

## Supplementary Results

Summary of the main findings according to each class of PCWDE.

### *Cutinases*

All six cutinase genes found in *Z. tritici* were confirmed in *Z. pseudotritici* and *Z. ardabiliae* and four were found in *Z. passerinii* (supplementary table S1). All cutinases were functionally assigned to the CAZy family Carbohydrate Esterases 5 (CE5). Estimated neutrality indices from the MKTs did not deviate significantly from neutral expectations for the pooled CE5 data set (supplementary table S3b). But at the level of individual genes, two of the six genes had neutrality indices significantly different from zero (fig. 3). CE5-ProtID-99331 was identified as being under significant diversifying selection ( $-\log_{10}NI = 0.356$ ,  $p = 0.011$ ;  $\alpha = 0.559$ ) while CE5- ProtID-18212 exhibited significant purifying selection (supplementary table S3a).

On the lineage specific level, we found a significant five-fold increase in dN / dS for CE5-ProtID-18212 of *Z. tritici* compared to its ancestors, indicating an increase in diversifying selection between species (supplementary table S4). All other cutinases showed dN /dS values not significantly different from the single rate model.

### *Cellulases*

All eleven cellulase genes were confirmed in *Z. tritici*, *Z. pseudotritici* and *Z. ardabiliae* and nine were found in *Z. passerinii* (supplementary table S1). The cellulases were assigned to five different CAZy families. Two of these families, Glycoside Hydrolases 5 (GH5) and GH61, were multi-gene families with six and two members, respectively. The individual members of these two families as well as the pooled data sets did not deviate from neutral expectations in the MKTs. GH45-ProtID-76589 was the only cellulase undergoing significant diversifying selection ( $-\log_{10}NI = 0.390$ ,  $p < 0.001$ ,  $\alpha = 0.593$ ). None of the cellulases showed evidence for significant purifying selection (fig. 3; supplementary tables S3a and S3b).

### *Hemicellulases*

Eighteen hemicellulase genes belonging to seven CAZy families exist in *Z. tritici*. Sixteen of the genes were confirmed in all species, but six could not be detected in *Z. passerinii* (supplementary table S1). CAZy families GH10, GH43 and GH51 were

multigene families with two, nine and three members, respectively. Multilocus MKTs on the pooled members resulted in evidence of significant purifying selection for CAZy family GH51, GH10 was neutral and no multilocus MKT could be calculated for GH43 because of heterogeneity among all pairwise comparisons (fig. 3; supplementary table S3b).

At the individual gene level, however, three of the nine GH43 genes (ProtIDs-73095, 98714, and 84076) and one of the three GH51 genes (ProtID-40215) were identified as being under significant purifying selection. Purifying selection was also indicated for the gene GH54-ProtID-70396. GH62-ProtID-68922 was the only hemicellulase gene showing significant diversifying selection ( $-\log_{10}NI = 0.686$ ,  $p < 0.001$ ,  $\alpha = 0.794$ ; fig. 3; supplementary table S3a).

The HYPHY analyses indicated widespread heterogeneity in selection pressure in this class of PCWDEs. Six of the 18 hemicellulases of *Z. tritici* showed significantly reduced dN / dS values compared to their ancestors, indicating increased purifying selection, e.g. due to substrate optimization on the new wheat host.

### *Pectinases*

Among the 13 pectinase genes in *Z. tritici*, all were confirmed in *Z. pseudotritici*, 11 were found in *Z. ardabiliae* and eight were found in *Z. passerinii*. The pectinases were distributed among eight CAZy families; five of these families included two members each (supplementary table S1). MKTs on the pooled members of CAZy families detected significant purifying selection for GH88 (fig. 3; supplementary table S3b). The pectinases are dominated by purifying selection and are the only class of enzymes without a gene showing significant diversifying selection. Eleven out of the 13 individual genes had negative NIs with five showing significant evidence of purifying selection (ProtIDs-77196, 25958, 33711, 50077, 65051, and 27839; fig. 3; supplementary table S3a).

On the phylogenetic level, one gene (GH115-ProtID-65051) showed a significant threefold reduction in dN / dS for *Z. tritici* (fig. 4; supplementary table S4).

**Supplementary table S1:** Identification of homologues and orthologues gene sequences coding for cell wall degrading enzymes in 19 whole genome assemblies of *Zymoseptoria tritici* and its closest relatives.

BLAST searches used search-algorithms of > 50% nucleotide identity and >50% sequence length identity with respect to 48 annotated CWDEs from the *Z. tritici* isolate IPO323 genome. Number of amino acids and average amino acid identity (AA\_ID) are given with respect to IPO323. Genes are listed by enzymatic families according to the Carbohydrate-Active EnZymes database (CAZy) and the protein identification number (Prot\_ID) from the Joint Genome Institute (JGI).

	JGI		<i>Z. tritici</i> (N=9)		<i>Z. pseudotritici</i> (N=5)		<i>Z. ardabiliae</i> (N=4)		<i>Z. passerinii</i> (N=1)			
	CAZy	PROT_ID	IPO323 amino acids	amino acids	% AA_ID	amino acids	% AA_ID	amino acids	% AA_ID	amino acids	% AA_ID	
<b>Cutinases</b>	CE5	77282	231	231	99.00	231	96.20	231	94.00	231	88.00	
	CE5	68483	216	216	99.00	216	97.00	216	97.00	214	82.00	
	CE5	35055	108	108	98.00	108	97.00	108	87.50	-	-	
	CE5	99331	239	239	99.00	239	94.20	141	87.65	-	-	
	CE5	43394	232	232	99.33	232	98.60	232	96.00	232	90.00	
	CE5	18212	202	202	99.89	202	99.00	202	95.00	202	84.00	
<b>Cellulases</b>	GH5	71664	495	495	99.78	495	95.20	495	93.00	495	88.00	
	GH5	88889	418	418	98.89	418	98.00	418	98.00	418	92.00	
	GH5	106779	417	417	99.78	417	98.00	417	96.50	-	-	
	GH5	83843	616	616	98.56	616	98.00	616	94.50	616	86.00	
	GH5	92097	364	364	98.86	364	89.59	364	87.81	363	81.73	
	GH5	111224	1085	1085	99.98	1085	93.45	1085	92.89	1084	85.27	
	GH7	100252	444	444	100.00	444	99.00	444	98.00	444	94.00	
	GH12	105871	246	246	98.78	246	92.40	246	91.00	245	80.00	
	GH45	76589	240	240	99.44	240	90.20	240	85.25	187	77.00	
	GH61	33254	320	320	98.50	320	92.00	284	89.50	-	-	
	GH61	103512	390	390	97.44	390	95.00	390	92.50	390	87.00	
	<b>Hemi-cellulases</b>	GH10	94846	370	370	99.00	370	96.00	370	94.00	369	84.00
GH10		61141	348	348	99.81	348	90.49	348	88.76	-	-	
GH11		60105	225	167	100.00	167	94.00	167	91.00	-	-	
GH43		98714	324	324	98.56	324	96.00	324	94.00	324	94.00	
GH43		105323	319	319	98.56	319	95.00	319	95.00	-	-	
GH43		96505	385	385	87.56	385	83.00	385	82.50	385	77.00	
GH43		84076	545	545	97.00	535	95.00	545	93.00	545	86.00	
GH43		75584	294	294	100.00	294	97.00	294	93.00	294	82.00	
GH43		96947	679	679	96.91	679	91.85	679	89.61	-	-	
GH43		73095	322	322	99.58	322	96.57	322	92.51	322	86.60	
GH43		30121	297	222	98.94	225	90.28	222	87.68	-	-	
GH43		105728	341	341	98.90	341	90.22	341	90.45	344	81.84	
GH51		71466	633	633	99.71	633	95.85	633	93.11	633	85.64	
GH51		111130	691	691	96.75	691	92.70	687	88.87	690	85.47	
GH51		40215	503	503	98.87	503	95.52	503	92.80	503	85.79	
GH54		70396	502	502	98.22	502	96.40	502	94.00	502	88.00	
GH62		68922	321	321	99.89	321	98.00	321	90.75	321	88.00	
GH93		95102	417	416	99.62	416	91.98	414	88.83	-	-	
<b>Pectinases</b>	CE8	66866	330	330	99.30	330	95.00	330	94.00	330	82.00	
	GH28	86032	416	416	99.22	416	97.00	416	93.00	416	86.00	
	GH28	77196	491	491	99.78	491	88.00	491	92.00	491	78.00	
	GH53	25958	332	332	98.78	332	97.60	-	-	-	-	
	GH53	51381	342	342	99.67	342	97.00	342	90.50	-	-	
	GH78	33711	798	798	99.27	798	94.27	798	94.15	-	-	
	GH78	69329	680	680	99.76	680	95.05	-	-	-	-	
	GH88	50077	380	380	97.72	380	94.20	380	93.23	380	78.28	
	GH88	57259	388	388	97.49	388	96.05	388	94.33	388	79.38	
	GH115	65051	1024	1024	99.17	1024	93.49	1024	91.84	1024	82.50	
	PL1	42327	342	342	98.11	342	91.80	342	91.00	342	83.00	
	PL1	85457	324	324	94.78	324	90.00	324	91.00	-	-	
	PL3	27839	215	215	100.00	215	96.40	215	95.50	215	89.00	
	<b>Average</b>					98.65		94.47		92.10		84.77









Supplementary Fig. S1











