, 2008). A bulk segregant analysis applied to yeast populations (Ehrenreich *et al.*, 2010) identified 21 QTLs associated with high-temperature growth in a cross of a heat-tolerant North American oak tree strain and a heat-sensitive West African palm wine strain (Parts *et al.*, 2011). A transcriptome analysis of 48 *Neurospora crassa* isolates from subtropical and tropical environments identified two genes involved in thermal adaptation, including an MRH4-like RNA helicase (Ellison *et al.*, 2011). Despite the progress illustrated by these studies, many gaps remain in our understanding of the genetic mechanisms underlying thermal adaptation in fungi (Robert *et al.*, 2015) and other organisms.

Zymoseptoria tritici (syn *Mycosphaerella graminicola*) is the fungal pathogen causing Septoria tritici blotch, currently the most damaging wheat disease in Europe (Jorgensen *et al.*, 2014; O'Driscoll *et al.*, 2014). The 39.7 Mb reference genome is among the best-assembled fungal genomes available. It contains ~11 000 predicted genes distributed across 21 chromosomes sequenced from telomere to telomere, with an average of ~21% repetitive DNA (Goodwin *et al.*, 2011). *Z. tritici* is a dimorphic fungus than can grow either as filamentous hyphae or yeast-like budding cells when grown *in vitro* (Nadal *et al.*, 2008). The pathogen is found in wheat-growing areas worldwide covering a wide range of temperature regimens (Eyal *et al.*, 1987; Zhan and McDonald, 2011; Jorgensen *et al.*, 2014; O'Driscoll *et al.*, 2014). An earlier study that included 138 *Z. tritici* strains sampled from diverse thermal environments on three continents found evidence for thermal adaptation and concluded that most of the thermal adaptation was due to genetic differentiation rather than phenotypic plasticity (Zhan and McDonald, 2011). Here, we sought to determine the genetic architecture of thermal adaptation and identify candidate genes based on QTL mapping of temperature sensitivity (TS) in wild-type strains sampled from the same thermal environment. This approach allowed us to identify and characterize genetic polymorphisms segregating within natural populations (Franks and Hoffmann, 2012; Robert *et al.*, 2015). Knowledge about co-occurring genetic variants conferring different levels of thermal adaptation allows us to predict how a population will be able to adapt to climate change. To determine the genetic architecture of thermal adaptation in *Z. tritici*, we used restriction site-associated DNA sequencing (RADseq) for genotyping, coupled with high-throughput digital image

analysis to measure growth rates under two temperatures (15 and 22 °C). We used QTL mapping to determine the genetic architecture of natural variation for TS and identify QTLs and their associated chromosomal segments. Comparisons of complete genome sequences of the parental isolates allowed us to identify polymorphisms in candidate genes for thermal adaptation.

MATERIALS AND METHODS

Mapping population, genotyping, genetic mapping and gene expression

Methods used for genetic mapping and gene expression were described earlier (Brunner *et al.*, 2013; Lendenmann *et al.*, 2014, 2015). Briefly, four Swiss wild-type strains were used to create two mapping populations, following an established protocol (Kema *et al.*, 1996). The mapping populations were composed of 263 (ST99CH3D1 (3D1: SRS383146) × ST99CH3D7 (3D7: SRS383147)) and 261 (ST99CH1A5 (1A5: SRS383142) × ST99CH1E4 (1E4: SRS383143)) retained progeny. BioProject numbers PRJNA256988 and PRJNA256991 contain the NCBI Short Read Archive accession numbers for the retained progeny of crosses 3D1 × 3D7 and 1A5 × 1E4, respectively. RADseq (Baird *et al.*, 2008) generated more than 7000 single-nucleotide polymorphism (SNP) markers for each cross. Resequencing of all four parents (Croll *et al.*, 2013) allowed identification of the retained RADseq SNPs in the parents and candidate genes responsible for the observed QTLs. R/qtl version 1.27-10 (Arends *et al.*, 2010) was used to construct two dense genetic maps (Lendenmann *et al.*, 2014). Candidate genes within QTL confidence intervals were characterized for their transcriptional profile, using RNA-Seq data from an *in planta* virulence time-course assay (Brunner *et al.*, 2013).

Phenotyping

Radial growth rates (mm per day) (Trinci, 1971; Lendenmann *et al.*, 2015) of single spore colonies grown at two temperatures (15 and 22 °C) and observed over three time points (8, 11 and 14 days post inoculation (dpi)) were used to calculate TS. Plate inoculation procedures and digital image analyses in ImageJ (Schneider *et al.*, 2012) were described earlier (Lendenmann *et al.*, 2014). Isolates were grown in Petri plates on potato dextrose agar (4 g l⁻¹ potato starch, 20 g l⁻¹ dextrose, 15 g l⁻¹ agar) and placed at a constant temperature of 15 or 22 °C with 70% humidity in the dark. TS for each isolate was calculated as the radial growth rate at 22 °C divided by the radial growth rate at 15 °C. Two absolute value traits (radial growth rate at 15 and 22 °C) and one relative value trait (relative growth rate coefficient = TS) were calculated for each isolate and used for QTL analysis. The 22 °C growth rate trait of this study was called growth rate (fungicide absent) in an earlier investigation mapping QTLs involved in fungicide sensitivity (Lendenmann *et al.*, 2015).

In addition to growth rate, two growth morphologies were scored for each strain. Although *Z. tritici* is considered a filamentous fungus that grows mainly as hyphae, a yeast-like morphology is often observed in culture on rich media, suggesting that *Z. tritici* is dimorphic (Mehrabi and Kema, 2006; Mehrabi *et al.*, 2006b; Nadal *et al.*, 2008). To determine if the yeast/hyphae dimorphism can be mapped, we conducted a visual inspection of the digital images to classify the colonies of each isolate as either hyphal- or yeast-like. Three people (ML, JP and ES) independently classified each isolate using all five technical replicates at 11 dpi for both temperatures. The scoring day of 11 dpi was chosen because the colonies were too small to make an accurate visual scoring at 8 dpi, while classifications at 14 dpi gave the same result. Progeny that exhibited only yeast-like colonies were scored as '0', whereas progeny exhibiting at least two hyphal colonies on a Petri dish were scored as '1' (Supplementary Figure S1). Only progeny with identical classifications from all three scorers were included in this analysis. At 15 °C, >98% of the progeny with a consensus score in cross 1A5 × 1E4 grew with a yeast-like morphology (binary value '0': 247 progeny/binary value '1': 3 progeny), hence this treatment was not included in the analysis. The binary counts were as follows: cross 3D1 × 3D7 at 22 °C binary value '0': 111 progeny/binary value '1': 90 progeny; at 15 °C binary value '0': 186 progeny/binary value '1': 52 progeny; cross 1A5 × 1E4 at 22 °C binary value '0': 104 progeny/binary value '1': 33 progeny.

Absolute and relative phenotypes as well as the yeast/hyphae scores for the retained progeny of each cross are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.cj432>.

QTL mapping

Simple interval mapping (SIM) was used for QTL analysis performed in R/qtl version 1.27-10 (Arends *et al.*, 2010) using R (R_Core_Team, 2012). A binary model was applied for the yeast/hyphae dimorphism phenotype. A total of 1000 genome-wide permutations were used to calculate the significant logarithm of odds (LOD) threshold. Further analyses considered only QTLs that showed P -values < 0.05 . Bayesian credible intervals (Manichaikul *et al.*, 2006) were used to calculate 95% confidence intervals for each QTL. Genetic positions (centiMorgans, cM) of markers were converted into physical positions (base pair, bp) by using the reference IPO323 genome (Goodwin *et al.*, 2011). QTLs were assumed to be different if their confidence intervals did not overlap. More detailed information on methods used for QTL mapping were described earlier (Lendenmann *et al.*, 2014).

Single spore colonies were also scored for their degree of melanization (Lendenmann *et al.*, 2014). Because melanin has been associated with protection against extreme temperatures as well as fungicide resistance (Butler and Day, 1998; Nosanchuk and Casadevall, 2006; Taborda *et al.*, 2008), we investigated pleiotropy among the growth rate traits, TS and melanization (11 dpi), and also looked for evidence of pleiotropic effects among temperature and fungicide sensitivity. This analysis was conducted by overlapping full SIM genome scans and using linear regression among the traits based on a general linear model in R with Pearson's correlation coefficient. Regression analysis between the yeast/hyphae dimorphism and growth rate at

each temperature was conducted using a one-way analysis of variance to calculate the phenotypic variance explained by the yeast/hyphae dimorphism phenotypes upon growth rate. A t -test was used to compare mean differences between the two morphologies in R. Marker allele effects were calculated using R/qtl to confirm the linear regressions.

Identification of candidate genes within QTL confidence intervals

Resequenced parents of each cross were aligned against the IPO323 reference genome, and sequence variants were called and annotated using the open-source tools SnpEff and SnpSift version 3.3 h (Cingolani *et al.*, 2012). Synonymous SNPs were omitted from any further investigation. A gene was considered a candidate for explaining the QTL if it contained at least one sequence variant within the boundaries of the 95% confidence interval. More details regarding methods used to identify candidate genes within QTL confidence intervals were provided earlier (Lendenmann *et al.*, 2014). Because heat-shock proteins have an important role in maintaining cellular functions under thermal stress (Feder and Hofmann, 1999), a hypergeometric test was used to determine whether there was a significant enrichment of proteins with an InterPro domain classified as HSPs within the QTL 95% confidence intervals (Tables 1 and 2). The QTL confidence intervals (Tables 1 and 2) were also investigated for the presence of HSPs as candidate genes.

RESULTS

Genetic architecture of TS

The general linear model of growth rate based on radial development of colonies over time provided an average fit (r^2) $> 98\%$ across both crosses and treatments (Supplementary Figure S2), justifying the

Table 1 Positions and effects of the five temperature sensitivity-associated QTLs identified in cross 3D1 × 3D7

Trait	Chromosome	LOD score at peak	P-value	Mean 3D1 allele (growth rate/temperature sensitivity)	Mean 3D7 allele (growth rate/temperature sensitivity)	Mean difference	Allele effect ^a	Percentage of variance explained by QTL (%)	Bayes confidence interval length (kb)	Heat-shock protein candidate genes (gene ID) ^b
Growth rate (15 °C)	2	4.89	0.001	0.301	0.347	0.046	3D7	9.2	996	68169, 90664
Growth rate (22 °C)	3	4.97	0.002	0.384	0.438	0.054	3D7	9.2	326	—
Growth rate (15 °C)	7	3.95	0.008	0.304	0.345	0.041	3D7	7.5	262	—
Temperature sensitivity	10	11.8	< 0.001	1.416	1.199	0.217	3D1	16.6	20	—
Growth rate (22 °C)	11	10.38	< 0.001	0.452	0.376	0.076	3D1	17.9	157	111223

Abbreviations: LOD, logarithm of odds; QTL, quantitative trait locus.

^a3D1 indicates that the 3D1 parent allele provided a higher phenotypic mean than the 3D7 parent allele. 3D7 indicates that the 3D7 parent allele provided a higher phenotypic mean than the 3D1 parent allele.

^bData refer to within the 95% Bayes confidence interval.

Table 2 Positions and effects of the five temperature sensitivity-associated QTLs identified in cross 1A5 × 1E4

Trait	Chromosome	LOD score at peak	P-value	Mean 1A5 allele (growth rate/temperature sensitivity)	Mean 1E4 allele (growth rate/temperature sensitivity)	Mean difference	Allele effect ^a	Percentage of variance explained by QTL (%)	Bayes confidence interval length (kb)	Heat-shock protein candidate genes (gene ID) ^b
Temperature sensitivity	1	5.66	< 0.001	1.281	1.127	0.154	1A5	9.9	248	—
Temperature sensitivity	2	4.31	0.005	1.265	1.129	0.136	1A5	7.7	3120	29054, 68169, 90664, 68653, 99 10
Temperature sensitivity	4	4.26	0.006	1.129	1.264	0.135	1E4	7.6	1442	108995, 109019, 99959
Growth rate (15 °C)	5	4.81	0.002	0.311	0.348	0.037	1E4	7.5	1366	72449, 104628, 42499
Growth rate (15 °C)	8	6.77	< 0.001	0.304	0.349	0.045	1E4	11.5	1437	—

Abbreviations: LOD, logarithm of odds; QTL, quantitative trait locus.

^a1A5 indicates that the 1A5 parent allele provided a higher phenotypic mean than the 1E4 parent allele. 1E4 indicates that the 1E4 parent allele provided a higher phenotypic mean than the 1A5 parent allele.

^bData refer to within the 95% Bayes confidence interval.

method used to measure growth rate for each isolate. The growth rate and TS phenotypes showed a continuous distribution consistent with a quantitative trait in both crosses. Transgressive segregation was found for all traits, with many progeny showing more extreme phenotypes than their parents (Figure 1). The high broad-sense heritability

($H^2 > 80\%$) found for average colony area indicates a low environmental variance among the five technical repeats (Supplementary Figure S2).

For the TS phenotype, one significant QTL was found in cross 3D1 × 3D7 and three were found in cross 1A5 × 1E4. These four QTLs were on chromosomes 1, 2, 4 and 10 with LOD values ranging from 4.3 to 11.8 (Figures 2 and 3 and Tables 1 and 2). For the absolute value phenotypes, cross 3D1 × 3D7 had four growth rate QTLs on chromosomes 2, 3, 7 and 11 and cross 1A5 × 1E4 had two growth rate QTLs on chromosomes 5 and 8 (Tables 1 and 2). There was a confidence interval overlap between the two crosses for the QTLs on chromosome 2, suggesting a shared QTL. This QTL overlap was already described in a previous study (Lendenmann *et al.*, 2015). On average, ~30% of total variance was explained by the significant QTLs for each trait in both crosses (Table 3). No significant QTLs were mapped on the accessory chromosomes 14–21. Detailed information for every mapped QTL can be found in Supplementary Tables S1 and S2.

QTLs associated with the yeast/hyphae dimorphism

For the yeast/hyphae dimorphism phenotype, we considered QTLs to be different if their confidence intervals did not overlap. In cross 3D1 × 3D7, we mapped five significant QTLs distributed on chromosomes 1, 3, 7 and 11 (Supplementary Figure S3 and Supplementary Tables S3 and S4), with two QTLs on chromosome 3 (Supplementary Table S3). In cross 1A5 × 1E4, two significant QTLs were identified on chromosomes 7 and 11 (Supplementary Figure S4 and Supplementary Tables S5 and S6). Both of these QTLs overlapped with QTLs of cross 3D1 × 3D7, suggesting these QTLs were shared in the two crosses (Supplementary Tables S3 and S5).

Figure 1 The distributions of growth rate and TS in both crosses. Positions of the parental phenotypes within each distribution are indicated by white dashed lines with corresponding parental names above their phenotypes.

Figure 2 LOD plots from SIM analysis over all chromosomes for growth rate at 15 and 22°C, as well as TS for cross 3D1 × 3D7. The dashed horizontal red line represents the significance threshold ($P=0.05$) obtained using 1000 genome-wide permutations. A full color version of this figure is available at the *Heredity* journal online.

Figure 3 LOD plots from SIM analysis over all chromosomes for growth rate at 15 and 22 °C, as well as TS for cross 1A5 × 1E4. The dashed horizontal red line represents the significance threshold ($P=0.05$) obtained using 1000 genome-wide permutations. A full color version of this figure is available at the *Heredity* journal online.

Table 3 Genetic architecture of each trait for both crosses

Trait	Chromosome with a QTL	Allele effect ^a	Total % variance explained by QTL(s)	Number of significant QTLs	Confirmation of transgressive segregation	Cross
Growth rate (22 °C)	2, 3, 11	3D1(1), 3D7(2)	34.5	3	Yes	3D1 × 3D7
Growth rate (15 °C)	2, 3, 7, 10, 11	3D1(1), 3D7(4)	48.2	5	Yes	
Temperature sensitivity	10	3D1(1)	16.6	1	No	
Growth rate (22 °C)	2, 4, 8	1A5(1), 1E4(2)	27.7	3	Yes	1A5 × 1E4
Growth rate (15 °C)	1, 5, 8	1E4(3)	25.2	3	No	
Temperature sensitivity	1, 2, 4	1A5(2), 1E4(1)	25.2	3	Yes	

Abbreviation: QTL, quantitative trait locus.

^a3D7/1E4 indicates that the parental 3D7/1E4 allele provided the higher phenotypic mean contribution than the parental 3D1/1A5 allele, while 3D1/1A5 indicates that the parental 3D1/1A5 allele provided the higher phenotypic mean contribution than the parental 3D7/1E4 allele. The number within brackets following the parental allele indicates the number of significant QTLs.

Identification and characterization of candidate genes within QTL confidence intervals

All annotated genes within each QTL 95% confidence interval were investigated to identify candidate genes affecting each trait. We excluded from consideration all genes that lacked sequence variation or that possessed only synonymous SNPs. For cross 3D1 × 3D7, a summary of the candidate genes for each trait is shown in Supplementary Tables S4 and S7. Supplementary Tables S6 and S8 summarize the candidate genes for cross 1A5 × 1E4.

On average, we found ~200 candidate genes per confidence interval, with a minimum of six candidate genes (chromosome 10, TS, cross 3D1 × 3D7) and a maximum of 1382 candidate genes (chromosome 1, growth rate (15 °C), cross 1A5 × 1E4). On average, ~40% of the candidate genes had no known function (Supplementary

Tables S4 and S6–S8). There was no evidence for enrichment of HSPs in the QTL confidence intervals relative to the rest of the genome. However, some HSPs were among the candidate genes found within the QTL confidence intervals (Tables 1 and 2).

A more detailed investigation was conducted on the three major QTLs ($\text{LOD} \geq 4.5$) containing ≤ 30 candidate genes in narrow confidence intervals (< 85 kb). The chromosome 10 QTL associated with TS in cross 3D1 × 3D7 had an LOD score of 11.8 and contained only six candidate genes (Supplementary Table S7). The chromosome 1 and 3 QTLs associated with the yeast/hyphae dimorphism at 15 °C in cross 3D1 × 3D7 contained 15 candidate genes each (Supplementary Table S4) with LOD scores of 4.5 and 10.2, respectively. The lists of candidate genes associated with each QTL are summarized in Table 4 and Supplementary Tables S9 and S10.

Table 4 Genes within the chromosome 10 temperature sensitivity QTL confidence interval containing ≤ 30 candidate genes for cross 3D1 × 3D7^a

Protein ID ^b	Gene ontology name	Gene ontology biological process	Gene ontology cellular component	Gene ontology molecular function	Number of non-Syn SNPs ^c	Number of other sequence variations ^{c,d}	Additional information	Highest RPKM mean ^e	RPKM Sd ^f
101405 (XP_003848843)	Catalytic activity	Acetyl-CoA metabolic process; acetate metabolic process; pyruvate metabolic process	Cytosol; mitochondrion; acetate CoA-transferase complex	Acetate CoA-transferase activity; acetyl-CoA hydrolase activity	4 (M)	0	—	71.4 (13 dpi)	17.4
105917 (XP_003848844)	5-Methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase activity	Methionine biosynthetic process; methylation	Not described	5-Methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase activity	3 (M)	2 Upstream (m), 1 UTR5 Prime (m)	—	148.0 (7 dpi)	24.0
111014 (XP_003849150)	Proteolysis	Proteolysis	Not described	Serine-type peptidase activity	7 (M)	2 Intron (m), 1 UTR5 Prime (m)	—	49.8 (7 dpi)	43.3
96183 (XP_003848845)	Not described	Not described	Not described	Not described	5 (M)	2 Intron (m)	—	0 (no specific day)	0.0
76249 ^g (XP_003849149)	Protein kinase activity	Protein phosphorylation; serine family amino-acid metabolic process	Not described	ATP binding; protein serine/threonine kinase activity	3 (M)	0	Name: MgPbs2 (hypothetical MAPKK) involved in the osmosensing signal-transduction pathway, activated under severe osmotic stress	139.2 (7 dpi) ^x	31.8
101408 (XP_003848847)	Integral to membrane	Transmembrane transport	Integral to membrane	Not described	2 (M)	0	Transporter—auxin efflux carrier-like with six predicted transmembrane regions	168.0 (56 dpi) ^x	24.3

Abbreviations: JGI, Joint Genome Institute; MAPKK, mitogen-activated protein kinase; NCBI, National Center for Biotechnology Information; QTL, quantitative trait locus; +UTR, untranslated region.
^aExcluding genes with no sequence variation or with only synonymous SNPs.
^bProtein IDs refer to IDs of the JGI and can be found on the JGI homepage of *Zymoseptoria tritici* under <http://genome.jgi.doe.gov/Mycogr3/Mycogr3.home.html>. Below the JGI protein ID is the GenBank accession number of the NCBI.
^cThe letter within brackets following the number of a specific sequence polymorphism refers to the likely impact of the sequence polymorphism according to SnpEff: H = high, M = moderate, L = low, m = modifier.
^dOther sequence variations include codon change plus insertion/deletion, codon insertion/deletion, frameshift, intron, splice site acceptor/donor, start gained/lost, stop gained/lost and UTRs.
^eA * indicates significant changes in transcript abundances over time in planta.
^fThe QTL peak is positioned closest to this gene or within the gene.

Evidence for pleiotropy

In a previous study (Lendenmann *et al.*, 2015), we described a shared QTL on chromosome 11 for melanization at 22 °C, growth rate at 22 °C and fungicide sensitivity in cross 3D1 × 3D7. We hypothesized that a rare mutation in *PKS1* (protein ID 96592) found in the 3D7 parent was responsible for this pleiotropy (Lendenmann *et al.*, 2014). In this study, we found evidence for pleiotropy between melanization and growth at 15 °C following the same pattern described earlier (Lendenmann *et al.*, 2015), namely the *PKS1* allele that increases melanization also slows growth at 15 °C. However, there was no evidence for pleiotropy between melanization and TS associated with the chromosome 11 QTL in cross 3D1 × 3D7 (Figure 4). On the other hand, we found strong evidence for pleiotropy among melanization at 15 °C, growth at 15 °C and TS based on a shared chromosome 10 QTL in cross 3D1 × 3D7. This QTL displayed the following pattern: the 3D7 allele that increases melanization also accelerates growth at 15 °C and decreases TS. There was some evidence for pleiotropy among melanization and TS in cross 1A5 × 1E4 due to a shared chromosome 2 QTL and there were significant correlations between melanization and growth at 15 °C for QTLs on chromosomes 5 and 8 (Figure 4).

In both crosses, we found no evidence for pleiotropy among TS and fungicide sensitivity.

The QTL peaks for the yeast/hyphae dimorphism overlapped with growth rate QTLs on chromosomes 3, 7, 10 and 11, providing evidence for pleiotropy in both crosses. There also was a strong correlation between growth rate and the yeast/hyphae dimorphism. In both crosses, the allele associated with faster growth was also associated with filamentous growth (Supplementary Tables S1, S3, S5 and S11 and Figures 5 and 6), with >17% of the total growth rate variance explained by the yeast/hyphae dimorphism and significant ($P < 0.001$) mean differences between the growth rates of the two morphologies (Figures 5 and 6). On the other hand, QTLs on chromosomes 1, 2, 4 and 8 did not overlap between the yeast/hyphae dimorphism and growth rate, indicating that growth morphology is not the sole determinant of growth rate (Figures 5 and 6). For example, in cross 3D1 × 3D7 there is a significant QTL for the yeast/hyphae dimorphism at 15 °C on chromosome 1, but there is no growth rate QTL at this position, whereas there is a significant QTL for growth rate at 15 °C on chromosome 2, but no corresponding QTL for the yeast/hyphae dimorphism (Figure 5b).

Figure 4 Evidence for pleiotropy among melanization, growth rate at 15 °C and TS for the chromosome 10 QTL in cross 3D1 × 3D7. Melanization is represented by gray values (11 dpi), with lower values indicating higher melanization (Lendenmann *et al.*, 2014). The 3D7 allele that increases melanization also accelerates growth at 15 °C (a) and decreases TS (c). Panels b and d, which show corresponding full genome scan overlaps for cross 1A5 × 1E4, indicate no pleiotropy among the same three traits. Each panel shows a full genome scan LOD overlap plot based on SIM analysis, with traits shown in different colors (red=melanization; green=growth rate at 15 °C or TS). Horizontal lines in the LOD plots represent the color-coded significance thresholds ($P = 0.05$) obtained with 1000 genome-wide permutations. Each panel includes the corresponding linear correlation among the two traits. The name above each QTL peak indicates which parental allele provided the higher phenotypic mean. Arrows point to the QTL peaks that could explain the observed positive or negative correlation among the two traits. A full color version of this figure is available at the *Heredity* journal online.

Figure 5 Evidence for pleiotropy among the yeast/hyphae dimorphism and growth rate phenotypes for the chromosome 3, 7, 10 and 11 QTLs in cross 3D1 x 3D7 (**a**=22 °C/**b**=15 °C). Each panel shows a full genome scan LOD overlap plot based on SIM analysis, with traits separated by colors (green=growth rate at 15 °C/22 °C, purple=yeast/hyphae dimorphism trait). Horizontal lines in the LOD plots represent the color-coded significance thresholds ($P=0.05$) obtained with 1000 genome-wide permutations. Each panel includes a corresponding box plot for the two traits. Mean growth rate values are presented either above or below the median line. The name above each QTL peak indicates which parental allele provided the higher phenotypic mean. Allele effects for nonsignificant, but visible pleiotropic peaks for either of the phenotypes were obtained by modeling the peaking marker of the significant peak for either of the phenotypes upon the corresponding opposite phenotype. For each pleiotropic peak, the allele providing faster growth also contributes to a more hyphal phenotype (**a** and **b**). A full color version of this figure is available at the *Heredity* journal online.

Figure 6 Evidence for pleiotropy among the yeast/hyphae dimorphism and growth rate phenotypes at 22 °C for the chromosome 7 and 11 QTL in cross 1A5 x 1E4. The figure shows a full genome scan LOD overlap plot based on SIM analysis, with traits separated by colors (green=growth rate at 15 °C/22 °C, purple=yeast/hyphae dimorphism trait). Horizontal lines in the LOD plots represent the color-coded significance thresholds ($P=0.05$) obtained with 1000 genome-wide permutations. In the box plot for the two traits, mean growth rate values are presented either above or below the median line. The name above each QTL peak indicates which parental allele provided the higher phenotypic mean. Allele effects for nonsignificant, but visible pleiotropic peaks for either of the phenotypes were obtained by modeling the peaking marker of the significant peak for either of the phenotypes upon the corresponding opposite phenotype. For each pleiotropic peak, the allele providing faster growth also contributes to a more hyphal phenotype. A full color version of this figure is available at the *Heredity* journal online.

DISCUSSION

Genetic architecture of TS

The TS phenotype we used to measure thermal adaptation reflects differences in growth rates at two temperatures. Strains with a $TS=1$ are 'generalists' that grow at the same rate at both temperatures. Strains with $TS>1$ grow faster at the higher temperature and strains with $TS<1$ grow faster at the lower temperature. Strains that grow

quickly at both temperatures (i.e. high growth rates and $TS \sim 1$) exhibit the greatest degree of thermal tolerance. Although the TS phenotypes of the parental strains used in both crosses were similar, the progeny from these parents exhibited a variance in TS comparable to the variance measured in natural field populations from around the world (Zhan and McDonald, 2011), indicating that the parents carried a high degree of genetic diversity for this trait. This finding also illustrates the potential for recombination to generate novel combinations of alleles that can affect thermal adaptation, providing a mechanism for a rapid evolutionary response to change in temperature. The presence of several visible but nonsignificant peaks on the genome scans, coupled with the finding of a maximum of 48.2% of the total phenotypic variance explained by the significant QTLs leads us to predict that additional QTLs will be revealed in future studies that include a greater number of markers and a larger number of offspring.

All four TS QTLs had allele means >1 , indicating faster growth at 22 °C than at 15 °C. An earlier analysis of Swiss populations of *Z. tritici* found many isolates with a $TS<1$, consistent with adaptation to growth at lower temperatures (Zhan and McDonald, 2011). These findings suggest that genes involved in adaptation to cooler temperatures were not variable in the four Swiss parents. We found no evidence for antagonistic pleiotropy (genetic trade-offs), but we postulate that testing our mapping populations under more extreme temperatures (>22 °C and <15 °C) could reveal such effects.

The finding of different TS QTLs in the two crosses likely reflects differences in the alleles found in the parents and may reflect different selective processes underlying thermal adaptation. For example, in cross 1A5 x 1E4 the significant TS QTLs on chromosomes 2 and 4 were due mainly to growth QTLs exhibited in the 22 °C environment. Pathogen strains that grow faster at warmer temperatures are likely to be favored during the asexual phase of reproduction that occurs during the summer (Ponomarenko *et al.*, 2011). The finding of different temperature-specific QTLs within the same cross suggests that different protein isoforms encoded by different alleles of the same gene could be better adapted to one temperature and maladapted to

another temperature or that the corresponding genes are differentially regulated at the two temperatures, a known mechanism for thermal tolerance (Schoville *et al.*, 2012).

All identified QTLs except the chromosome 10 TS QTL in cross 3D1×3D7 had large confidence intervals containing more than 10 genes. These QTLs could contain more than one segregating explanatory variant. Future studies using targeted backcross mapping (Sinha *et al.*, 2008) could be used to determine if multiple linked QTLs exist within any of these QTLs.

QTLs associated with the yeast/hyphae dimorphism

We identified seven QTLs associated with the yeast/hyphae dimorphism, with two of these QTLs shared between the crosses. On average, ~35% of the overall variation in growth morphology was explained by these QTLs. These findings indicate that the yeast/hyphae dimorphism is inherited as a quantitative trait in *Z. tritici*. The yeast/hyphae dimorphism QTLs correlate with the growth QTLs, indicating pleiotropic effects. QTL alleles associated with faster growth were also associated with filamentous growth in both crosses. We consider it likely that the filamentous morphology is responsible for the faster growth, as the yeast morphology results in a more localized growth of *Z. tritici* colonies (Mehrabani and Kema, 2006). However, it is also possible that morphology is a threshold trait that is triggered through faster growth. Our results also indicate that growth rate is not determined solely by the colony morphology, as we found three growth QTLs that were not associated with the dimorphism phenotype. In *Z. tritici* the switch between morphologies can be induced *in vitro* by changing from a nutrient-rich medium, which favors yeast-like growth, to a poor medium that favors hyphal growth (Mehrabani and Kema, 2006; Mehrabi *et al.*, 2006b). We found that temperature significantly affected dimorphism in our mapping populations, with more progeny exhibiting hyphal growth at 22 °C compared with 15 °C. While diverse environmental signals can affect dimorphism, the main signaling pathways appear well conserved, with the cAMP-dependent protein kinase A and mitogen-activated protein kinase pathways (Nadal *et al.*, 2008) having major roles. In *Z. tritici* both of these pathways were shown to affect the dimorphism phenotype (Cousin *et al.*, 2006; Mehrabi and Kema, 2006; Mehrabi *et al.*, 2006a, b; Gohari *et al.*, 2014). It is not known if the yeast-like morphology plays any role in the natural history of *Z. tritici*. Filamentous growth is obvious during all phases of its life cycle, including biotrophic, necrotrophic and saprotrophic phases of growth and development, but a yeast-like morphology has never been reported *in planta*. Regardless of the purpose of the yeast phase in *Z. tritici*, our findings illustrate that *Z. tritici* offers a new model for elucidating the mechanisms responsible for the yeast/hyphal dimorphism in fungi.

Identification and characterization of candidate genes within QTL confidence intervals

Earlier gene disruption studies in the IPO323 reference strain identified 10 genes (*MgTpk2*, *MgGpa1*, *MgGpa3*, *MgGpb1*, *MCC1*, *MVE1*, *MgSlt2*, *MgHog1*, *MgFus3* and *ZtWor1*) that affected the yeast/hyphae growth morphology (Cousin *et al.*, 2006; Mehrabi and Kema, 2006; Mehrabi *et al.*, 2006a, b, 2009; Choi and Goodwin, 2011a, b; Gohari *et al.*, 2014). We did not find any of these genes in the 95% confidence intervals of the dimorphism QTLs identified in our study, indicating that our QTL mapping approach identified novel gene candidates associated with the yeast/hyphae dimorphism in *Z. tritici*.

Out of the 15 candidate genes found in the 3D7×3D1 chromosome 1 QTL for the yeast/hyphae dimorphism at 15 °C, gene 88644 encodes

a Ca²⁺/calmodulin-dependent protein kinase, which is part of a kinase family involved in various signaling cascades. In the dimorphic ascomycete *Sporothrix schenckii*, calmodulin-dependent protein kinases were shown to be involved in the dimorphism (Valle-Aviles *et al.*, 2007). Hence, we consider gene 88644 as the most likely candidate to explain this chromosome 1 QTL.

The 3D7×3D1 chromosome 3 QTL for the yeast/hyphae (15 °C) dimorphism contains 15 candidate genes. Among these, gene 56742 encodes a guanine nucleotide exchange factor, a family of proteins involved in various intracellular signaling pathways. In a previous study in *Ustilago maydis*, a guanine nucleotide exchange factor was shown to be involved in dimorphism (Muller *et al.*, 2003), hence we consider this the most likely candidate gene explaining this QTL.

Evidence for pleiotropy

The mitogen-activated protein kinase kinase candidate gene (JGI ID: 76249 (MgPbs2)) in the chromosome 10 TS QTL of cross 3D1×3D7 is the ortholog of the *S. cerevisiae* gene *PBS2*. *Pbs2* phosphorylates the Hog1 protein (a mitogen-activated protein kinase) in *S. cerevisiae* as well as in *Aspergillus nidulans* (Panadero *et al.*, 2006; Duran *et al.*, 2010). *HOG1* is part of the HOG pathway that is induced upon osmotic stress. Recent studies indicate that other stress responses, including thermal stress, are also governed by the HOG pathway. For example, the HOG pathway provides a mechanism for thermal tolerance in *S. cerevisiae*, which accumulates glycerol under cold stress to protect against freezing (Panadero *et al.*, 2006). In *Aspergillus fumigatus*, the HOG pathway is thought to affect the fluidity of plasma membranes, providing protection under thermal stress (Ji *et al.*, 2012). Changes in cell membrane fluidity are thought to be a common mechanism for microorganisms to adapt to changing temperatures (Leach and Cowen, 2014). A mutant study (Mehrabani *et al.*, 2006b) of the *Z. tritici* ortholog *HOG1*, MgHog1 (JGI ID: 76502), found higher melanization associated with greater hyphal growth as well as larger colony sizes, the same pattern of phenotypes exhibited by the chromosome 10 TS QTL. Many of the chromosome 10 QTL pleiotropic effects observed in this study were found at 15 °C but not at 22 °C. This suggests that *PBS2*, and the HOG pathway, may be induced at lower temperatures in *Z. tritici*, providing a plausible mechanism to explain phenotypic plasticity.

Pleiotropy also explains the shared QTL peak on chromosome 11 in cross 3D1×3D7 for melanization (11 dpi), growth rate and the yeast/hyphae dimorphism for both temperatures (Lendenmann *et al.*, 2014). In this case, we hypothesize that *PKS1*, in particular the postulated quantitative trait nucleotide at amino-acid position 1783 (Lendenmann *et al.*, 2014), is the source of the observed trait variance. This hypothesis is supported by analyses showing that the RADseq marker closest to the *PKS1* gene explains >7% of the variance for each of these traits.

CONCLUSIONS

We elucidated the genetic architecture of thermal adaptation in a pathogenic fungus and identified multiple QTLs as well as candidate genes underlying this trait. To our knowledge, this is the first study to use QTL mapping to identify genes involved in thermal adaptation in a plant pathogen. Our findings suggest that thermal adaptation in *Z. tritici* involves not only changes in growth rate but also morphological adaptations, including degree of melanization and changes in growth morphology. This suggests that *Z. tritici* has evolved many strategies for thermal adaptation, in part explaining its global distribution across a wide temperature range and leading us to predict that it will adapt rapidly to global warming. Future investigations

aiming to functionally validate the candidate genes responsible for the mapped QTLs are underway. Additional population genetic diversity studies are needed to analyze the variation for these genes found in global populations and may further elucidate the potential of *Z. tritici* to adapt to changes in temperature. These studies point the way towards understanding the potential response of fungal pathogens to global warming.

DATA ARCHIVING

Sequence data of parental strains and progeny have been submitted to NCBI: NCBI BioSample accession numbers of parental strains: SRS383146 (ST99CH3D1), SRS383147 (ST99CH3D7), SRS383142 (ST99CH1A5) and SRS383143 (ST99CH1E4). NCBI BioProject accession numbers of progeny strains: PRJNA256988 (cross: ST99CH3D1 × ST99CH3D7) and PRJNA256991 (cross: ST99CH1A5 × ST99CH1E4).

Progeny phenotypes have been submitted to Dryad: DOI:10.5061/dryad.cj432.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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