

## SUPPLEMENTARY INFORMATION

**Table S1. Genome sequencing strategy.**

Genome	<i>F. verticillioides</i>			<i>F. oxysporum</i>		
	Reads	Physical Coverage (Fold)	Sequence Coverage *	Reads	Physical Coverage *	Sequence Coverage (Fold)
4kb Plasmid	66,479	3	2.44	444,726	15	4.44
10kb Plasmid	144,437	17	1.11	102,176	9	0.88
40kb Fosmid	70,511	34	1.08	110,003	37	0.96
Plasmid reads (Syngenta)	235,835		3.83			
<b>Total</b>	<b>517,262</b>	<b>54</b>	<b>8.46</b>	<b>656,905</b>	<b>106</b>	<b>6.28</b>

\* Q20 base coverage

**Table S2. *F. oxysporum* assembly anchored to the optical maps**

<b>Optical Contigs</b>	<b>Map estimated size (kb)</b>	<b>Scaffolds</b>	<b>Scaffold size (kb)</b>
I	7,451	14, 1, 27	6,853
II	6,444	6, 10, 31	5,575
III	5,497	47, 18, 32, 7, 25	5,631
IV	5,419	8, 4	5,212
V	5,205	26,2	4,913
VI	5,143	9, 33, 41, 21, 53, 42	4,585
VII	4,376	5, 13	4,346
VIII	3,992	3, 29	3,983
IX	3,434	11, 17	3,304
X	3,422	20, 15, 45	2,896
XI	2,929	35, 12	2,336
XII	2,855	19, 23	2,232
XIII	2,426	16, 39	1,750
XIV	2,269	22, 51, 36	1,645
XV	2,053	37, 38, 24, 28	2,434
<b>Total</b>	<b>62,917</b>		<b>57,697</b>

**Table S3. Enriched gene families in three *Fusarium* genomes.**

Species	Transcription	Carbohydrate-active enzymes <sup>2</sup>					Transporters <sup>3</sup>		
	Factors <sup>1</sup>	GH	GT	PL	CBM	Total	ABC	Partial ABC	PDR
<i>Fusarium graminearum</i>	716	242	102	21	37	402	54	0	19
<i>Fusarium verticillioides</i>	683	300	107	23	46	476	57	0	18
<i>Fusarium oxysporum</i>	881	363	124	24	44	555	67	5	25
<i>Aspergillus fumigatus</i>	562	262	100	14	23	399	42	2	15
<i>Neurospora crassa</i>	420	171	74	4	14	263	28	0	7
<i>Magnaporthe grisea</i>	461	231	92	5	36	364	41	1	8
<i>Saccharomyces cerevisiae</i>	295	46	68	0	2	116	22	0	10
<i>Candida albicans</i>	308	58	69	0	1	128	20	0	9
<i>P value*</i>	2.6E-06	0.01	0.0004	0	2E-11	0.001	7E-9	0.34	5E-7

\*The *p*-value (Z score) is calculated using the distribution of gene families in *Fusarium* compared to three other Ascomycete fungi (*Aspergillus fumigatus*, *Neurospora crassa* and *Magnaporthe grisea*)

**Table S4. Classification of transcription factors in three *Fusarium* species**

TF Family Name <sup>a</sup>	<i>F. graminearum</i>	<i>F. verticillioides</i>	<i>F. oxysporum</i>	<i>F. oxysporum</i> -LS
APSES	4	3	3	0
AT-rich interaction region	3	3	3	0
Bromodomain transcription factor	3	3	3	0
C2H2 zinc finger	76	67	73	10
CCR4-Not complex component, Not1	1	1	1	0
Centromere protein B, DNA-binding region	3	2	6	0
Cold-shock protein, DNA-binding	1	0	0	0
DDT	1	1	1	0
Forkhead	4	4	5	0
GATA type zinc finger	7	6	8	1
Grainyhead/CP2	1	1	1	0
HMG	39	36	39	5
Heat shock factor (HSF)-type, DNA-binding	3	0	5	2
Helix-turn-helix type 3	1	1	1	0
Helix-turn-helix, AraC type	8	4	4	0
Helix-turn-helix, Psq	0	0	1	0
Heteromeric CCAAT factors	7	6	7	0
Homeobox	13	10	13	0
Homeodomain-like	41	43	63	15
Lambda repressor-like, DNA-binding	2	4	6	2
MADS-box	3	2	4	1
Mating-type protein MAT alpha 1	1	1	1	0
Myb	17	20	22	0
NDT80/PhoG like DNA-binding	3	3	4	0
Negative transcriptional regulator	2	2	2	0
Nucleic acid-binding, OB-fold	48	46	42	3
RFX DNA-binding domain	1	1	1	0
SART1	1	1	1	0
SGT1	1	1	1	0
Transcription factor TFIIIS	2	1	2	0
Transcription factor jumonji	6	6	8	2
Winged helix repressor DNA-binding	28	28	35	3
YL1 nuclear protein	1	1	1	0
Zinc finger, BED-type predicted	1	1	1	0
Zinc finger, CCHC-type	12	14	21	6
Zinc finger, DHHC-type	5	3	5	0
Zinc finger, GRF-type	1	0	2	0
Zinc finger, MIZ-type	2	2	2	0
Zinc finger, PARP-type	0	1	0	0
Zinc finger, NF-X1-type	2	0	1	0
Zinc finger, Rad18-type putative	3	3	3	0
Zn2Cys6	313	311	370	27
bHLH	16	15	46	18
bZIP	21	19	55	24
p53-like transcription factor	6	4	6	0
ssDNA-binding transcriptional regulator	2	2	2	0
<b>Total TFs (Genome Ratio %)</b>	<b>716 (5.37)</b>	<b>683 (4.82)</b>	<b>881 (4.97)</b>	<b>119 (4.86)</b>

<sup>a</sup>Individual TFs were classified into families based on the DNA binding domain in each TF. <sup>b</sup>The ratio of TFs to the total proteome. <sup>c</sup>This total number may not be same to the sum of TF numbers for each TF family because some TFs have more than one type of DNA-binding motif.

Table S5. Comparison of pectin lyase enzymes

PL family	1	3	4	9	11	20	Total
<i>Aspergillus fumigatus</i>	6	3	3	0	0	1	13
<i>Aspergillus niger</i> CBS 513.88	6	0	2	0	0	0	8
<i>Neurospora crassa</i> OR74A	1	1	1	0	0	1	4
<i>Magnaporthe grisea</i> 70-15	2	1	1	0	0	1	5
<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	11	7	3	2	1	0	24
<i>Fusarium verticillioides</i>	11	7	3	2	0	0	23
<i>Fusarium graminearum</i>	9	7	3	1	0	1	21

**Table S6. The five families of *Fusarium* ABC transporters\*.**

	<i>F. graminearum</i>	<i>F. verticilloides</i>	<i>F. oxysporum</i>
ABCA	FGSG_08373.3	FVEG_02321.3 FVEG_12730.3+	FOXG_03449.2 FOXG_15400.2+
ABCB	FGSG_01885.3 FGSG_10911.3 <b>FGSG_03805.3</b> FGSG_05527.3 <b>FGSG_03032.3</b> FGSG_02749.3 FGSG_04336.3 FGSG_00541.3 FGSG_03323.3 FGSG_06881.3 FGSG_08823.3 FGSG_02786.3 FGSG_12704.3 FGSG_06771.3 FGSG_07516.3 FGSG_11988.3	FVEG_07368.3 FVEG_11590.3 <b>FVEG_12943.3</b> FVEG_06970.3 <b>FVEG_10569.3</b> FVEG_08945.3 FVEG_11327.3 FVEG_00998.3 FVEG_08641.3 FVEG_05103.3 FVEG_02402.3 FVEG_13095.3 FVEG_04890.3 FVEG_05216.3 FVEG_12783.3	FOXG_04248.2 FOXG_12846.2 <b>FOXG_16894.2</b> FOXG_09366.2 <b>FOXG_11983.2</b> FOXG_10296.2 FOXG_13902.2 FOXG_00515.2 FOXG_09840.2 FOXG_01943.2 FOXG_03534.2 FOXG_15633.2 FOXG_07972.2 FOXG_02052.2 FOXG_15452.2 FOXG_16892.2 FOXG_11845.2 FOXG_04837.2 FOXG_12952.2 FOXG_04763.2
ABCC	FGSG_06565.3 FGSG_09611.3 FGSG_00669.3 <b>FGSG_11028.3</b> FGSG_07325.3 FGSG_05571.3 FGSG_09017.3 FGSG_08308.3 <b>FGSG_06141.3</b> FGSG_09707.3 FGSG_02316.3 FGSG_02139.3 FGSG_04440.3 FGSG_10995.3 FGSG_10547.3 FGSG_00046.3	FVEG_05573.3 FVEG_04167.3 FVEG_00524.3 <b>FVEG_12505.3</b> FVEG_07940.3 FVEG_06924.3 FVEG_06686.3 FVEG_01831.3 <b>FVEG_04778.3</b> FVEG_06313.3 FVEG_11763.3 FVEG_07622.3 FVEG_03149.3 FVEG_09941.3 FVEG_10533.3+ FVEG_00329.3 FVEG_06048.3+ FVEG_07254.3 FVEG_03281.3 FVEG_03317.3	FOXG_02392.2 FOXG_06317.2 FOXG_00990.2 <b>FOXG_17424.2</b> FOXG_01579.2 FOXG_09320.2 FOXG_09082.2 FOXG_02981.2 <b>FOXG_07857.2</b> FOXG_08360.2 FOXG_13025.2 FOXG_04493.2 FOXG_08622.2 FOXG_05625.2 FOXG_10860.2 FOXG_11953.2 FOXG_04142.2 FOXG_04825.2
ABCD	FGSG_06012.3 FGSG_01526.3 FGSG_07383.3	FVEG_04642.3 FVEG_09605.3 FVEG_07897.3	FOXG_07718.2 FOXG_11187.2 FOXG_01535.2

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 ABCG

FGSG_02847.3	FVEG_13230.3	FOXG_15760.2
FGSG_09329.3	FVEG_03775.3	FOXG_05904.2
FGSG_11272.3	FVEG_11813.3	FOXG_13381.2
FGSG_03735.3	FVEG_10580.3	FOXG_11989.2
FGSG_04580.3	FVEG_11089.3	FOXG_13653.2
FGSG_08312.3	FVEG_01835.3	FOXG_02985.2
FGSG_08309.3	FVEG_01830.3	FOXG_02979.2
<b>FGSG_10577.3</b>	<b>FVEG_13032.3</b>	<b>FOXG_17147.2</b>
FGSG_08830.3	FVEG_02410.3	FOXG_03541.2
FGSG_02870.3	FVEG_13167.3	FOXG_15712.2
FGSG_10935.3	FVEG_11615.3	FOXG_12868.2
FGSG_05076.3	FVEG_06058.3	FOXG_08610.2
FGSG_01388.3	FVEG_09464.3	FOXG_11327.2
FGSG_05589.3	FVEG_06901.3	FOXG_16340.2
FGSG_08027.3	FVEG_09324.3+	FOXG_07314.2
FGSG_03882.3	FVEG_13012.3	FOXG_06754.2
FGSG_11240.3	FVEG_10503.3	FOXG_06510.2
FGSG_10706.3		FOXG_07190.2
		FOXG_11897.2
		FOXG_17197.2
		FOXG_10675.2
		FOXG_16543.2
		FOXG_04577.2
		FOXG_09300.2

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\*Shaded genes are orthologous for all three *Fusarium* sp. Red color highlights the transporters unique to *Fusarium*

**Table S7. Annotation of the *Fusarium* G protein subunits**

Protein Class	Nc Protein	NCU Number	Foxy Number	Fg Number	Fv Number
G alpha Subunit	GNA-1	NCU06493	FOXG_09359.2	FGSG_05535.3	FVEG_06962.3
G alpha Subunit	GNA-2	NCU06729.3	FOXG_05338.2	FGSG_09988.3	FVEG_02792.3
G alpha Subunit	GNA-2	NCU06729.3	FOXG_08807.2		
G alpha Subunit	GNA-3	NCU05206.3	FOXG_06321.2	FGSG_09614.3	FVEG_04170.3
G beta Subunit	GNB-1	NCU00440.3	FOXG_11532.2	FGSG_04104.	FVEG_10291.3
G gamma Subunit	GNG-1	NCU00041.3	FOXG_02181.2	FGSG_07235.3	FVEG_05349.3
RACK1 Homologue	CPC-2	NCU05810.3	FOXG_05557.2	FGSG_09870.3	FVEG_02582.3
Krh1/Gpb1	No Hits	No Hits	No Hits	No Hits	No Hits
Krh2/Gpb2	No Hits	No Hits	No Hits	No Hits	No Hits



Table S8. Predicted G protein coupled receptors (GPCRs) \*

Species	<i>Neurospora crassa</i>	<i>Magnaporthe grisea</i>	<i>Aspergillus nidulans</i>	<i>Fusarium graminearum</i>	<i>Fusarium oxysporum</i>	<i>Fusarium verticillioides</i>
<b>Pheromone Receptors</b>	NCU00138	MG06452	AN7743	FGSG_07270	FOXG_02147	FVEG_05310
	NCU05758	MG04711	AN2520	FGSG_02655	FOXG_10633	FVEG_09280
<b>cAMP Receptor-Like Proteins</b>	NCU00786	MG11962	AN3765	FGSG_05239	FOXG_08375	FVEG_06210
	NCU04626	MG06257	AN8262	FGSG_09693 (5)	FOXG_08466	FVEG_06299
	NCU09427	MG06738		FGSG_07716	FOXG_04274	FVEG_07395
				FGSG_01861	FOXG_10838	FVEG_13831(4)
				FGSG_03023	FOXG_14393(5)	FVEG_13224(2)
					FOXG_15087(5)	FVEG_12377
				FOXG_15757(4)		
				FOXG_15051(2)		
				FOXG_13170		
<b>Carbon Sensors</b>	NCU06312	MG08803 MG00258	No Hits	FGSG_05006	FOXG_09179	FVEG_06780
<b>Stm1-Related Proteins</b>	NCU00300	MG04698	AN5720	FGSG_05579	FOXG_09314	FVEG_06916
	NCU09195	MG02855	AN10166	FGSG_08496	FOXG_03300	FVEG_02168
<b>Microbial Opsins</b>	NCU10055	MG09015	AN3361	FGSG_07554	FOXG_15406	FVEG_12735
	NCU01735			FGSG_03064	FOXG_15870(5)	FVEG_09519
				FGSG_01440	FOXG_12142	FVEG_10716
				FOXG_11274		
<b>Homologues of <i>Magnaporthe grisea</i> PTH11</b>	NCU06531	MG05871	AN5639	FGSG_03707	FOXG_15891	FVEG_08756
	NCU00700	MG10473	AN1930	FGSG_02155	FOXG_04477	FVEG_13472
	NCU08624	MG07553	AN2587	FGSG_05821	FOXG_09718	FVEG_09407
	NCU05854	MG06755	AN7774	FGSG_03897(6)	FOXG_11987	FVEG_02076
	NCU07649	MG09022	AN0178 (4)	FGSG_07839	FOXG_12029	FVEG_07442
	NCU07591	MG07565	AN7232	FGSG_00994	FOXG_10751	FVEG_07166 (5)
	NCU09201	MG07946	AN7395	FGSG_08408	FOXG_04316	FVEG_06016
	NCU07538	MG11006	AN5059	FGSG_04529	FOXG_04048 (5)	FVEG_06583 (5)
	NCU05189	MG09070	AN2249	FGSG_11381	FOXG_03203	FVEG_08029
	NCU05307	MG07806	AN9444	FGSG_07663	FOXG_14725	FVEG_10345
	NCU08447	MG03584	AN9036	FGSG_04731	FOXG_08949	FVEG_13718
	NCU02903	MG05214	AN5664	FGSG_02374	FOXG_07137	FVEG_13358
	NCU09823	MG09863	AN11159	FGSG_05039	FOXG_16067	FVEG_05766
	NCU09796	MG10407	AN8328 (4)	FGSG_03464	FOXG_15611	FVEG_02749 (15)
	NCU04106	MG10571	AN1738	FGSG_07757	FOXG_08656	FVEG_10611 (6)
	NCU07769	MG01867	AN3395 (5)	FGSG_03005	FOXG_09988	FVEG_13015
	NCU04931	MG09455	AN6415 (6)	FGSG_04825 (6)	FOXG_14513 (6)	FVEG_14001
	NCU08718	MG10050	AN8951	FGSG_04159	FOXG_11475	FVEG_11908
	NCU09022	MG05352	AN1540 (5)	FGSG_06541	FOXG_02583	FVEG_10583
	NCU05101	MG07420	AN9306	FGSG_03800	FOXG_03452 (6)	FVEG_05605
	NCU08431	MG10442	AN8971	FGSG_02614	FOXG_16509	FVEG_07611
	NCU08429	MG02160	AN10886	FGSG_07136	FOXG_01664	FVEG_08525 (6)
	NCU06891	MG02001	AN10369 (5)	FGSG_03237	FOXG_05380 (15)	FVEG_09326
	NCU05187	MG10257	AN2386 (6)	FGSG_00101 (6)	FOXG_16884	FVEG_00078 (6)
	NCU05829	MG01905	AN10357	FGSG_07489	FOXG_17564	FVEG_03296 (4)
		MG07987	AN7400	FGSG_10028 (17)	FOXG_09687	FVEG_03544 (4)
		MG10438	AN4452(5)	FGSG_03504	FOXG_09958	FVEG_03509 (3)
		MG06171	AN6413	FGSG_09370	FOXG_04850 (4)	FVEG_13899
		MG07851	AN8943	FGSG_07655 (5)	FOXG_12335	FVEG_13048
		MG04935	AN4378(16)	FGSG_03932 (6)	FOXG_02421	FVEG_08692 (6)
		MG05386	AN2575(5)	FGSG_10085 (6)	FOXG_17561	FVEG_13491
		MG09865	AN5069	FGSG_11343	FOXG_14891 (6)	FVEG_07449
		MG05514	AN5312	FGSG_03688	FOXG_07081	FVEG_05876 (5)
		MG06535	AN2683 (6)	FGSG_01989	FOXG_14645 (4)	FVEG_11641 (6)
		MG01190	AN9387 (5)	FGSG_07601	FOXG_13506	FVEG_13696
		MG10581	AN0011 (5)	FGSG_11161	FOXG_16469 (4)	FVEG_14101
		MG03009	AN4213 (4)	FGSG_03164	FOXG_05656 (3)	FVEG_10934 (5)
		MG10747	AN7270(5)	FGSG_04693 (6)	FOXG_05684 (6)	FVEG_08880
		MG03935	AN7523 (6)	FGSG_03151 (6)	FOXG_10677	FVEG_10980
		MG04682	AN9266(4)	FGSG_03409	FOXG_08793 (5)	FVEG_13747 (6)
	MG09416	AN1557(4)	FGSG_11529	FOXG_09789	FVEG_11209 (6)	
	MG02692	AN3349(8)	FGSG_03009	FOXG_13475	FVEG_07701 (6)	
	MG07857	AN2649	FGSG_09352	FOXG_04322	FVEG_10789 (6)	
	MG00826	AN2726 (6)	FGSG_07841	FOXG_03973 (5)	FVEG_08553	

		MG06624	AN0171	FGSG_02134	FOXG_16863	FVEG_01609 (4)
		MG00435	AN3257	FGSG_05722	FOXG_16533 (3)	FVEG_00216
		MG08653	AN5943	FGSG_10630 (6)	FOXG_17122 (5)	FVEG_03914
		MG10706	AN7406(3)	FGSG_04749	FOXG_05864	FVEG_10190
		MG04170	AN3241	FGSG_07792	FOXG_12370 (5)	FVEG_08564 (6)
		MG08525	AN2044	FGSG_02981	FOXG_13042 (6)	FVEG_00008
		MG00277	AN3348	FGSG_05793	FOXG_12111 (6)	FVEG_03741 (6)
		MG02365	AN3886(5)	FGSG_03588	FOXG_15848 (5)	FVEG_07488 (5)
		MG06595	AN8548	FGSG_02401	FOXG_04515	FVEG_12807 (2)
		MG06084	AN11079(3)	FGSG_03959	FOXG_07468	FVEG_04399 (5)
		MG09437	AN4642	FGSG_03823	FOXG_13775 (5)	FVEG_10593 (6)
		MG01890	AN8512	FGSG_11598	FOXG_04594	FVEG_08784(4)
		MG01871	AN8984	FGSG_01833	FOXG_14426	FVEG_11719
		MG03794	AN0857	FGSG_03277	FOXG_15055	FVEG_12016
		MG09667	AN8151	FGSG_11385	FOXG_01279	FVEG_10615 (5)
		MG09061	AN6924	FGSG_03336 (6)	FOXG_06047	FVEG_10905 (6)
			AN6946 (5)	FGSG_00966	FOXG_09944 (6)	FVEG_10682 (6)
			AN2108	FGSG_10946	FOXG_11631	FVEG_11941 (5)
			AN2389(3)	FGSG_02853	FOXG_09918 (3)	FVEG_13612
			AN8661	FGSG_02569	FOXG_12943	FVEG_13238 (5)
			AN1317 (5)	FGSG_01806	FOXG_14011 (6)	FVEG_08439
			AN8727	FGSG_04500	FOXG_15477 (2)	FVEG_09408 (6)
			AN0500 (5)	FGSG_04865	FOXG_14208	FVEG_13145 (5)
			AN0751	FGSG_03215	FOXG_03205	FVEG_12373
			AN6622	FGSG_11351	FOXG_12528 (4)	FVEG_10044 (4)
			AN6458 (6)	FGSG_11080 (5)	FOXG_13490	FVEG_10829 (6)
				FGSG_03091	FOXG_15682	FVEG_12935 (3)
				FGSG_00201	FOXG_17114 (5)	FVEG_09946 (5)
				FGSG_10983	FOXG_07138 (6)	FVEG_09132 (5)
				FGSG_02334 (6)	FOXG_03847	FVEG_08689 (2)
				FGSG_03192	FOXG_07024 (5)	FVEG_10131
				FGSG_03962	FOXG_04018 (6)	FVEG_09357 (5)
				FGSG_04815	FOXG_12023 (6)	FVEG_08466
				FGSG_10958	FOXG_12208	FVEG_11704 (5)
				FGSG_02844	FOXG_07055 (5)	FVEG_06368 (2)
				FGSG_03310	FOXG_02918 (4)	FVEG_07719 (5)
				FGSG_06439	FOXG_16629	
				FGSG_02274	FOXG_02572	
				FGSG_02402	FOXG_10585 (5)	
				FGSG_02818 (5)	FOXG_15140 (3)	
				FGSG_03104	FOXG_12296 (6)	
				FGSG_03476	FOXG_11781 (4)	
				FGSG_09749	FOXG_09542 (6)	
				FGSG_13429	FOXG_10856 (5)	
				FGSG_13487	FOXG_11694	
				FGSG_11662	FOXG_10697 (6)	
				FGSG_12419(5)	FOXG_09792 (5)	
				FGSG_13972 (6)	FOXG_10478 (6)	
				FGSG_04023	FOXG_03932 (6)	
				FGSG_13461(4)	FOXG_08308	
				FGSG_12205	FOXG_10038	
				FGSG_12474 (3)	FOXG_15596 (2)	
				FGSG_12463(3)	FOXG_15595 (3)	
				FGSG_12490(6)	FOXG_17695 (4)	
<b>Homologues of <i>Homo sapiens</i> mPR-like GPCRs</b>	NCU04987 (6) NCU03238	MG05072 MG09091 MG04679	AN4932 AN10630 AN5151	FGSG_04051 (6) FGSG_01064	FOXG_00325 FOXG_12880 FOXG_12495 (6) FOXG_14044(6) FOXG_17118(6) FOXG_14172 (6) FOXG_11596(4)	FVEG_01198 FVEG_11630(6) FVEG_10223(4)
<b>Rat Growth Hormone Releasing Factor- Related Protein</b>	NCU03253(5)	MG00532	AN6680 AN5508	FGSG_00527 (5)	FOXG_00532	FVEG_00981 (5)
<b>GprK/AtRGS1- like GPCRs</b>	NCU09883	MG13926 MG11693	AN7795	FGSG_04628	FOXG_13617	FVEG_11041 FVEG_03266 (3)
<b>FlbA</b>	NCU08319	MG14517	AN5893	FGSG_06228 FGSG_03597	FOXG_08482 FOXG_16355 FOXG_06767	FVEG_08855 FVEG_06192

FOXG\_07329  
FOXG\_06495  
FOXG\_09613  
FOXG\_07099  
FOXG\_17640  
(6.5E-39)

\* Number of TMs in parenthesis

Table S9. Characterization of the secondary metabolite biosynthesis gene clusters<sup>a</sup>

	PKS	Cluster -id	Size (bp)	Genes	Preferential expression <sup>b</sup>	Known cluster	
Non-reducing PKS	FGSG_04588	FG3_26	38179	17	in planta		
	FGSG_03964	FG3_25	29205	9	poor		
	FGSG_02395	FG3_34	29521	8	poor	ZeaA	
	FGSG_09182	FG3_15	21303	8	sexual	PGL1	
	FVEG_03695	FV3_16	19281	7	Fumonisin		
	FOXG_05816	FO2_8	19694	7			
	FGSG_02324	FG3_33	25247	10	in culture	aurofusarin	
	FVEG_03379	FV3_12	18788	7	Fumonisin		
	FOXG_04757	FO2_19	18624	8			
Reducing PKS	Clade I	FGSG_03340	NONE				
		FOXG_11954	NONE				
		FVEG_10535	NONE				
		FVEG_05537	FV3_32	24127	8	Fumonisin	
		FVEG_08425	FV3_41	33102	13		
		FOXG_02884	FO2_31	19644	7		
		FVEG_01736	FV3_10	19430	7		
		FVEG_00316	FV3_21	49188	16	Fumonisin	Fumonisin
		FGSG_01790	FG3_45	17914	5	sexual	
		FVEG_13715	FV3_37	9568	5		
		FOXG_14850	NONE				
		FOXG_15886	NONE				
	Clade II	FGSG_10464	FG3_35	27100	11	sexual	
		FVEG_12610	FV3_27	22802	5		
		FOXG_15296	FO2_23	22800	5		
		FVEG_11932	FV3_5	35392	11	Fumonisin	
		FOXG_03945	FO2_7	42649	16	transposon insertion	
		FOXG_14587	FO2_13	32469	10		
		FVEG_10497	FV3_36	28933	9	Fumonisin	
		FOXG_01189	FO2_14	26645	8		
		FVEG_11086	FV3_17	27587	9	Fumonisin	Fusarin C
		FGSG_07798	FG3_19	26974	9	in planta	Fusarin C
	Clade III	FOXG_02741	FO2_30	22204	9		
		FVEG_09961	FV3_19	12742	5		
		FVEG_00079	FV3_24	14557	4	Fumonisin	
		FGSG_04694	FG3_1	14340	4		
		FVEG_12523	FV3_28	23139	7	Fumonisin	
		FOXG_15248	FO2_22	25367	8		
		FGSG_08208	FG3_20	31544	7	sexual	
FGSG_08795		FG3_28	22447	6	sexual		
FVEG_01914		FV3_8	21577	5			
FOXG_03051		FO2_33	21645	5			
FGSG_12126	FG3_34	29521	8	poor	ZeaB		
FGSG_10548	FG3_38	17838	4	poor			
FOXG_10805	FO2_21	28119	9				

	FGSG_05794	FG3_12	19827	6	constitutively expressed	
TS		FG3_13	19701	12	constitutively expressed	
		FG3_21	27016	12	in planta	trichothecene
		FG3_29	21271	10		
		FV3_33	21271	9	Fumonisin	
		FO2_17	18689	8		
		FG3_40	29834	11	sexual	
	FG3_44	26907	8	poor		

<sup>a</sup>. Light blue shaded SMB clusters share orthologous PKS based on the PKS phylogeny (Figure S5) and are also conserved in the flanking genes identified in the cluster. None = no cluster identified with the required four SMB related genes within a 20 kb window.

<sup>b</sup>. The co-expression of the clusters under various experiment conditions using published *Fg* microarray data (13). The clusters in green are specifically expressed in *planta*. The clusters in red are specifically expressed during sexual development; and the clusters in brown are expressed in culture. Fumonisin indicates co-expression of *Fv* gene clusters under culture conditions conducive to fumonisin production in a liquid medium.

**Table S10. Pair-wise comparison among three *Fusarium* species.**

	<i>F. oxysporum</i> <sup>a</sup>	<i>F. verticillioides</i>	<i>F. graminearum</i>
<i>F. oxysporum</i>		90%	80%
<i>F. verticillioides</i>	37,302 kb (90%) <sup>b</sup>		80%
<i>F. graminearum</i>	29,018 kb (80%)	28,755 kb (79%)	

<sup>a</sup> Average sequence identity

<sup>b</sup> Total length of syntenic regions (percentage of the genome)

**Table S11. Transposable elements in three *Fusarium* genomes.**

a.

	Class/LTR retrotransposons			DNA transposons						
	SINE	LTRs	Total	Pogo	hAT	Helitron	MITEs	Impala	Others	Total
<i>Fg</i>	360	7,632	8,552	1,076	345	0	0	0	2520	1,421
<i>Fv</i>	248	55,000	56,075	372	999	0	0	0	0	2,018
<i>Fol</i>	159,408	274,097	433,505	491,352	629,444	163,307	21,180	14,563	342,368	1,506,939

b.

	Class/LTR retrotransposons			DNA transposons						
	SINE	LTRs	Total	Pogo	hAT	Helitron	MITEs	Impala	Others	Total
<i>Fol_Cons</i>	46.01%	50.98%	49.15%	20.78%	18.54%	29.84%	5.38%	0%	22.76%	5.19%
<i>Fol_LS</i>	53.99%	49.02%	50.85%	79.22%	81.46%	70.16%	94.62%	100%	77.24%	94.81%

a). Each type of TE in *Fg*, *Fv* and *Fol* genomes is reported as a total number of bases in each genome.

b). Each type of TE in *Fol* is further located into either *Fol* LS regions or the conserved portion of the genome (*Fol* Cons) and is reported as the percentage distribution between these two regions.

**Table S12. Over-represented GO categories of genes encoded in *Fol* LS regions**

Go	name	FDR	FWER	p-Value	#Test	#Ref	#nonAnnotTest	#nonAnnotRef
GO:0005576	extracellular region	0.00349266	0.0135177	7.05E-05	19	70	576	6682
GO:0044421	extracellular region part	8.84E-04	0.00154564	1.05E-05	12	23	583	6729
GO:0005625	soluble fraction	4.48E-04	6.72E-04	3.55E-06	12	20	583	6732
GO:0005615	extracellular space	8.87E-04	0.00177332	1.20E-05	10	15	585	6737
GO:0004568	chitinase activity	0.00413742	0.0174305	9.25E-05	8	12	587	6740
GO:0006032	chitin catabolic process	0.00495101	0.0232431	1.39E-04	8	13	587	6739
GO:0006046	N-acetylglucosamine catabolic process	0.00495101	0.0232431	1.39 E-04	8	13	587	6739
GO:0060089	molecular transducer activity	0.00349266	0.0138736	7.61E-05	23	97	572	6655
GO:0016310	phosphorylation	0.00315689	0.0102075	5.20E-05	36	189	559	6563
GO:0004672	protein kinase activity	0.00315689	0.00937104	4.43E-05	29	135	566	6617
GO:0004871	signal transducer activity	0.00349266	0.0138736	7.61E-05	23	97	572	6655
GO:0008986	pyruvate, water dikinase activity	3.65E-06	3.65E-06	2.49E-08	9	3	586	6749
GO:0006094	gluconeogenesis	0.00258178	0.00643367	4.10E-05	9	14	586	6738
GO:0046364	monosaccharide biosynthetic process	0.00579484	0.0316186	1.75E-04	9	18	586	6734
GO:0019319	hexose biosynthetic process	0.00579484	0.0316186	1.75E-04	9	18	586	6734
GO:0016781	phosphotransferase activity, paired acceptors	3.65E-06	3.65E-06	2.49E-08	9	3	586	6749
GO:0006090	pyruvate metabolic process	0.00579484	0.0316186	1.75E-04	9	18	586	6734
GO:0004231	insulysin activity	1.00E-07	2.51E-08	1.46E-10	9	0	586	6752
GO:0000003	reproduction	0.00579484	0.0327718	1.88E-04	16	57	579	6695
GO:0003006	reproductive developmental process	2.32E-04	2.90E-04	1.90E-06	9	8	586	6744
GO:0022414	reproductive process	0.00315689	0.00996744	4.71E-05	13	33	582	6719
GO:0007548	sex differentiation	1.64E-06	8.20E-07	6.73E-09	9	2	586	6750
GO:0004040	amidase activity	0.0011712	0.00263172	1.81E-05	10	16	585	6736
GO:0005871	kinesin complex	0.00695187	0.0408539	1.99E-04	4	1	591	6751



**Table S13. Annotation of secreted proteins**

Species	Total	Anchored		Small		
	Secreted	CPGRP	GPI	Cysteine-rich	GH	NEP
<i>Fusarium graminearum</i>	1386	47	88	97	138	4
<i>Fusarium verticillioides</i>	1549	46	86	95	172	4
<b><i>Fusarium oxysporum</i></b>	<b>1803</b>	<b>46</b>	<b>91</b>	<b>126</b>	<b>210</b>	<b>7</b>
<b><i>p-value</i> *</b>	<b>2E-05</b>	<b>0.85</b>	<b>3E-05</b>	<b>1e-100</b>	<b>6E-04</b>	<b>0</b>
<i>F. oxysporum</i> LS specific	253	0	0	26	31	3

\* *p*-value is computed using T-test comparing genes in Fol to other two *Fusarium* genomes. Except GPI anchored proteins, genes Fol LS regions contribute to all other gene family expansions.

**Table S14: Enriched glycosyl hydrolases (GHs) in *Fol*.**

Family	<i>F. oxysporum</i>	<i>F. verticillioides</i>		<i>F. graminearum</i>		Putative activities
	Num <sup>1</sup>	Num	% <sup>2</sup>	Num	%	
GH5	23	19	21%	13	77%	Activity against arabinogalactans
GH43	32	24	36%	18	75%	Arabinanases; $\beta$ -1,3-galactanases
GH79	6	2	200%	1	500%	$\beta$ -glucuronidase
GH28	15	9	67%	6	150%	Polygalacturonases, against pectin
GH35	7	4	75%	3	133%	$\beta$ -galactanases
GH32	14	7	100%	5	180%	Invertases/endo- and exo-inulinases
GH18	30	20	50%	19	58%	Chitinases
GH76	12	9	33%	7	71%	Activity against fungal mannans

<sup>1</sup>The number of the each glycoside hydrolase (GH) gene family is characterized by search against a library of catalytic and carbohydrate-binding modules of carbohydrate-active enzymes (see also [www.cazy.org](http://www.cazy.org)) using blastp.

<sup>2</sup>The increase in *Fol* with respect to the number of genes in the other two genomes.

**Table S15. *Fol* carbohydrate-active enzyme (CAZY) genes examined for the presence of transcripts during plant infection.**

Predicted gene	CAZY family	Putative activity	Chr(s)	Copy no.	Transcript in <i>planta</i> <sup>1</sup>
FOXG_08862	GH28	exo-polygalacturonase pgx4	2a	1	Yes
FOXG_13051	GH28	endo-polygalacturonase pg5	9	1	Yes
FOXG_14695	GH28	endo-polygalacturonase pg1	12	1	Yes
FOXG_12535	GH28	endo-polygalacturonase	3,6	4	Yes
FOXG_14951	GH61	endoglucanase	2b,3	2	Yes
FOXG_14383	GH32	sucrose-6-phosphate hydrolase	1,15	2	Yes
FOXG_14385	GH32	inulinase	1,15	2	yes
FOXG_02657	GH79	$\beta$ -glucuronidase	8	1	yes
FOXG_06568	GH79	$\beta$ -glucuronidase	3,6	3	no
FOXG_04665	GH78	$\alpha$ -L-rhamnosidase	4	1	yes
FOXG_15491	GH78	$\alpha$ -L-rhamnosidase	15	2	no
FOXG_09503	GH64	$\beta$ -1,3-glucanase	9	1	yes
FOXG_12407	GH64	$\beta$ -1,3-glucanase	3,6	2	no
FOXG_14366	GH76	$\alpha$ -1,6-mannosidase	1,15	2	no
FOXG_06755	GT1	UDP-glucose sterol transferase	3,6	4	no
FOXG_07014	GT4	trehalose phosphorylase	6	1	no
FOXG_07432	GT4	glycosyltransferase	3,4	2	no
FOXG_12436	GT8	glycosyltransferase	2,15	3	no
FOXG_00921	GH18	chitinase 18-3	1	1	yes
FOXG_14329	GH18	chitinase 18-8	1,15	2	yes
FOXG_15172	CBM12	chitin-binding domain	1,15	2	yes

<sup>1</sup>As determined by reverse transcriptase PCR analysis of cDNA obtained from tomato plants infected with *Fg* f. sp. *lycopersici* strain 4287 three days after inoculation (see also Figure S17). Plant CAZYS from GH families encoded in LS regions are coloured pink, those from GT families are coloured green and fungal CAZYS are labelled light blue.

**Table S16. Gene-specific primers used for RT-PCR and product sizes.**

<b>Gene</b>	<b>gDNA (bp)</b>	<b>cDNA (bp)</b>	<b>Forward primer (5'→3')</b>	<b>Reverse primer (5'→3')</b>
FOXG_00921	372	311	TTAGTGATGAGTGGGCGGATG	TGGTAGATGAATGCGGACAGC
FOXG_02259	450	395	CCAATGAGCTTCCCGACCTC	CTGGTAGCGGAGAGTCAAGC
FOXG_02657	374	317	AATACCTCTGGGCAATCGAACT	GTCATCGGCGGTAGCATTAGA
FOXG_04665	456	281	TCTGGCATGATGTGGTCGTCT	GAGTATGCGGTCTGCGTGTC
FOXG_06568	472	297	AGTCTAGGCACCCGTGTTTGT	GCCCAGAAGAATCCAGTCAAC
FOXG_06755	476	288	TGCGATATCTGTCCCGATAGG	TTTGAGGATGCCGTTATGAGTG
FOXG_07014	670	474	GCCTTCAAACATGGTCGAAAGT	GCGAAAGCCAACTTGTAGGAG
FOXG_07432	505	444	CTCCTCGGGCGGTTACAAAG	TCATGGATAGACCGTCGTTGG
FOXG_08862	387	328	GTACAGCATTGCCTCGCCAC	CGGGTTTCTCATTTCGCAGGTT
FOXG_09503	219	219	TCTGCGAGGGCTTGAAGCGT	CCCATTCTGCCTGCGTGTTG
FOXG_12407	482	355	GATCAGCTTCTCTGCGAGGG	TGTCTCATCTTAATACCGTGTAC
FOXG_12436	495	414	GTTCGACATAAGGATAATACGGA	TATTCGGAGCCCAGATACTTG
FOXG_12535	426	368	CTGGAAAGACTCTGGACCTGA	TGTGTCCGCCAGTTTGTGCG
FOXG_13051	389	341	GCCTGGTCGCCTCCGTACTION	TCTTCTTGCCGCCGTTGCTG
FOXG_14329	391	330	AGCCCAACGAGCCGTCAAGT	AACTGCCGCCAGCGTATGAG
FOXG_14366	470	362	CGTCTGGAATAAGGTTGTCAAG	CCCGCTTTTCTGCTATTGTGA
FOXG_14383	400	400	GGCTCCTCCGCTTGCTCCT	TGCTGCCATCTTTGCTAGTCG
FOXG_14385	407	407	GAGCAAGAGAAACCGCACGAT	ATCACGCCCAAAGTCGAGCC
FOXG_14695	328	277	GCAGCGTCACTGACTACTCC	GTTAGAACCTTCGCCATCCCA
FOXG_14951	365	365	GCTGGCTCGTTCAGATCCCT	CGGGAGCAGCAGCGGAGAT
FOXG_15172	443	443	GACGCACGCAAGAACGCCTA	ACGCTGTGACACCGCATGGA
FOXG_15491	437	379	CGATTTGGCATGATGTGGTTGT	GCCTCTGGTGACACGTATTC
FOXG_16779	439	381	GCCCAGCAGCAGTTTACATCT	TGTCCGCAGAGCCTAGATAGA
FOXG_01569				
Actin 1	478	429	GAGGGACCGCTCTCGTCGT	GGAGATCCAGACTGCCGCTCAG

Table S17. Lipid metabolism-related gene expansion in *Fol* LS regions\*

Species	<i>F. oxysporum</i>	<i>F. verticillioides</i>	<i>F. graminearum</i>	<i>Neurospora crassa</i>	<i>Magnaporthe grisea</i>	<i>Aspergillus nidulans</i>
<b>Homologues</b>	FOXG_00325	FVEG_01198	FGSG_04051 (6)			
<i>Homo sapiens</i>				NCU04987 (6)	MG05072	AN4932
<b>mPR-like GPCRs</b>	FOXG_12880	FVEG_11630(6)	FGSG_01064	NCU03238	MG09091	AN10630
	FOXG_12495 (6)	FVEG_10223(4)			MG04679	AN5151
	FOXG_14044(6)					
	FOXG_17118(6)					
	FOXG_14172 (6)					
	FOXG_11596(4)					
<b>Perilipin-like gene (CAP20)</b>	FOXG_07916	FVEG_04839	FGSG_05177	NCU03370	MGG_11916	AN10518
	FOXG_06858					
	FOXG_07131					
	FOXG_07164					
	FOXG_15915					
	FOXG_16152					
	FOXG_16227					
	FOXG_16658					
	FOXG_16721					
<b>Phosphoinositide-specific phospholipase C (PLC)</b>	FOXG_07611	FVEG_04539	FGSG_05898	NCU001266	MGG_02497	AN6389
	FOXG_12081			NCU06245		
	FOXG_06661			NCU09655		
	FOXG_07227			NCU02175		
	FOXG_15032					
	FOXG_06598					
	FOXG_14448					
	FOXG_07102					
<b>Lipase - carboxylic esters</b>	FOXG_15813	FVEG_13281	FGSG_09122	NCU09575	MGG_02497	AN6389
	FOXG_17621	FVEG_12978				
	FOXG_13461	FVEG_13937				
	FOXG_14397					
	FOXG_14401					
	FOXG_14404					
	FOXG_15281					
	FOXG_15077					
	FOXG_15080					
	FOXG_15083					
<i>FlibA</i>	FOXG_08482	FVEG_08855	FGSG_06228	NCU08319	MGG_14517	AN5893
	FOXG_16355	FVEG_06192	FGSG_03597			
	FOXG_06767					
	FOXG_07329					
	FOXG_06495					
	FOXG_09613					
	FOXG_07099					
	FOXG_17640 (6.5E-39)					
<b>Kinases (cdc2)</b>	FOXG_09513	FVEG_07140	FGSG_05393	NCU01266	MGG_02444	AN0664
	FOXG_03267	FVEG_02139	FGSG_08468			
	FOXG_09498	FVEG_07125	FGSG_05406			
		FVEG_10803	FGSG_03132			
	FOXG_17471					
	FOXG_06983					
	FOXG_07291					
	FOXG_06877					
	FOXG_06534					
	FOXG_06733					
	FOXG_03267					
	FOXG_16245					
	FOXG_16169					
	FOXG_06842					
	FOXG_16211					
	FOXG_16133					

\*Yellow highlights the orthologous genes among three *Fusarium* genomes, while all others are Fol LS expansions.

**Table S18. Proteins involved in chromatin silencing pathways\*.**

**1. DNA methyltransferase homologues**

**a. RID homologues (DMT domain of unknown enzymatic function)**

Species	Locus	Name	Chromosome	EST
<i>Fg</i>	FGSG_08648.3	FgRID	2	none
<i>Fv</i>	FVEG_02018.3	FvRID	6	none
<i>Fol</i>	FOXG_03151.2	FolRID	8	none

**b. DIM-2-type DNA methyltransferase homologues**

Species	Locus	Name	Chromosome	EST
<i>Fg</i>	FGSG_10766.3	FgDIM2	3	none
<i>Fv</i>	FVEG_11429.3	FvDIM2	7	incomplete
<i>Fol</i>	FOXG_12693.2	FolDIM2	9	none

**2. Histone H3 Lysine9 methyltransferase DIM-5 homologues**

Species	Locus	Name	Chr	EST
<i>Fg</i>	FGSG_02778.3	Fg Dim5	1	incomplete
<i>Fv</i>	FVEG_08911.3	Fv Dim5	5	complete
<i>Fol</i>	FOXG_10263.2	FolDim5	7	complete

**3. Heterochromatin Protein 1 (HP1) homologues**

Species	Locus	Name	Chr	EST
<i>Fg</i>	FGSG_08763.3	FgHP1	2	none
<i>Fv</i>	FVEG_01876.3	FvHP1	6	complete
<i>Fol</i>	FOXG_03020.2	FolHP1	8	complete

**4. RNA-dependent RNA Polymerase homologues**

Species	Locus	Name	Chr	EST
<i>Fg</i>	FGSG_06504.3	FgQde1	4	incomplete
<i>Fv</i>	FVEG_05646.3	FvQde1	3	none
<i>Fol</i>	FOXG_02461.2	FolQde1	5	none
<i>Fg</i>	FGSG_08716.3	FgSad1	2	none
<i>Fv</i>	FVEG_01945.3	FvSad1	6	none
<i>Fol</i>	FOXG_03081.2	FolSad1	8	none
<i>Fg</i>	FGSG_01582.3	FgRdp3	1	none
<i>Fv</i>	FVEG_09667.3	FvRdp3	1	incomplete
<i>Fol</i>	FOXG_11123.2	FolRdp3	1	incomplete

**5. Dicer homologues**

Species	Locus	Name	Chr	EST
<i>Fg</i>	FGSG_09025.3	FgDcl1	4	none
<i>Fv</i>	FVEG_06696.3	FvDcl1	7	none
<i>Fol</i>	FOXG_09093.2	FolDcl1	9	none
<i>Fg</i>	FGSG_04408.3	FgDcl2	2	none
<i>Fv</i>	FVEG_11254.3	FvDcl2	9	incomplete
<i>Fol</i>	FOXG_13826.2	FolDcl2	10	incomplete

**6. Argonaute homologues**

Species	Locus	Name	Chr	EST
<i>Fg</i>	FGSG_08752.3	FgQde2	2	Incomplete
<i>Fv</i>	FVEG_01865.3	FvQde2	6	Incomplete

<i>Fol</i>	FOXG_03010.2	FolQde2	8	Incomplete
<b><i>Fol</i></b>	<b>FOXG_12456.2</b>	<b>FolQde2-2</b>	<b>3</b>	<b>none</b>
<b><i>Fol</i></b>	<b>FOXG_14081.2</b>	<b>FolQde2-3</b>	<b>6</b>	<b>none</b>
<b><i>Fol</i></b>	<b>FOXG_16455.2</b>	<b>FolQde2-4</b>	<b>14</b>	<b>none</b>
<i>Fg</i>	FGSG_00348.3	FgSms2	1	none
<i>Fv</i>	FVEG_00803.3	FvSms	1	none
<i>Fol</i>	FOXG_00711.2	FolSms2	1	none

\* Loci were identified by using *Fg* or *Neurospora crassa* predicted and known protein sequences as baits in tblastn searches of the *Fusarium* genomes with default settings. All genes except for the three non-orthologous *QDE2* homologues of *Fol* are located on non-LS chromosomes. The non-orthologous *Fol QDE2* proteins (shown on bold) are more closely related to *Ajellomyces capsulatus*, *Aspergillus* spp. and *Pyrenophora tritici-repentis*.

**Table S19. Pearson correlation coefficient ( $r$ ) of codon usage between chromosome pairs.**

	Chr1	Chr2	Chr4	Chr5	Chr7	Chr8	Chr9	Chr10	Chr11	Chr12	Chr13	Chr3	Chr6	Chr14
Chr2	0.999													
Chr4	0.999	0.999												
Chr5	0.999	1.000	0.999											
Chr7	0.999	0.999	1.000	1.000										
Chr8	0.999	0.999	1.000	1.000	1.000									
Chr9	0.999	0.999	0.999	0.999	0.999	0.999								
Chr10	0.999	0.999	1.000	0.999	1.000	0.999	0.999							
Chr11	0.994	0.996	0.998	0.997	0.997	0.997	0.997	0.998						
Chr12	0.994	0.996	0.997	0.996	0.996	0.997	0.996	0.997	0.999					
Chr13	0.993	0.996	0.997	0.996	0.996	0.997	0.996	0.997	0.999	0.999				
Chr3	0.992	0.992	0.990	0.992	0.990	0.991	0.990	0.991	0.989	0.989	0.989			
Chr6	0.992	0.993	0.991	0.993	0.991	0.992	0.991	0.992	0.990	0.990	0.990	1.000		
Chr14	0.987	0.990	0.988	0.990	0.987	0.988	0.987	0.988	0.987	0.986	0.988	0.997	0.997	
Chr15	0.993	0.992	0.991	0.993	0.992	0.992	0.991	0.992	0.988	0.989	0.987	0.998	0.998	0.993



Table S20. Amino acids with significant codon bias in the *Fol* LS chromosomes

Amino acid	Codon	Number of codons in conserved regions (%)		Number of codon in <i>Fol</i> LS regions (%)		P-value*
Q	CAA	166721	53.74%	22461	48.79%	1.00E-86
	CAG	143544	46.26%	23576	51.21%	1.00E-86
	total	310265		46037		
C	TGT	87368	45.16%	11409	42.75%	1.00E-13
	TGC	106112	54.84%	15281	57.25%	1.00E-13
	Total	193480		26690		
A	GCA	127397	24.79%	19537	24.72%	0.35
	GCC	135711	26.40%	22477	28.44%	1.00E-30
	GCG	94290	18.35%	15659	19.81%	1.00E-20
	GCT	156573	30.46%	21357	27.02%	1.00E-80
	total	513971		79030		
G	GGC	131445	29.97%	22031	32.31%	1.00E-30
	GGG	72143	16.45%	13128	19.25%	1.00E-70
	GGA	123164	28.09%	17921	26.28%	1.00E-20
	GGT	111775	25.49%	15107	22.16%	1.00E-78
	Total	438527		68187		
V	GTC	129752	32.55%	18820	32.07%	0.02
	GTA	64982	16.30%	10286	17.53%	1.00E-10
	GTG	89308	22.41%	14147	24.11%	1.00E-19
	GTT	114564	28.74%	15430	26.29%	1.00E-30
	Total	398606		58683		
T	ACC	118093	34.71%	16778	34.20%	0.06
	ACA	129291	38.01%	17878	36.44%	1.00E-07
	ACT	117785	34.62%	15832	32.27%	1.00E-18
	ACG	93106	27.37%	15351	31.29%	1.00E-60
	Total	340182		49061		
E	GAA	153574	46.16%	23891	45.15%	1.00E-05
	GAG	179115	53.84%	29019	54.85%	1.00E-05
	Total	332689		52910		

\* P-value

determined using Fisher's exact test to examine the significance of codons used between conserved versus LS regions. Pink indicates codons with increased usage among the *Fol* LS genes. Blue indicates the codons with decreased usage among the *Fol* LF genes.

**Table S21 Distance matrix of *Fol* LS homologous genes among selected fungal genomes\***

	<i>Fo</i> LS genes	<i>Fs</i> homo	<i>Fo</i> conserved homo	<i>Fv</i> homo	<i>Fg</i> homo	<i>M. grisea</i> homo	<i>N crassa</i> homo	<i>A. nidulans</i> homo	<i>A. oryzae</i> homo
<i>Fo</i> LS genes	-	0.436	0.487	0.542	0.538	0.682	0.721	0.714	0.703
<i>Fs</i> homologs	1058	-	0.148	0.195	0.177	0.260	0.292	0.300	0.294
<i>Fo</i> _conserved homologs	1180	358	-	0.111	0.115	0.238	0.265	0.269	0.269
<i>Fv</i> homologs	1313	473	269	-	0.082	0.201	0.209	0.227	0.229
<i>Fg</i> homologs	1304	430	278	199	-	0.194	0.203	0.229	0.222
<i>M. grisea</i> homologs	1654	630	576	487	470	-	0.099	0.126	0.126
<i>N crassa</i> homologs	1747	707	643	506	491	241	-	0.153	0.154
<i>A. nidulans</i> homologs	1731	727	651	550	555	305	372	-	0.080
<i>A. oryzae</i> homologs	1703	713	651	554	537	305	374	194	-

\* The distance is measured by the presence and absence of homologous gene in each genome through blastp search (1e-20). Only genes encoded in the conserved regions of *Fol* genome are used for the search within *Fol* genome. Below diagonal: Total character differences; Above diagonal: Mean character differences.

**Table 22: Peptides from Six5, Six6 and Six7 identified with mass spectrometry.**

Protein	Peptide	Peptide mass (MS) <sup>1</sup>	Amino acid sequence(MS/MS) <sup>1</sup>
Six5	AANGKIDGDTFYNLCINNGGADSTCFDCSK	spot 17	p12 <sup>2</sup>
	IDGDTFYNLCINNGGADSTCFDCSK	spot 17	
	CINNGGADSTCFDCSK	spot 17	spot 17
	NVIYCDA	spot 17	p12 <sup>2</sup>
	FAGIYLK	spot 17	spot 17 <sup>3</sup>
	TSPAYWFADR	spot 17	spot 17
	SYLWAQTSPAYWFADR	spot 17	
	RDHQYCACQSGSGDSIDIDATTQLQNDNSK	spot 17	
Six6	SFCVANPR	spot 16	spot 16
	ITDTPCQPR	spot 16	spot 16
	EKITDTPCQPR	spot 16	spot 16
	DTLPVSTCPAGQKYDR	spot 16	
	EGCTTTSVNPAGYHHLGTIVYDINK	spot 16	
	EGCTTTSVNPAGYHHLGTIVYDINKNPIEVDK	spot 16	
	ISYFGEPGNVNEGIGGSTS YFSSDNFQFSK	spot 16	
Six7	TTFVEVR	spot 21	spot 21
	QGQCFSTTGSTPPRPPAAR	spot 21	spot 21
	EVTFDITQNVNTFTSAASTPWTEGVGLSNIR	spot 21	
	VFGTAEAQVLLPDAPGTSR	spot 21	

<sup>1</sup> spot numbers in the 2-D gel with pH range 3-10 as published in Houterman *et al.* (2007)<sup>76</sup>.

<sup>2</sup> p12: Found among previously unidentified peptides in 12 kDa protein band reported in Rep *et al.* 2004<sup>77</sup>.

<sup>3</sup> also found in spot 18 of the gel with pH range 6-11<sup>76</sup>.

**Table S23: SIX genes used in this study.**

Gene	Accession	Reference <sup>1</sup>	Length <sup>2</sup>	Cys <sup>3</sup>	Introns	Position on chromosome 14 <sup>4</sup>
<i>SIX1</i>	AJ608702	Rep <i>et al.</i> 2004 <sup>77</sup>	263 [189]	8	0	scaffold 36: 116409-116848
<i>SIX2</i>	AJ608703	Houterman <i>et al.</i> 2007 <sup>76</sup>	212 [172]	8	0	scaffold 36: 108933-109802
<i>SIX3</i>	AM234063	Houterman <i>et al.</i> 2007 <sup>76</sup>	144	2	0	scaffold 36: 5017-5484
<i>SIX5</i>	FJ767863	unidentified #2 PH2007 <sup>76</sup>	102	6	3	scaffold 36: 3273-3407
<i>SIX6</i>	FJ755835	unidentified #3 PH2007 <sup>76</sup>	195	7	0	scaffold 22: 761399-762125
<i>SIX7</i>	FJ755836	unidentified #5 PH2007 <sup>76</sup>	201	3	0	scaffold 51: 65216-65875
<i>ORX1</i>	AM236600	Houterman <i>et al.</i> 2007 <sup>76</sup>	588	2	7	scaffold 22: 88830929-833119

<sup>1</sup> PH2007: previously described as unidentified, in Houterman *et al.*, 2007<sup>76</sup>.

<sup>2</sup> In amino acids, without predicted signal peptide; between brackets without predicted prodomain.

<sup>3</sup> Number of cysteine residues.

<sup>4</sup> In strain 4287 (<http://www.broad.mit.edu/>).

**Table S24. Double resistant colonies per million ( $10^6$ ) colony forming units.**

receiving strain	Experiment				
	1 (a)	2 (b)	3 (b)	4 (c) average ± st. dev.	5 (c) average ± st. dev.
Fol 7	0.13	0.69		0.04 ± 0.03	0 ± 0
Fol 8	0.12			0.06 ± 0.04	0 ± 0
Fol 9	0.08	11.16			
Fom H1*	0.05	0			
Fom H2*	30			0 ± 0	0 ± 0
Fom H3*	10	6.98		1.17 ± 0.33	6.98 ± 4.25
Fo H1*	1.77	2.73			
Fo H2*	0.35		0.84	1.13 ± 0.10	
Fo H3*	1.85	0	2.1	0.79 ± 0.45	
Foc H1	0	0			
Foc H2	0				

empty box: not determined

- (a): Mixed spores were used to incubate four different co-incubation plates.  
Spores from the co-incubation were pooled before plating on selective medium.
- (b): Mixed spores were used to incubate only one co-incubation plate.
- (c): Mixed spores were used to incubate three different co-incubation plates.  
Spores from the co-incubation plates were plated separately on selective medium and the average number of double resistant colonies was calculated.
- \* double resistant colonies from this experiment were used for further analyses.

**Table S25A. Chromosomal –specific primers based Foxy insertion sites in each chromosome**

Primer name*	Sequence	Band in Fol007**	Selected as markers
chr1sc1fwd1L	ATATGAAAGGGGTCAAGGA	no (smaller band)	
chr1sc1fwd2K	GATCTAATTGAGTTATGAGCC	no	
chr1sc1fwd3L	TCCTGAGAACTTTCTCAGC	YES	X
chr1sc27fwd1K	TTACCGAGCAGGAGACGAGC	YES	X
chr2sc6fwd1L	AAAAGAATTGCTCGCGCTCT	YES	X
chr2sc6fwd2K	GAATTCTCTCGGCATCACT	YES	
chr2sc10fwd1L	GAATTCCTTGTAGAGGTGCT	YES	X
chr2sc31fwd1K	TCTACCCGGGTCTTCCAACG	YES	X
chr3sc7fwd1L	GTACCGATAATATGCCATAC	no	
chr3sc7fwd2K-chr6	GTATGCCACTCAAAACAATC	YES (also anneals to chr6sc33)	X
chr3sc18fwd1L	GAGCAGATGGGCGTTCCTA	YES	
chr3sc18fwd2K	GTGTACGAATCTTACAATGAC	YES	
chr3sc25fwd1L	ATTTACAGGGTTGCGCGAAC	YES	X
chr4sc4fwd1K	GGCACTATCAACTGTTAATC	YES	X
chr4sc4fwd2L	AGTCCCTTGCGTCCAACCGA	YES (weak)	
chr4sc8fwd1K	TAGATCCTCCCCTGGTGTCC	YES	X
chr5sc2fwd1L	ACCTCCCGAGAAGGTTATCA	YES	X
chr5sc2fwd2K	CAAACCTAATACTCAACGG	YES	
chr5sc2fwd3L	CCCATGCAGCTTCAAAGTCG	no	
chr5sc26fwd1K	AAAGTTGGCGAACTTAGGGA	YES	X
chr6sc9fwd1L	ATTCATTGTACATGCAGCC	YES (weak)	X
chr6sc9fwd2K-chr3	CATCAACCAGTTATGACATC	YES	
chr6sc21fwd1L	ACAAGACGAAAAGGCATGG	YES (also anneals to chr3sc18)	X
chr7sc5fwd1K	GAGGAGATCAAGGACATTTT	YES	X
chr7sc5fwd2L	TCAGCGGGTTCGAGGGCAAT	no (smaller band)	
chr7sc13fwd1K	GGAAAGTCTCCGTGGCTTG	YES	
chr7sc13fwd2L	CTTGTCATTTCTGAGCCTTG	YES	X
chr8sc3fwd1K	ACCTTGAGGAAATAAACTG	no	

chr8sc3fwd2L	CACTTCGAATACGTAGGCTG	YES	X
chr8sc3fwd3K	ACGTGCAGTGCATTCATGGC	YES	X
chr9sc11fwd1L	TATGCTGTTCCCCAGTCGCT	YES	X
chr9sc11fwd2K	TGAAGTGGACTAAGGAGGAG	no (smaller band)	
chr9sc17fwd1L	CTCCTTACTTCAACGGCGTC	no	
chr10sc15fwd1L	GCATAAACGCCGAGCCTCCA	YES	X
chr10sc20fwd1K	GTTCTGAATGTCCTGAGGAA	YES (weak)	X
chr10sc20fwd2L	GGACCTATCAAGTTCCGTGT	no	
chr11sc12fwd1K	GGGCATAATTAAGCAGTGAT	YES	X
chr11sc12fwd2L	GGACGGAGGGTAACAGGTAC	YES	
chr11sc12fwd3K	GCGAAAACCTTGCTTTTAAG	YES	X
chr12sc19fwd1L	GTTATAACGAACTGCGGTGG	YES (weak)	
chr12sc19fwd2K	GGGCATTTATATGGTTCAGT	YES	X
chr12sc23fwd1L	GAACGCTCGCGTGATGAAGC	YES	X
chr13sc16fwd1K	TACTGCTTGCCTCGGGGATG	YES	X
chr13sc16fwd2L	AGGCATCGTTTAATCGTTGG	YES	X
chr14sc22fwd1K	ATTCATAATCTGGCGGCTC	no	
chr14sc36fwd1L	ACTATTAACAATCACGAGGAC	YES	X
chr15sc24fwd1L	ACCGCACAAGACTCAACTAT	YES (weak)	X
chr15sc28fwd1K	TTTAGGGCCCACGTGCAGAC	YES	X
HYG-F	GTTGGTCAAGACCAATGCGGAG		
HYG-R	TGCACAGGGTGTACGTTGC		
BLE-F	TCCTTCCCATCCCTTATTCC		
BLE-R	CACGAAGTGCACGCAGTTG		
Foxy-primer	GAGAGAATTCTGGTCGGTG		X

\* The number behind 'chr' refers to the chromosome and the number behind 'sc' refers to the super contig on which the primer was designed. Two primers also anneal to a second supercontig on another chromosome (both on chr. 3 and 6).

\*\* Used in combination with Foxy-primer as a reverse primer.

**Table S 25B Primers based on Foxy insertions covering the duplicated region in Chr3/6**

Primer name*	Sequence	Anneals on:		Used in	Band present in: **					
		chr 3	chr 6	suppl. fig.	FoI007	Fo-47	1B	1C	2A	2B
chr3sc32fwd1K	GTATGCCACTCAAAACAATC	SC 32	SC 33		X					
chr3sc18fwd3L	TTGCAGGAGAATATCTAGAG	SC 18	SC 21		X			X	X	
chr3sc18fwd4K	TATCAAGAGATATGCAGAGC	SC 18	SC 21		X			X	X	
chr3sc18fwd1L	GAGCAGATGGGCGTTCCCTA	SC 18	SC 21	S20 A	X			X	X	
chr3sc18fwd5K	ACAGGAAGAGCACCAACAAA	SC 18	SC 21		X			X	X	
chr6sc21fwd1L	ACAAGACGAAAAAGGCATGG	SC 18	SC 21	S20 A/B	X			X	X	
chr3sc18fwd6K	AGTGATAGAAAGTTGAGAAAG	SC 18	SC 21		X			X	X	
chr3sc18fwd7L	TGCAGGGTGCCTAGATTG	SC 18	SC 21		X			X	X	
chr3sc18fwd8K	TGCATGCAGGATAATCAATC	SC 18	SC 21		X			X	X	
chr3sc18fwd9L	TATGCGTGAAGTTCTTCGGC	SC 18	SC 21, 41		X	X	X	X	X	X
chr3sc18fwd2K	GTGTACGAATCTTACAATGAC	SC 18	SC 21, 41	S20 A	X			X	X	
chr3sc18fwd11L	TCTAATCAAACGCTTCAGTC	SC 18	SC 21, 41		X			X	X	

\* the number behind 'chr' refers to the chromosome and the number behind 'sc' refers to the super contig on which the primer was designed.

\*\* used in combination with Foxy-primer as a reverse primer.