Article

Cadmium exposure impairs development, detoxification mechanisms and gene expression of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae)

Shuaiqi Zhao [1], Wanglong Miao [1], Sheng Sheng [1,2], Xin Pan [1], Ping Li[1,2], Weihong Zhou [1,2*], Fuan Wu [1,2*]

1 Jiangsu Key Laboratory of Sericultural Biology and Biotechnology, School of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang 212100, China.

2 Key Laboratory of Silkworm and Mulberry Genetic Improvement, Ministry of Agriculture and Rural Affairs, The Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212100, China.

*Corresponding author: zhouweihong2020@just.edu.cn (W.-H.Z.); fuword@163.com (F.-A.W.)

Supplementary Material #1

Glyphodes pyloalis Walker was divided into 6 groups in the 2nd instar, and fed with mulberry leaves soaked in Cd concentrations at 0, 5, 15, 30, 60, 100 and 120mg/kg, respectively. The body weights of the *G. pyloalis* larvae (n=80) from 2nd to 5th instar of each group were recorded, and their corresponding Cd concentrations and larval weights were analyzed using probit regression analysis. Based on the regression analysis, IC_{10} and IC_{50} of Cd exposure on the larval weights was 3.89 mg/kg and 51.69 mg/kg, respectively.

Heavy metal concentration (mg/kg)	Larval body weight gain (mg)	Weight suppression rate (%)
0	33.4±0.48a	0a
5	27.7±1.11b	17.07b
15	26.2±2.44bc	21.56bc
30	23.2±1.14c	30.54c
60	15.8±1.051d	52.69d
100	12.9±1.24de	61.38de
120	8.8+0.7115e	73.65e

Different lowercase letters on the same column indicate significant differences between the Cd-treated groups (P<0.05).

Table S1. Primers used for PCR in *G. pyloalis*

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
CYP12A2	AAGACCAGGTTCGGCAAGAG	GTCGTCAACCTGGCGTTAGA
CYP4G112	TGTACGATCCTCGCGATGTG	TGAGGAGACCGTTTCCAAGC
CYP6AB51	CAGGAGTCAGGCCATGGAAG	ATCCCTCAAAACCAGCTCCG
CYP4504V2	TCGTTTCAAAAGCTTGGCCG	TGGCGCAAAGATAAGTCCGT
GST-epsilon4	TTCAGGCCGAGTTCATCGAC	TGTTCCCGTAAGTGTCTGCT
CarEs1	TATCGCACCCATCTCGTTCG	AGTCGTTGACCAGCTTCCTG
AKP	AGATGGTACCAGACTCGGCT	CGCCAACGATTTTAGCCTCG
ACP	TGTTCGTCGATGGTGGGAAG	GCCACTGAACCGACCACATA
Ugt2B1	ACCTGTCCTACCAACTTGCG	CTCTGCTCCTGGTGGAACTG
UGT40AL1	GCTATAACCTGCCCGAGGAC	CCGGTCGTGGTAGATCGAAG
UGT2B30	TGACCTGATTGCTCGGGAAC	AGGCTTGGCTTTAGGCACAT
Mn-SOD	CAAACACCACGCCACTTACG	CTTTAACTTGTGCGTGCGCT
Rpl32	ACTGGCGTAAACCCAGAGGT	GGTAGCATGTGACGGGTCTT

Table S2. Quality of *G. pyloalis* clean reads

Sample	Raw Reads(M)	Raw Bases(G)	Clean Reads(M)	Clean Bases(G)	Q30 (%)	GC (%)
CK_1	50.97	7.65	50.35	7.4	89.4	48.1
CK_2	51.18	7.68	51.12	7.49	96.94	46.22
CK_3	49.81	7.47	49.19	7.19	89.83	46.48
LD_1	48.48	7.27	47.88	7.1	88.47	47.3
LD_2	48.94	7.34	48.28	7.17	88.84	47.57
LD_3	49.29	7.39	48.61	7.15	89.95	45.88
HD_1	45.69	6.85	45.11	6.69	87.71	46.85
HD_2	50.08	7.51	49.47	7.3	89.01	47.04
HD_3	49.21	7.38	48.53	7.15	89.74	45.31

Table S3. Functional annotation of the *G. pyloalis* transcriptome

Database	NR	Swiss-Prot	KEGG	Pfam	eggNOG	KOG	GO
Number of annotated unigenes	17,292	11,020	5,056	10,867	15,424	9,989	10,173
Percentage of annotated unigenes	57.68 %	36.76 %	16.87 %	36.25 %	51.45 %	33.32 %	33.93 %

Table S4. Summary of SSR searing in the *G. pyloalis* transcriptome.

Searching Item	Number
Total number of sequences examined	29,978
Total size of examined sequences (bp)	41,173,146
Total number of identified SSRs	4,479
Number of sequences containing more than 1 SSR	3,631
Number of SSRs present in compound formation	208

Table S5. List of DEGs related to detoxification and metabolism of *G. pyloalis* larvae

Group	Gene_id	Description	Log_2fold change	Up/Dowm
HD vs. CK	TRINITY_DN11850_c0_g1_i1_2	*CYP12A2*	1.93	Up
HD vs. CK	TRINITY_DN11966_c0_g1_i2_2	*CYP4G112*	1.41	Up
HD vs. CK	TRINITY_DN13233_c0_g1_i2_3	*CYP6AB51*	-4.06	Down
HD vs. CK	TRINITY_DN12719_c6_g2_i1_3	*CYP4504V2*	-1.68	Down
HD vs. CK	TRINITY_DN14393_c1_g1_i3_2	*GST-epsilon4*	1.55	Up
HD vs. CK	TRINITY_DN13325_c0_g1_i2_2	*CarEs1*	4.69	Up
LD vs. CK	TRINITY_DN10895_c0_g1_i1_1	*AKP*	1.17	Up
LD vs. CK	TRINITY_DN13247_c0_g1_i5_1	*ACP*	1.21	Up
HD vs. CK	TRINITY_DN13922_c0_g3_i1_2	*Ugt2B1*	2.86	Up
HD vs. CK	TRINITY_DN10956_c0_g1_i1_3	*UGT40AL1*	-1.50	Down
HD vs. CK	TRINITY_DN12887_c1_g1_i1_3	*UGT2B30*	-1.48	Down
HD vs. CK	TRINITY_DN12845_c0_g2_i5_3	*Mn-SOD*	-1.31	Down

Table S6 Primers used to synthesize siRNA

Primer Names	Sequence (5'-3')
Gp*CYP12A2*-Oligo-1	GATCACTAATACGACTCACTATAGGGCCAAGCCTCTCAAGAATATTT
Gp*CYP12A2*-Oligo-2	AAATATTCTTGAGAGGCTTGGCCCTATAGTGAGTCGTATTAGTGATC
Gp*CYP12A2*-Oligo-3	AACCAAGCCTCTCAAGAATATCCCTATAGTGAGTCGTATTAGTGATC
Gp*CYP12A2*-Oligo-4	GATCACTAATACGACTCACTATAGGGATATTCTTGAGAGGCTTGGTT
GFP-Oligo-1	GATCACTAATACGACTCACTATAGGGGGGATGTCTCACATCTTGTTT
GFP-Oligo-2	AAACAAGATGTGAGACATCCCCCCTATAGTGAGTCGTATTAGTGATC
GFP-Oligo-3	AAGGGATGTCTCACATCTTGTCCCTATAGTGAGTCGTATTAGTGATC
GFP-Oligo-4	GATCACTAATACGACTCACTATAGGGACAAGATGTGAGACATCCCTT

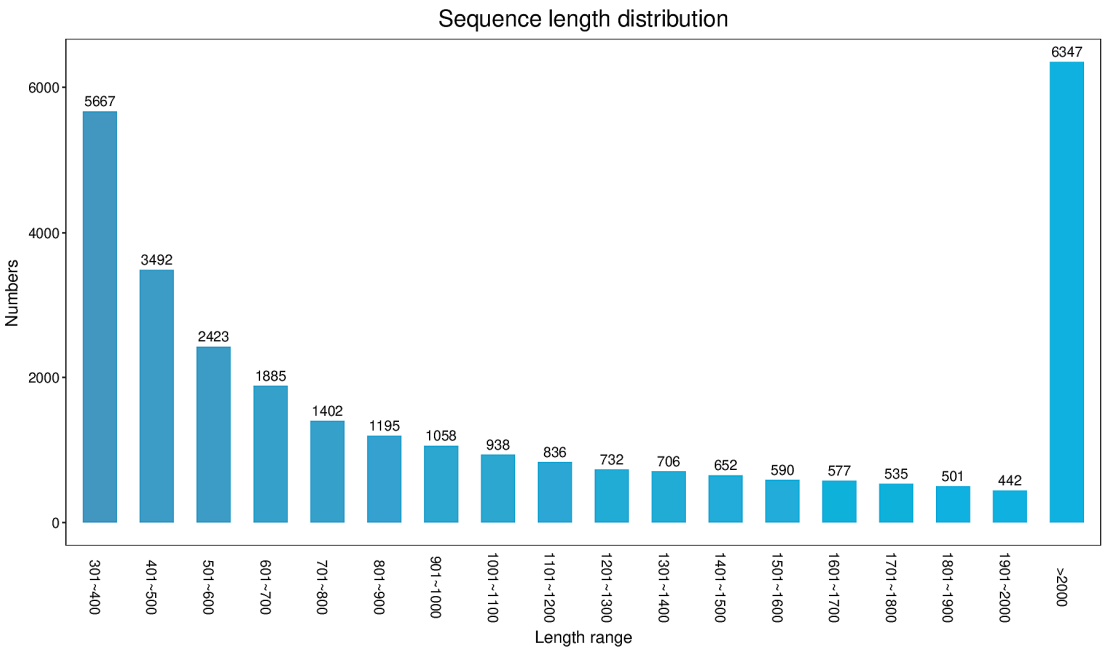


Figure S1. Length distribution of *G. pyloali*

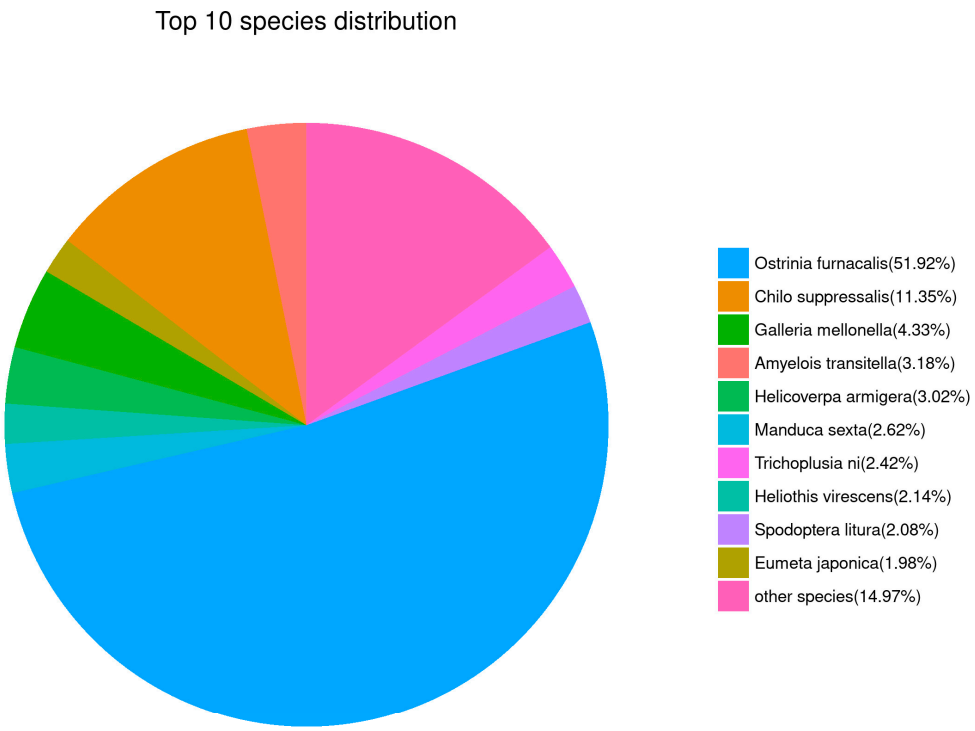
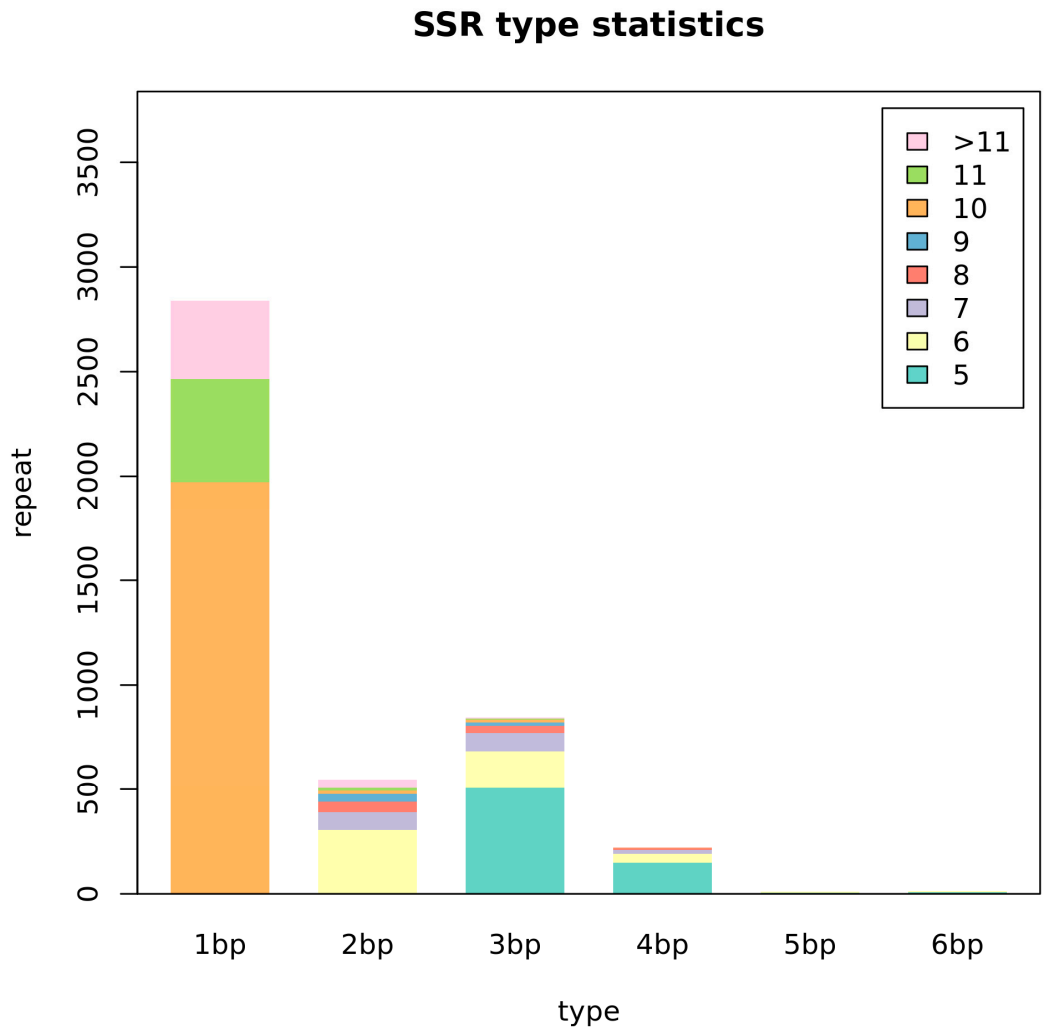


Figure S2. Species classification based on the NR database.



FigureS3. The repeat type frequency of SSR motifs in tested *G. pyloalis* transcriptome.

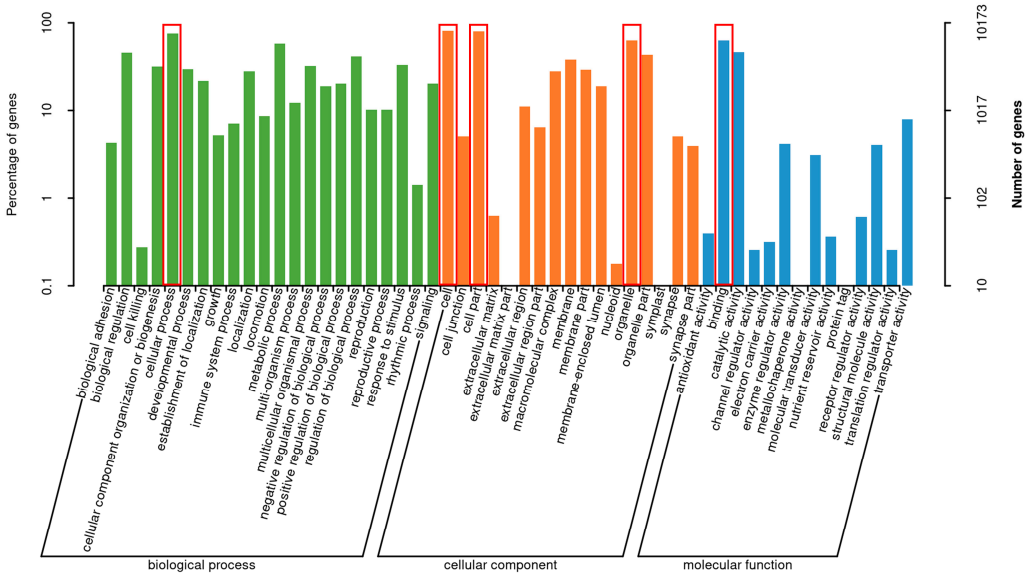


Figure S4. Classification of the gene ontology for the transcriptome of *G. pyloalis* 10,173 (33.93%) of the total unigenes were categorized into 53 function groups. The x-axis represents the different gene functions, and the y-axis denotes the number of DEGs. The red box represents top five abundant levels of GO terms.