

Supplementary Information

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Characterization and expression of the cytochrome P450 gene family in diamondback moth, *Plutella xylostella* (L.)

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19 **This file includes:**

20 **Table S1~S2**

21 **Figures S1~S5**

22

23 **Table S1** Cytochrome P450 genes in *P. xylostella*.

No.	Gene CDS ID	Cytochrome P450 name	Protein length	Scaffold location	Gene start site	Gene end site	Gene length	No. of exon
1	Px000631.1	CYP341G1	448	scaffold_106	535778	541121	5344	8
2	Px000833.1	CYP367A4	556	scaffold_109	1288791	1297112	8322	10
3	Px000834.1	CYP367B5	500	scaffold_109	1303983	1311031	7049	10
4	Px000924.2	CYP340V1v1	494	scaffold_11	200786	212000	11215	10
5	Px000926.1	CYP340V2v1	424	scaffold_11	223579	232025	8477	8
6	Px001368.1	CYP367B5v2	215	scaffold_1179	13947	16730	2784	4
7	Px002079.1	CYP340AF1	458	scaffold_130	925350	930821	5472	9
	Px002080.1							
8	Px002082.2b	CYP340T1	503	scaffold_130	950638	958947	8310	10
9	Px002082.2a	CYP340S1v1	493	scaffold_130	961179	978354	17176	10
10	Px002083.1	CYP340Y1	479	scaffold_130	1000916	1010810	9895	10
11	Px002092.2	CYP4S10	507	scaffold_130	1235035	1247351	12317	10
12	Px002515.1	CYP305B1	488	scaffold_14	52466	59966	7501	8
13	Px002723.1	CYP6CV2	505	scaffold_140	953589	955357	1769	2
14	Px002776.1b	CYP340V1v2	346	scaffold_141	688428	693615	5188	7
15	Px002776.1a	CYP340V2v2	403	scaffold_141	697194	704079	7538	7
16	Px002858.1	CYP304F9v2	363	scaffold_144	157394	161092	3699	6
17	Px003086.1	CYP6BD11	527	scaffold_148	1607990	1626691	18702	6
18	Px003246.1	CYP6AE45	520	scaffold_15	2271373	2275194	3822	2
19	Px003485.1	CYP9G11v1	466	scaffold_154	219752	226769	7018	10
20	Px003987.1	CYP367A5v2	162	scaffold_1625	416	2553	2138	3
21	Px004291.1	CYP302A1v1	515	scaffold_17	1656307	1660850	4544	9
22	Px004643.1	CYP6AB28	512	scaffold_18	104121	106032	1912	2
23	Px004650.2	CYP367A5	534	scaffold_18	201458	209934	8477	10
24	Px004651.2	CYP367B4v2	484	scaffold_18	216878	225966	9089	12
25	Px004867.2	CYP340W1v2	426	scaffold_185	426584	441472	14889	8
26	Px004951.2	CYP4DK1v1	505	scaffold_187	507838	516624	8787	11
27	Px005514.4	CYP366C1	508	scaffold_20	363581	372702	9122	10
28	Px005515.2	CYP366C2	525	scaffold_20	377563	387967	10405	11
29	Px005621.2	CYP4DK1v2	452	scaffold_201	206890	210613	3724	10
30	Px005900.1	CYP6BG6	524	scaffold_21	1278550	1281643	3094	3
	Px005901.1							
31	Px005902.4	CYP6BG4	519	scaffold_21	1287549	1289300	1752	2
32	Px006053.5b	CYP4M22	503	scaffold_214	48369	56675	8307	8
33	Px006053.5a	CYP4M23	508	scaffold_214	57398	61660	4263	9
34	Px006271.1	CYP367B4	500	scaffold_220	512431	519173	6743	10
35	Px006272.1	CYP367A5v3	162	scaffold_220	529897	531867	1971	4
36	Px006839.1	CYP340X1	466	scaffold_240	366112	376657	10546	10
37	Px007099.1	CYP4G78	563	scaffold_25	1012110	1018813	6704	10

No.	Gene CDS ID	Cytochrome P450 name	Protein length	Scaffold location	Gene start site	Gene end site	Gene length	No. of exon
38	Px007213.1	CYP340U1v1	559	scaffold_254	6413	12905	8305	12
39	Px007214.1	CYP340U2	298	scaffold_254	26018	34339	8322	6
40	Px007338.1	CYP333B15	503	scaffold_26	560386	564771	4386	9
41	Px007339.2	CYP333B16	502	scaffold_26	566773	570392	3620	9
42	Px007344.4	CYP333A5	508	scaffold_26	724089	727671	3583	9
43	Px007583.1	CYP307A1	539	scaffold_27	494517	502133	7617	2
44	Px008026.1	CYP4G77	560	scaffold_286	50304	54942	4639	7
45	Px008489.1	CYP6CN1	496	scaffold_30	1210747	1217884	7138	3
46	Px008532.1	CYP340U1v2	415	scaffold_30	2113381	2124856	11476	9
47	Px008537.1	CYP340W1v1	495	scaffold_30	2251972	2261032	9061	9
48	Px009062.1	CYP304F-1	146	scaffold_32	145580	151208	5630	3
49	Px009291.1	CYP304F8	478	scaffold_329	282849	296335	13487	8
	Px009292.1							
	Px009293.1							
50	Px009407.1	CYP428A1v2	549	scaffold_331	315475	322803	7329	11
51	Px009572.1	CYP338C1	472	scaffold_34	928224	931458	3235	2
52	Px009934.1	CYP365A2	456	scaffold_358	28588	34920	6333	8
53	Px009935.1	CYP365A5	169	scaffold_358	44166	52002	7837	4
54	Px010671.1	CYP339A3	493	scaffold_393	121828	127053	5226	10
55	Px010920.1	CYP340Z1	456	scaffold_403	93788	106621	12834	11
56	Px011058.5	CYP314A1	525	scaffold_410	156902	168437	11536	9
57	Px011481.1	CYP15C1	441	scaffold_436	180461	185272	4812	8
58	Px011483.1	CYP429A1	437	scaffold_436	191369	194940	3572	8
59	Px011722.1	CYP301A1	519	scaffold_45	130150	145519	15370	8
60	Px011724.2	CYP301B1	550	scaffold_45	156061	170376	14316	12
61	Px011849.1	CYP302A1v2	476	scaffold_455	200185	204370	4186	8
62	Px012360.1	CYP304F-2	153	scaffold_484	199097	201835	2739	3
63	Px012411.1	CYP18B1	565	scaffold_49	30802	37118	6317	7
64	Px012412.2	CYP18A1	522	scaffold_49	60494	63210	2717	7
65	Px012658.1	CYP49A1	503	scaffold_50	610535	617485	6951	8
66	Px013017.1	CYP6CT3	518	scaffold_52	1254475	1256852	2378	5
67	Px013063.1	CYP340S1v2	418	scaffold_522	117690	128766	11077	8
68	Px013091.1	CYP428A1	469	scaffold_526	48482	58721	10240	9
69	Px013145.1	CYP6BF1	514	scaffold_53	266651	268456	1806	2
70	Px013454.1	CYP6FM1	549	scaffold_552	47611	49492	1882	2
71	Px013707.2	CYP341A12	509	scaffold_579	90391	101147	10757	10
72	Px013884.1	CYP9G11v2	517	scaffold_59	1385465	1396016	10552	10
73	Px013958.1	CYP321E1	500	scaffold_6	239033	241012	1980	2
74	Px014069.1	CYP315A1	461	scaffold_600	90089	99740	9652	7
75	Px014216.1	CYP6BG5	521	scaffold_613	47293	49077	1785	2
76	Px014217.1	CYP6BG3	523	scaffold_613	58716	60633	1918	2
77	Px014440.2	CYP354A8	517	scaffold_629	18899	24482	5584	10

No.	Gene CDS ID	Cytochrome P450 name	Protein length	Scaffold location	Gene start site	Gene end site	Gene length	No. of exon
78	Px014651.1	CYP6AN14	514	scaffold_65	303284	306224	2941	2
79	Px014978.1	CYP9G10	520	scaffold_68	366776	371019	4244	9
80	Px014981.1	CYP9G4	519	scaffold_68	524542	532510	7969	10
81	Px014982.1 Px014984.1	CYP9G2	521	scaffold_68	534795	541015	6221	10
82	Px015762.1	CYP4AU6	474	scaffold_74	272693	278565	5873	10
83	Px015922.1	CYP303A1	500	scaffold_75	703659	709650	5992	3
84	Px016740.1	CYP365A3P	527	scaffold_83	203727	211127	7401	10
85	Px017253.1	CYP6AE46	516	scaffold_9	1531564	1533352	1789	2

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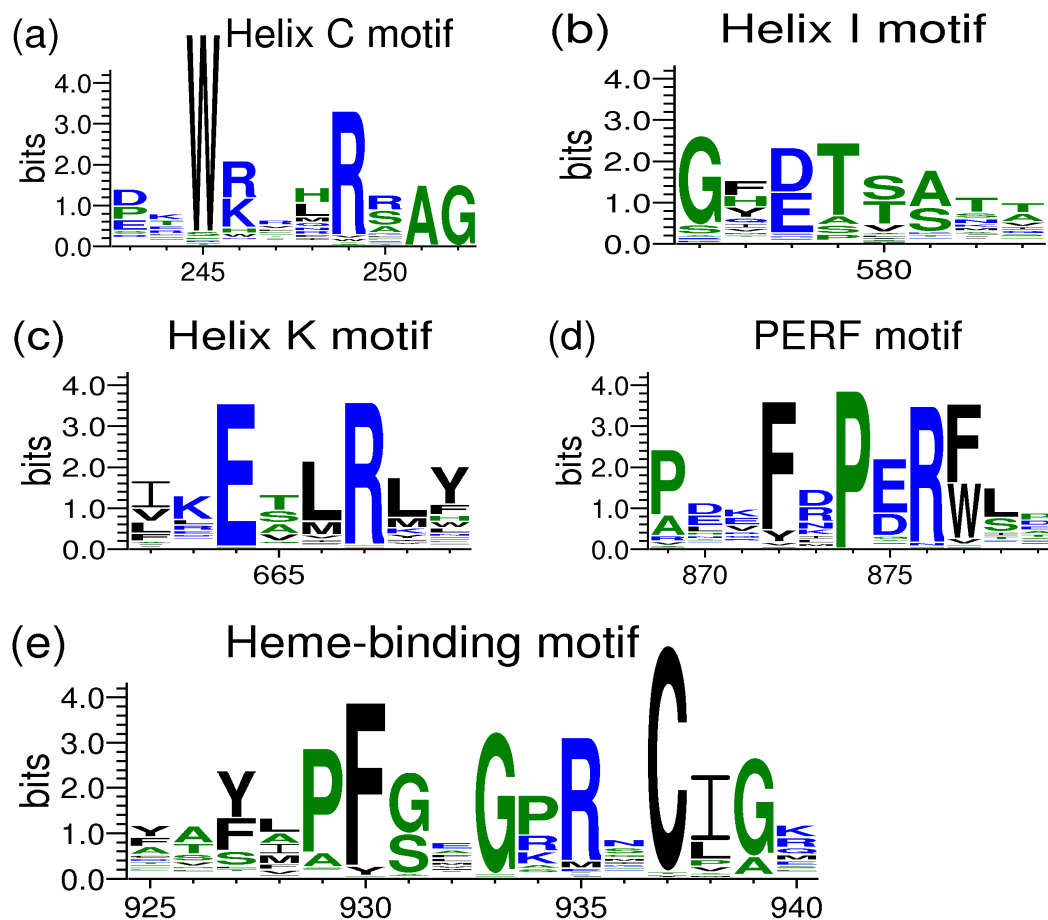
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26 **Table S2 Primers used for qRT-PCR.**

27

No.	P450s	Sense primer	Anti-sense primer	Sense primer T _m (°C)	Anti-sense primer T _m (°C)	Product length (bps)
1	CYP307A1	CTCGTGGTCAATAGTTTGGATAGGA	GAAGAGTTGGTGGAAACCGCAT	61.8	61.4	99
2	CYP314A1	CCAATGAAGGAAGGGTTTAGGC	TGACGATGAGGAAGAGGAGGG	62	61.3	128
3	CYP315A1	CAGGTGCTGTTGGCTAAGTTGC	CGTCAGGTGCGTACATGAAGGT	62.5	62.4	94
4	CYP18A1	GCGAGGTCCACGAGTTGATTG	GACATGGTGATGCCACAGATGAC	62.7	62.2	112
5	CYP4G77	GTCTCGCTTTCTTGGACCTGC	GGTGTCTGTGACCCTCGAACAT	61.4	61.8	110
6	CYP4G78	CGCAGAACGGAACCTCACAACAT	CCAAGGAGGCACAGCACGA	62.5	62.5	118
7	CYP301A1	GCGGCTAGGATGCCTGTCTT	GCCAGTAGGGTGCCTTCAGTTC	61.8	62.7	119
8	CYP6BF1	CATAACGGGAGACAGCATTGACA	TGGAAGCCAGCTTGAAGAAC	62.5	61.8	117
9	CYP6BG4	CGACAGAACCTCACGCCACT	TCCTCACTTCTACTGCGTCACTCA	60.7	61.7	133
10	CYP6BG3	TTTGAAGTGTCGGTATTAAGAGGCT	GAAGAACGCAGGTATTGTGGG	61.2	61.3	111
11	CYP4M22	CAAGAGTTGCGTGAGATATTCGG	GAAGCGTCTCCTTGATACAGCA	61.7	59.7	100
12	CYP4M23	CTTCGCTTGTTTCTCCTGTGT	GCATACTCGCCATTTCTCA	61.1	62.4	100
13	CYP6BD11	ACCTCCGCCGCTTCACTCT	CGCACTGGTCCACAAACTTCAT	63.3	62.3	142
14	CYP305B1	GATAAGCCTAATGACTTCAACCCAG	TGATTCGCTAACGCATCTCCT	60.9	61.7	126
15	CYP6CN1	AGGATTTCTTCTACGGCATGGTTC	TTGTCTCCCTCGTGCTTCTTCA	63	62.3	139
16	CYP9G10	AGAAGGAGGGAAAACAGTGAAA	CAGGCTCTGGGTAATACTGTTTCG	59.7	60.9	95
17	CYP9G4	GCTGTCCGCATTCATACCTTTC	CCGCGTCTTCTCGCACTTC	61.4	61.5	133
18	CYP9G11v1	CGTGAGCGAGACCATGCAGTA	CTTTCTTAGCCTCCATAAGCAGTTGT	62	62.3	83
19	CYP6CV2	ATGACAAGGTGGCGAAACTGG	GCTCGTGGATGAGGAAGGACA	62.2	62.2	117
20	CYP301B1	GAGTATTCACCTCTTCTCCAGTT	CACATTCGCTTCCCGTATCCA	62.9	63.1	113

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31 **Figure S1 Five conserved domains of the cytochrome P450s in *P. xylostella*.** The

32 horizontal axis is the position of amino acids over the entire length of the alignment

33 (1021); the vertical axis represents the information content in bits. Seventy-seven

34 *PxP450* protein sequences were aligned by ClustalX2, and their conserved motif

35 logos were generated using the online WebLogo program. (a) The helix C motif

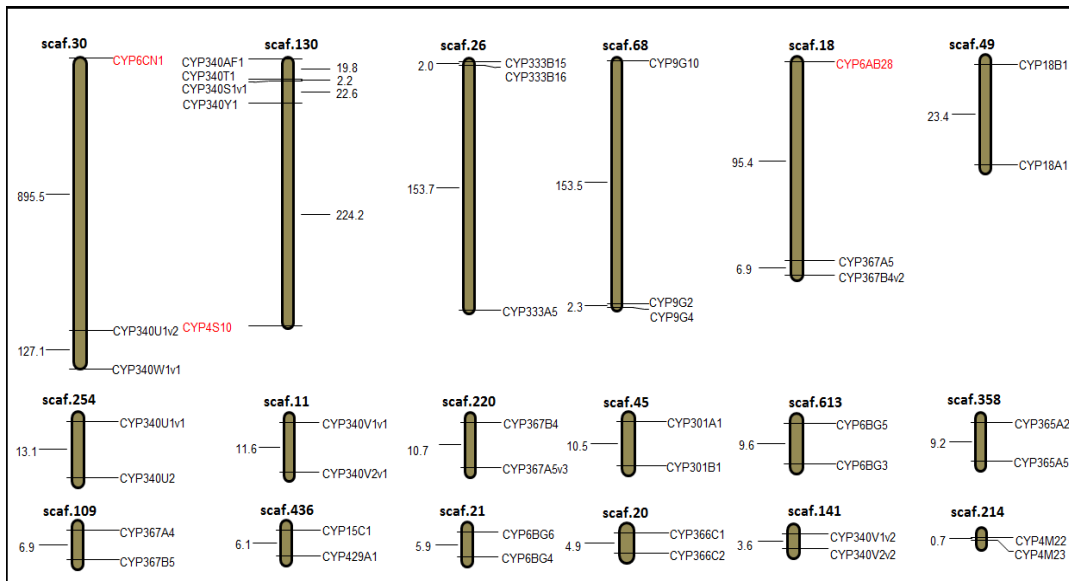
36 (WxxxR) sequence logo. (b) The helix I motif (Gx[ED]T[TS]) sequence logo. (c)

37 The helix K motif (ExLR) sequence logo. (d) The PERF motif (PxxFxp[ED]R)

38 sequence logo. (e) The heme-binding motif (PFxxGxRxCx[GA]) sequence logo.

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43 **Figure S2 Scaffold localization of cytochrome P450s in *P. xylostella*.** The scaffold

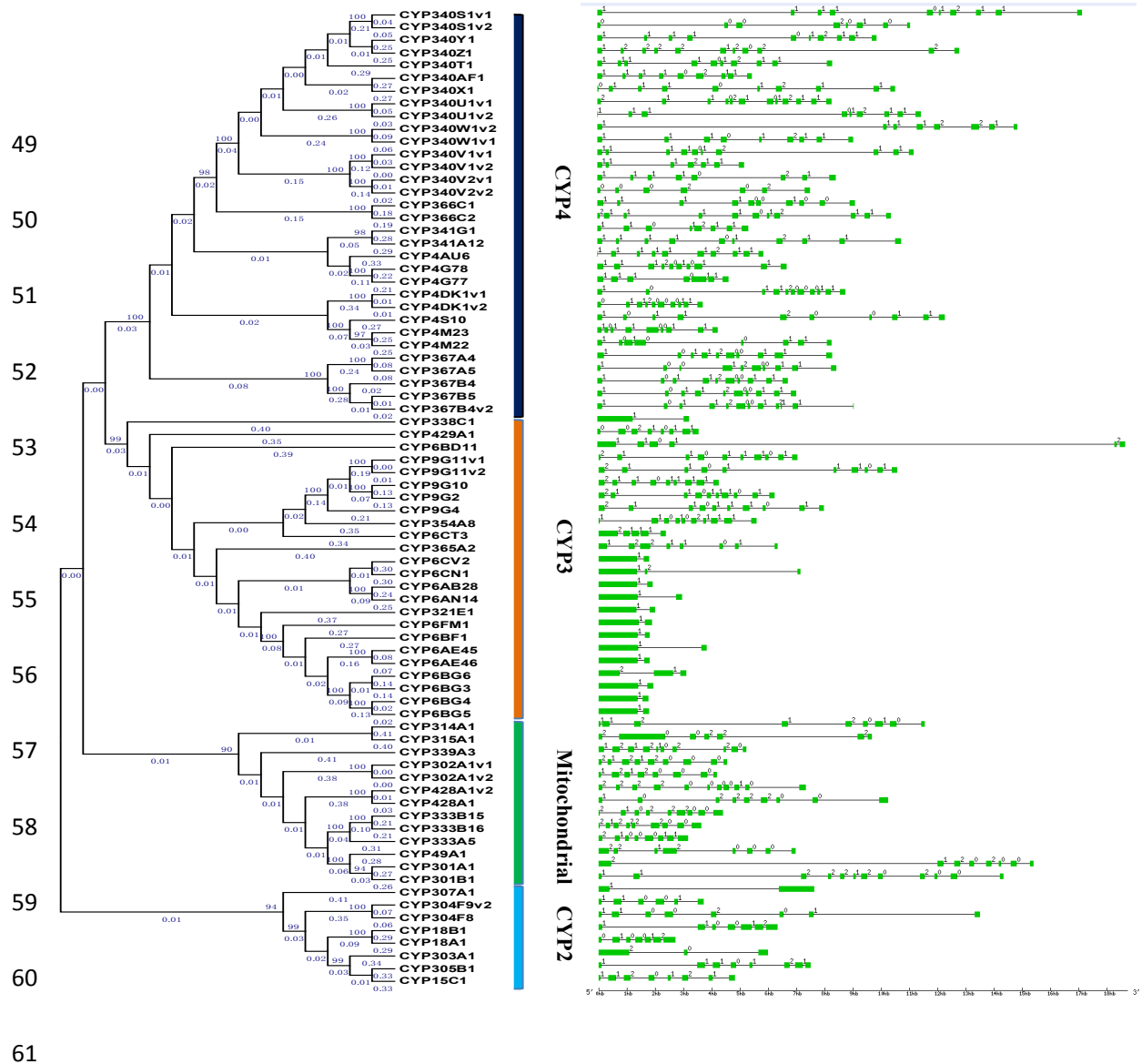
44 numbers are indicated at the top of the each bar. The P450 gene names and their

45 distance (kilobases, kb) between two nearby genes are displayed on the right and left

46 of each bar. Tandemly duplicated genes that are supposed to be separated by 0 - 5

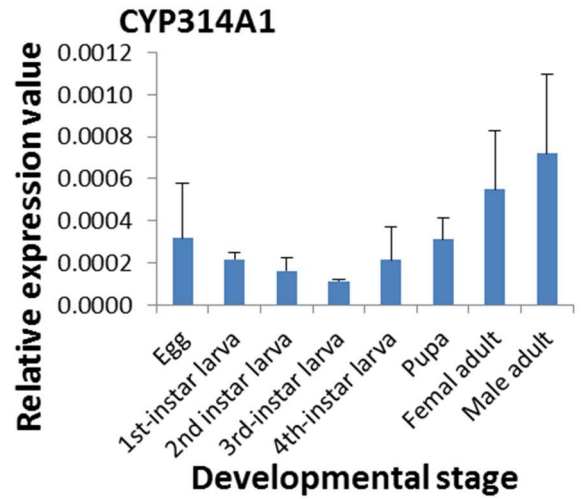
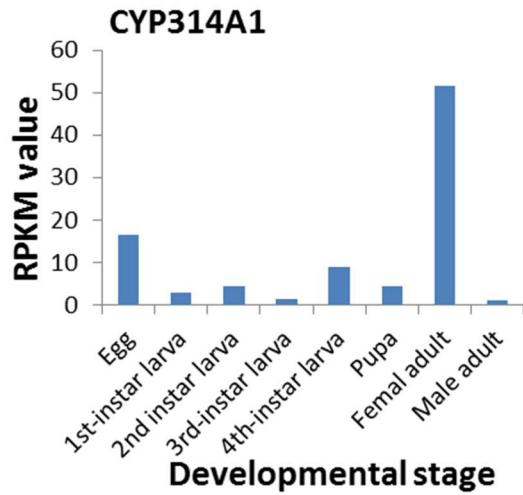
47 genes are in black, and genes that are not tandemly distributed are in red.

48

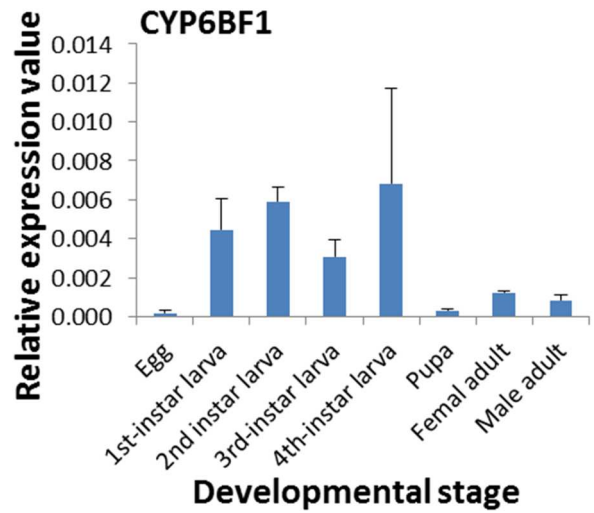
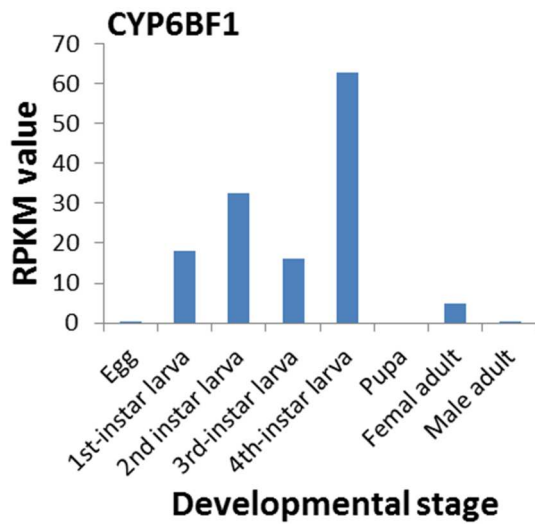


62 **Figure S3 Phylogenetic tree and gene architecture of cytochrome P450s in *P.***
 63 ***xylostella*.** On the left is the phylogenetic relationship between more than 300 amino
 64 acid residues of the P450s. Only Bootstrap values larger than 90% are displayed. On
 65 the right is the gene architecture. The green bar represents exons, and black lines
 66 represent introns. The digits on the black line represent the intron phase, and three
 67 types of intron phases exist in eukaryotic genes and are represented by phases 0, 1, 2.
 68 The scaled line on the right bottom measures the gene length.

69

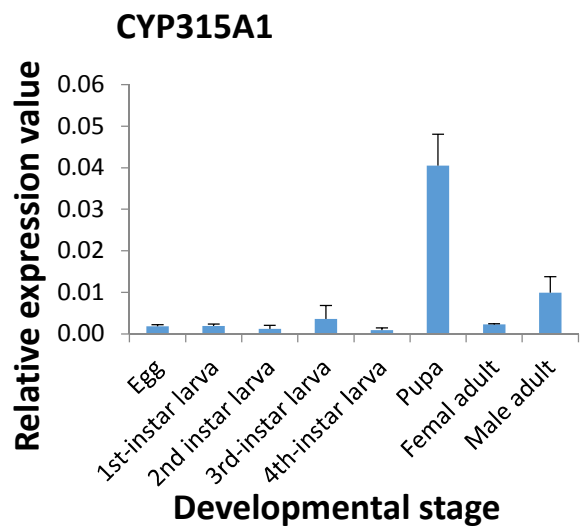
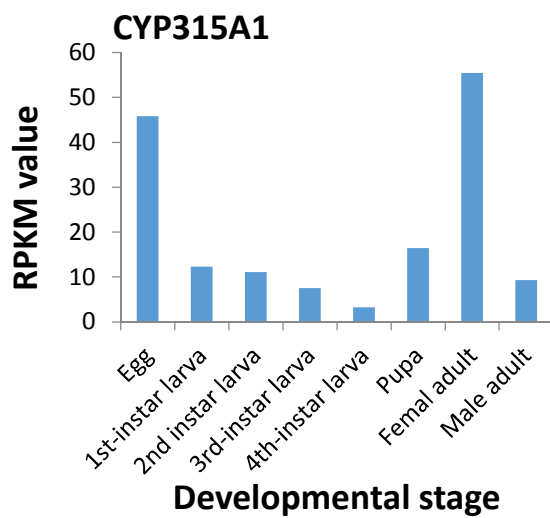


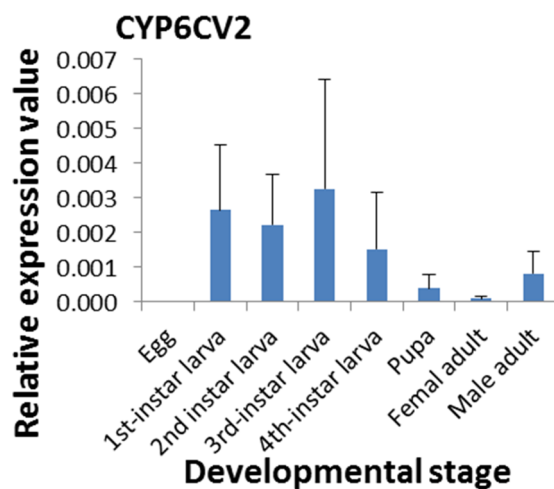
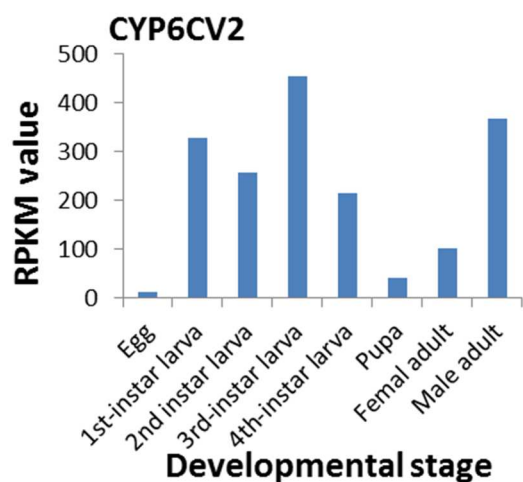
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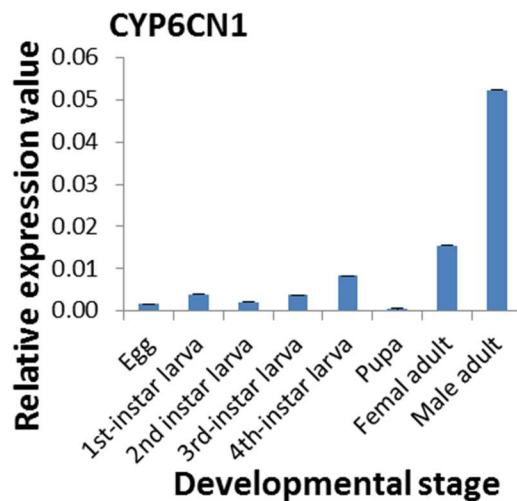
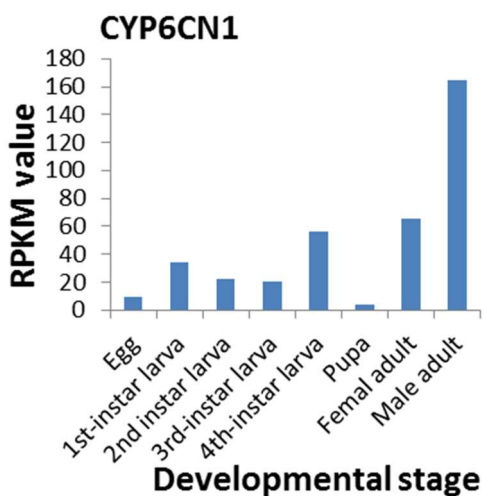
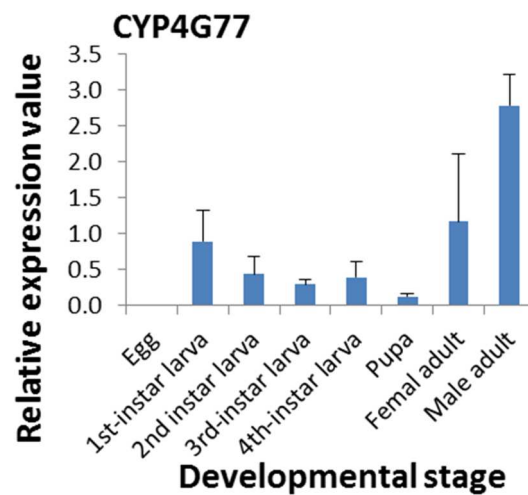
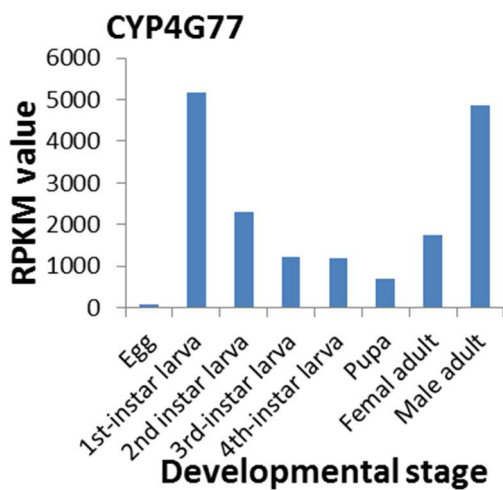
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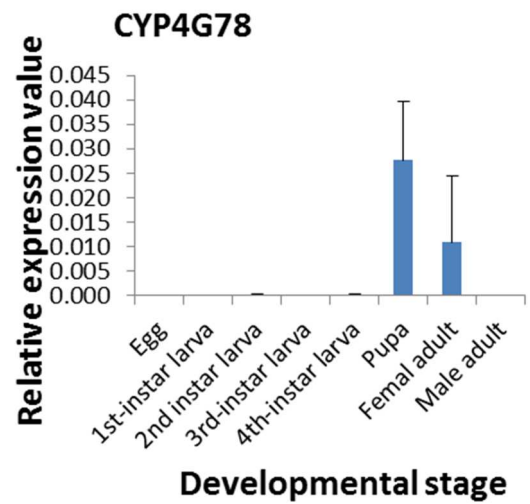
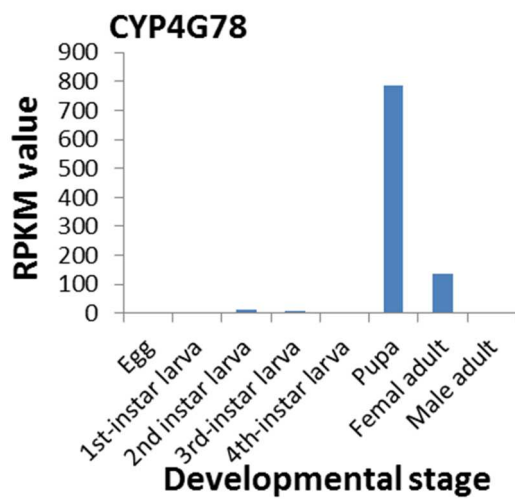
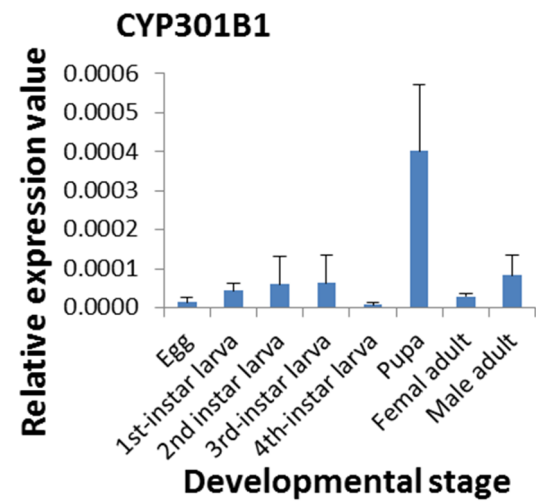
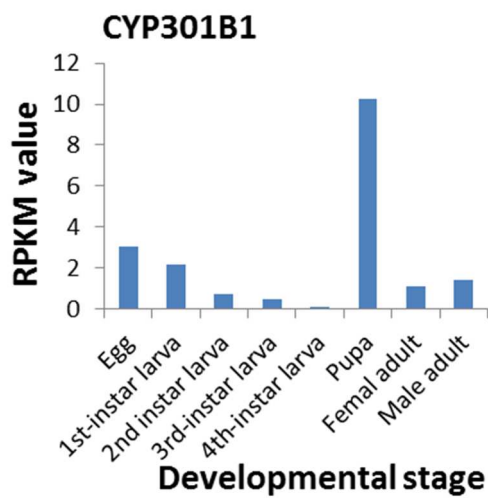
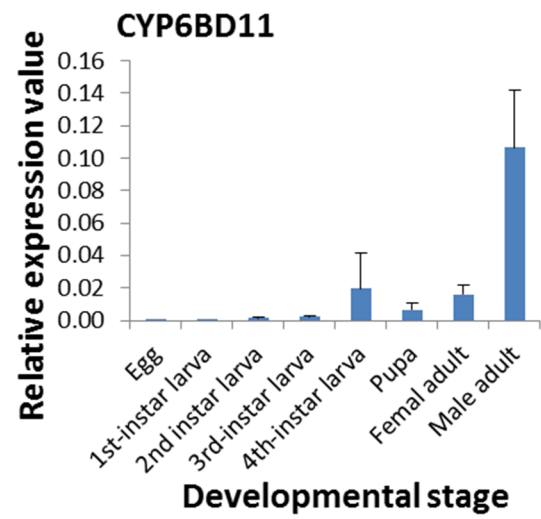
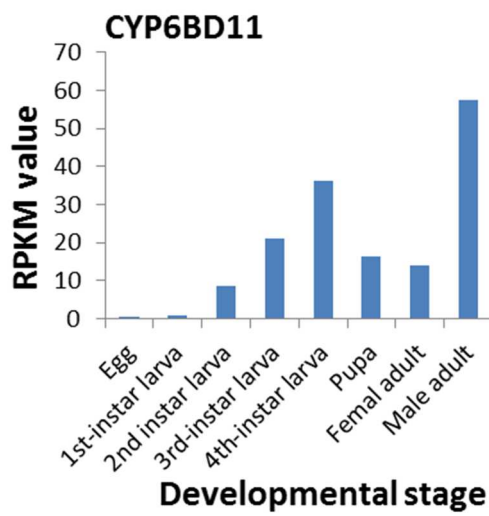
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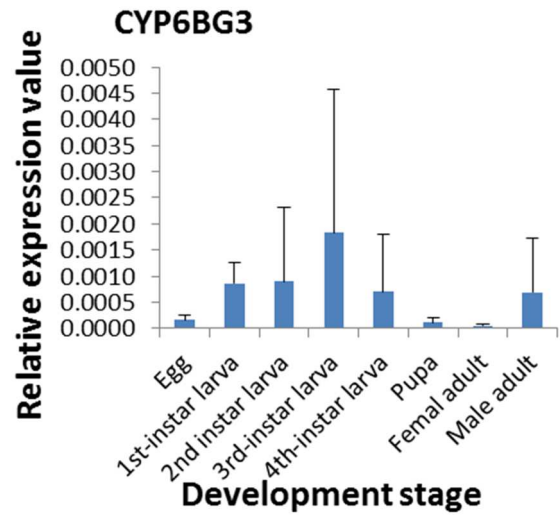
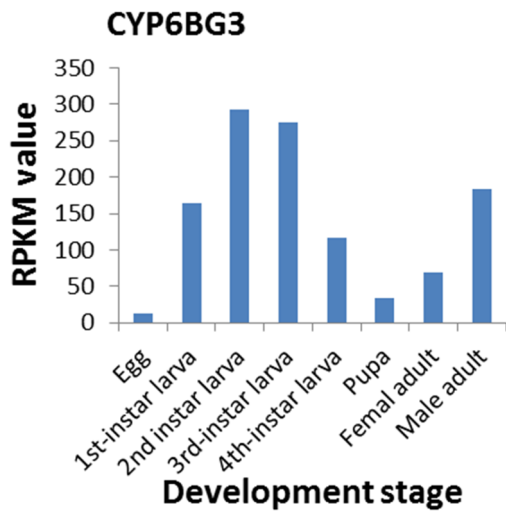


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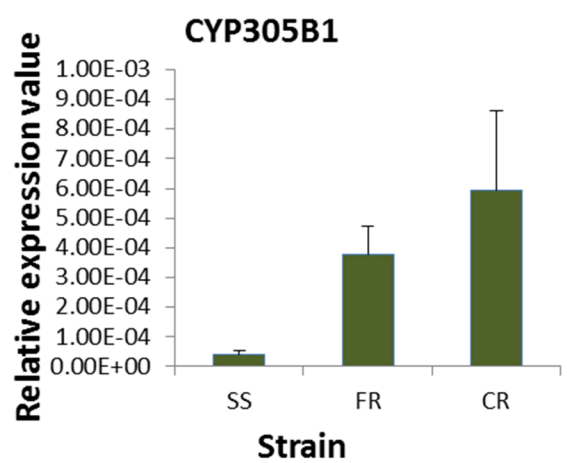
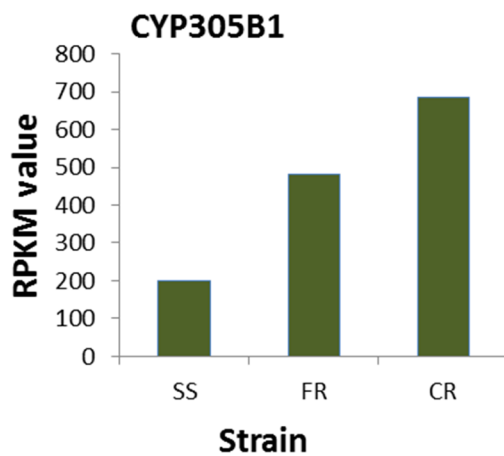
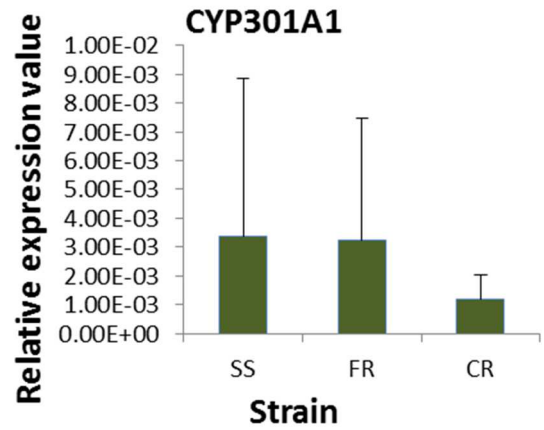
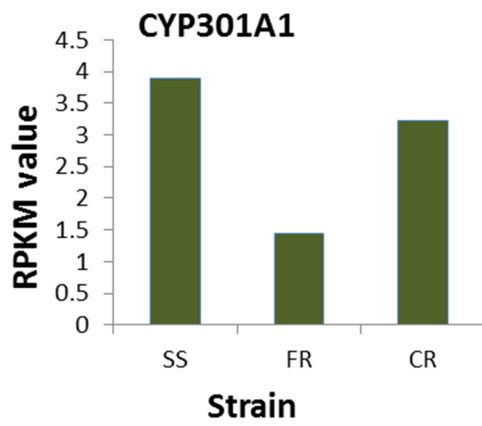
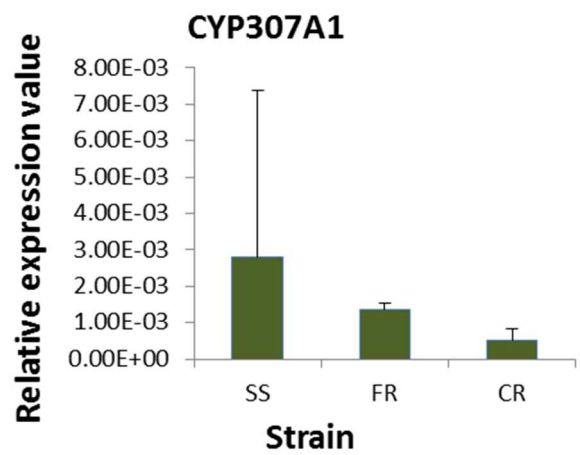
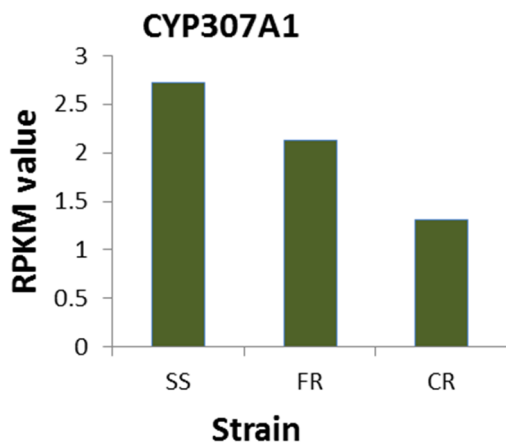


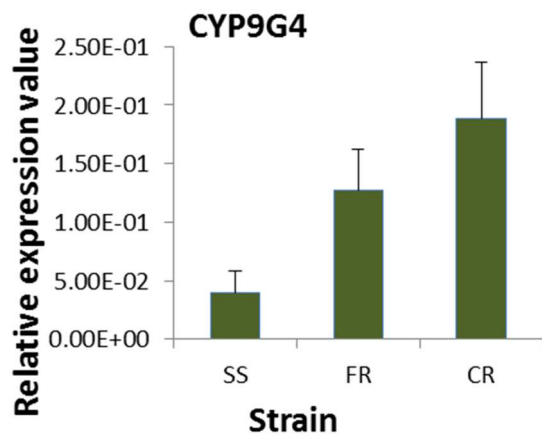
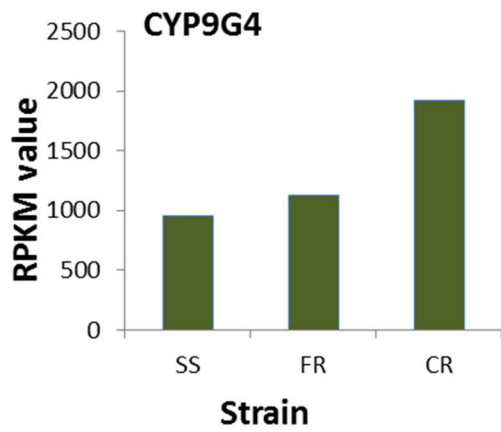
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77 **Figure S4 Stage-specific gene expression analysis of 10 *P. sylostella* P450s based**

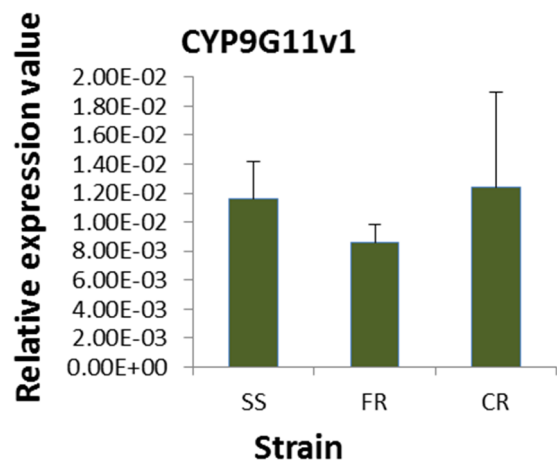
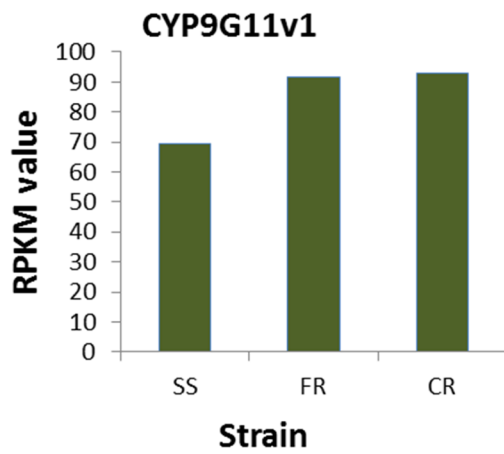
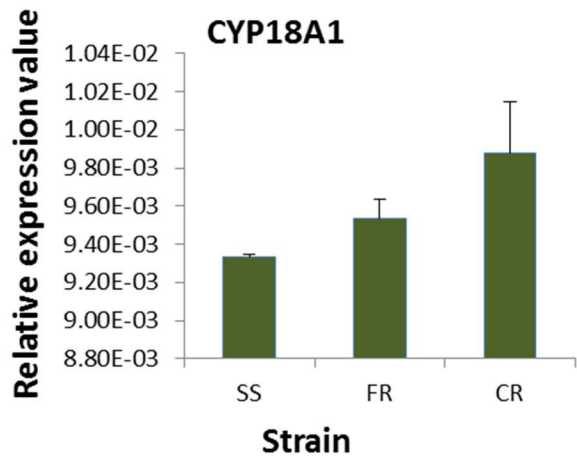
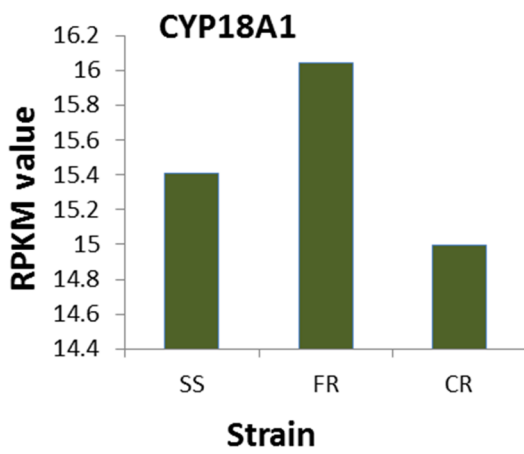
78 **on RNA-Seq (left) and qRT-PCR validation (right).**

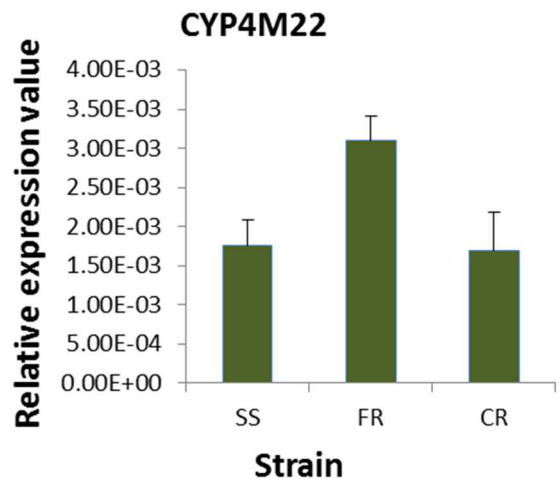
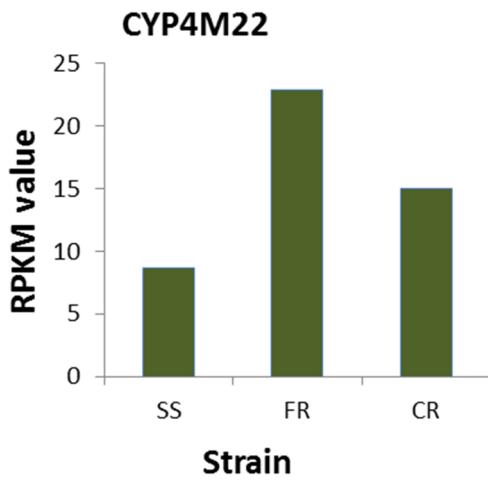
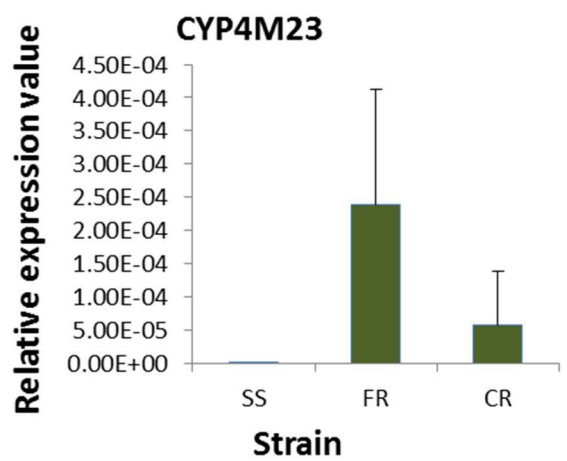
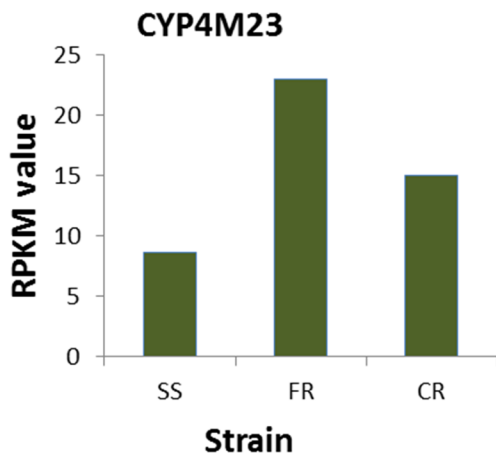
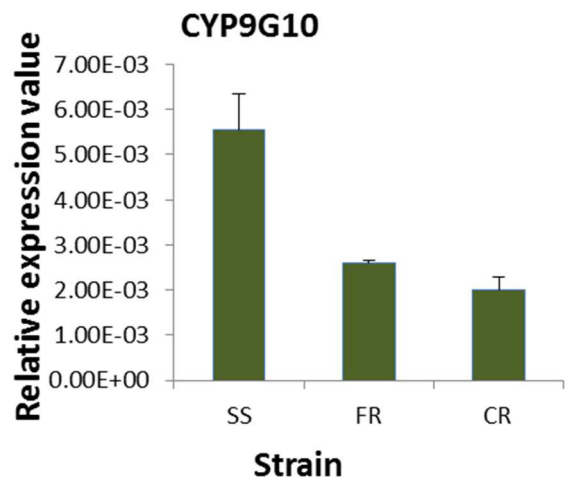
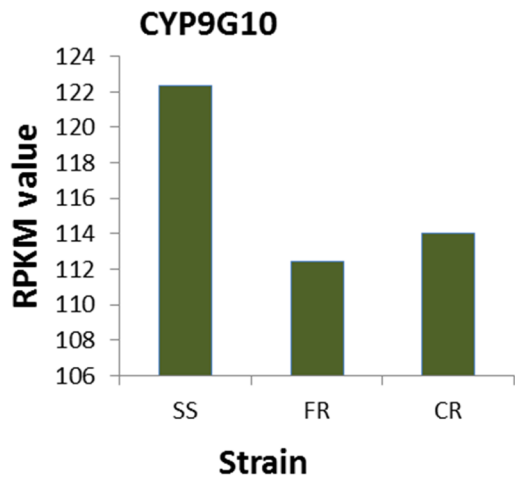
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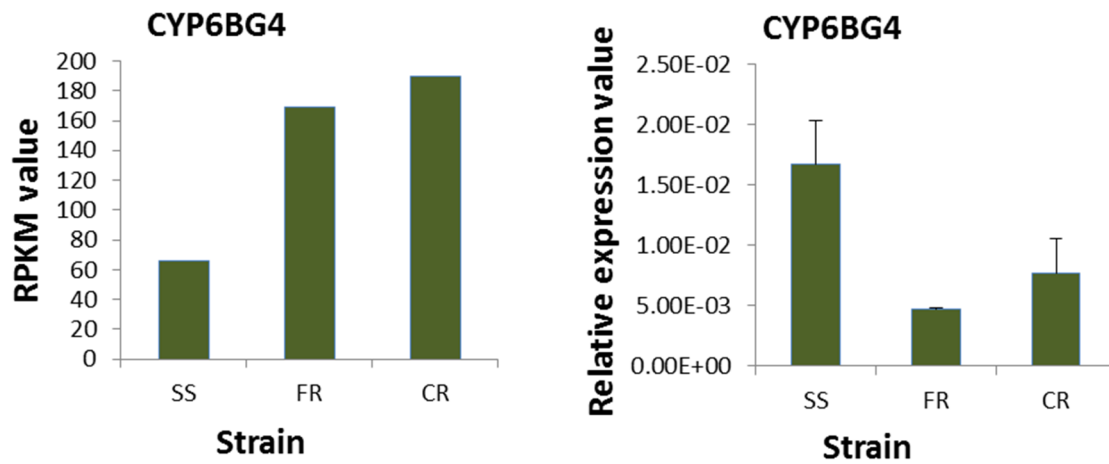




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84 **Figure S5 Strain-specific gene expression analysis of 10 *P. xylostella* P450s based**
85 **on RNA-Seq (left) and qRT-PCR validation (right).** SS represents 3rd-instar larva
86 from the susceptible strain; FR 3rd-instar larva from the fipronil-resistant strain; and
87 CR 3rd-instar larva from the chlorpyrifos-resistant strain.