**Cry1Ab-expressing rice did not influence expression of fecundity-related genes in the
wolf spider *Pardosa pseudoannulata***

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Short title: Cry toxins did not affect spider gene expression

Abstract

The impact of *Bacillus thuringiensis* (*Bt*) toxin proteins on non-target predatory arthropods is not well understood at the cellular and molecular levels. Here, we investigated the potential effects of Cry1Ab expressing rice on fecundity of the wolf spider, *Pardosa pseudoannulata*, and some of the underlying molecular mechanisms. The results indicated that brown planthoppers (BPHs) reared on Cry1Ab-expressing rice accumulated the Cry toxin and that reproductive parameters (pre-oviposition period, post-oviposition stage, number of eggs, and egg hatching rate) of the spiders that consumed BPHs reared on *Bt* rice were not different from those that consumed BPHs reared on the non-*Bt* control rice. The accumulated Cry1Ab did not influence several Vitellin (Vt) parameters, including stored energy and amino acid composition, during one generation. We considered the possibility that the Cry toxins exert their influence on beneficial predators via more subtle effects detectable at the molecular level in terms of gene expression. This led us to transcriptome analysis to detect differentially expressed genes in the ovaries of spiders exposed to dietary Cry1Ab and their counterpart control spiders. Eight genes, associated with vitellogenesis, vitellogenin receptor activity, and vitelline membrane formation were not differentially expressed between ovaries from the treated and control spiders, confirmed by qPCR analysis. We infer that dietary Cry1Ab expressing rice does not influence fecundity, nor expression levels of Vt-associated genes in *P. pseudoannulata*.

Introduction

Transgenic rice expressing the insecticidal Cry endotoxins from *Bacillus thuringiensis* (*Bt*) effectively control Lepidoptera pests, including the rice leaf folder *Cnaphalocrocis medinalis* Guenee, thus reducing economic losses and chemical pesticide pollution in rice agroecosystems [1-2]. Several single-transgene rice lines expressing individual *Bt* proteins have been developed, including Cry1Aa, Cry1B, Cry1Ab, Cry1Ac, Cry2A, indicating the potential for broad application of *Bt* rice in agricultural production [3-6]. However, *Bt* protein was detected by ELISA in organisms at higher trophic levels [7-8], possibly leading to unintended negative effects in non-target organisms responsible for biocontrol services. A series of investigations has been conducted to assess potential risks of transgenic *Bt* rice to non-target herbivores and their natural enemies, including parasitoids of pests in rice agroecosystems at population levels. For example, no adverse effects of *Bt* rice on fitness parameters (developmental duration, survival rate and fecundity) of non-target herbivorous insects, such as brown planthoppers (BPHs), leafhoppers, and soil collembolans, have been identified [9-11]. Similar results also were obtained in studies of fitness parameters of predatory *Pardosa pseudoannulata*, *Ummeliata insecticeps*, *Cyrtorhinus lividipennis*, *Propylea japonica* and frogs [12-16]. For parasitoid species, results of multiple field surveys were inconsistent with respect to impacts on species richness, diversity, evenness, and dominance indices between *Bt* and non *Bt* fields [17].

A few studies revealed potential risk of *Bt* rice to non-target organisms. For instance, the reproductive rate and catalase activity of the collembolan, *Folsomia candida*, were lower in

test groups maintained on *Bt* rice compared to the control groups maintained on a near-isogenic non-*Bt* rice line. The authors inferred that *Bt* rice may impose environmental stress on collembolans [18]. The activities of three stress response related enzymes, acetylcholine esterase (increased except for day 7), glutathione peroxidase (increased), and superoxide dismutase (decreased) were influenced in the spiders *Ummeliata insecticeps* and *Pardosa pseudoannulata* exposed to dietary Cry1A-reared fruit flies. Again, the authors inferred that Cry proteins originating in transgenic crops have the potential to impact non-target arthropods at the physiological and biochemical levels via food chains. These studies at the level of food chains and food webs help us appreciate how Cry proteins can move within rice agroecosystems and influence the fitness of non-target beneficial predators [19]. Nonetheless, detailed risk analyses require a deeper, mechanistic understanding of the potential risks that *Bt* rice poses to non-target organisms and to long-term sustainability of rice production. In particular, research into the influence of *Bt* toxins at the molecular level is necessary.

The wolf spider *P. pseudoannulata* is one of the dominant insect predators on Chinese farmland, providing some control of insect pests, especially BPHs [20]. Previous studies reported that dietary *Bt* proteins were not deleterious to spider fecundity, registered at the organismal level within a single generation. Nonetheless, more sensitive investigations, designed to detect possible multi-generational effects of *Bt* toxins at the level of gene expression are needed to assess risks associated with long-term reliance on transgenic crops in large-scale agroecosystems. Here, we examined the potential effect of Cry1Ab-expressing

rice on the fecundity of a non-target beneficial predator at the organismal level in terms of reproductive parameters and at the molecular level in terms of differentially expressed genes (DEGs).

Materials and Methods

Rice sample preparation

Seeds of the transgenic Shanyou 63 rice expressing the Cry1Ab protein and its non-transgenic parental wild-type Shanyou 63 rice were obtained from the Life Science College, Hunan Normal University. Both rice lines were grown under nylon netting ($3\times 2\times 1\text{m}^3$) to exclude insects and predators from neighboring fields and managed separately on the experimental farm, Hunan Agricultural University. No insecticides were used during the experimental period.

BPH sample preparation

BPHs were first reared on normal rice Shanyou 63 and allowed to colonize naturally. Newly independent 2nd-instar nymphs were transferred to transgenic and control rice lines. After 15 days on either rice line, BPHs were collected and used as spider diets [21].

Spider rearing

P. pseudoannulata spiderlings were obtained from the eggs of female spiders collected from the non-*Bt* rice field on the experimental farm. Each spiderling was individually placed

in a tube (12x100mm) with a moist cotton ball to provide water and supplied with 16 BPHs daily. At maturity, the female and male spiders were allowed to mate for three hours and to continue feeding on BHPs until egg deposition. The egg sacs were collected from females for vitellin (Vt) characterization. Another group of spiderlings was raised under identical conditions to produce a sufficient number of experimental spiders. The number of eggs laid and egg hatch rate for each female were recorded individually. All the spider-rearing tubes were maintained in a climate-chamber (30⁰C, 70% RH and 10L:14D photoperiod).

Quantification of Cry1Ab in spiders

Adult spiders consuming BPHs for 3, 6, 9 or 12 days were collected individually into 1.5-ml Eppendorf tubes and frozen at -80⁰C for quantification of Cry1Ab protein content using ELISA on a QualiPlate kit for Cry1Ab/Cry1Ac (EnviroLogix, Portland, ME). For each treatment (five spiders per replicate, three biological replicates), spider samples were weighed, homogenized individually in 1 ml 10 mM PBS buffer and centrifuged for 5 min at 12,000×g. The supernatant was used to determine Cry1Ab concentration. Spectrophotometric measurements were taken using a microplate reader (BioTek, ELX 800) at 450 nm. Purified Cry1Ab (EnviroLogix, Portland ME) toxin at concentrations of 0, 2, 4, 8, 16, and 24 ng/L was used as quantitative standards. Ideally, the ELISA data would be confirmed by western blot analysis, however, the antibody available does not detect degraded Cry1Ab fragments. Additional necessary antibodies to detect toxin protein fragments are not available, an important shortcoming we plan to address directly.

Potential effect of Cry1Ab accumulation on Vt characteristics

Thirty newly formed egg sacs (ten egg sacs per replicate, three biological replicates) from the test or control group were dissected in 10 mM PBS buffer (pH 7.4), weighed, and homogenized in the PBS buffer. Vt was extracted according to Gong et al [22]. We quantified sugars [23], lipids [24] and soluble proteins [25]. We determined the Vt amino acid composition using an amino acid analyzer (Hitachi 835-50, Japan) according to the manufacturer's instruction.

RNA isolation and sequencing

This work was done by Oebiotech Enterprise, Shanghai. RNA was extracted from a mix of 35 ovaries prepared from experimental or control spiders. The integrity and quality of total RNA were checked using a NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies Inc, Rockland, DE, USA), limiting the standard to $1.8 \leq OD_{260}/OD_{280} \leq 2.1$ and further confirmed by agarose gels. RNA sequencing libraries were constructed and sequenced on an Illumina Hiseq 2000. Clean reads were assembled using the *de novo* transcriptome assembler Trinity after removing adaptor sequences, and low-quantity reads (reads with ambiguous bases "N" and duplicate sequences). Trinity combines relatively short, clean reads into longer continuous sequences, contigs, and assembles the contigs into longer unigenes. The unigenes were processed to remove redundancy, leading to non-redundant unigenes, which were used in the bioinformatics analysis reported here.

Functional annotation

A sequence similarity search was performed against five common databases (E-value<10⁻⁵) [26]. All unigene sequences were aligned by Blast searching against protein databases, Nr (non-redundant protein database, NCBI), Swissport, COG (Clusters of Orthologous Groups) and KEGG (Kyoto Encyclopedia of Genes and Genomes), retrieving proteins with the highest sequence similarity to the given unigenes along with their protein functional annotations. The Blast2GO program was used to get GO annotation of all unigenes [27-30]. Three arthropod species databases (*Tetranychus urticae*, *Drosophila melanogaster*, and *Stegodyphus mimosarum*) were interrogated to assess identity of the obtained unigenes.

Analysis of gene expression

We used the RPKM (number of reads per kb of exon region per million mapped reads) method to quantify gene expression level [31]. We used DEGseq software (<http://www.bioconductor.org/packages/2.6/bioc/html/DEGseq.html>) to screen for DEGs based on a statistical analysis of negative binomial distribution to quantify the gene expression level relative to a base mean value [32]. A threshold for false discovery rate (FDR) of <0.01 was used to determine significant differences in gene expression. GO enrichment analysis was performed based on GO classification. The hypergeometric enrichment test was used to calculate *p* value of DEGs in some special branch of GO classification and *p*-value correction was performed using the Benjamini-Hochberg method [33]. A GO term with a

FDR value less than 0.0001 was considered significantly enriched. This work was done by Oebiotech Enterprise, Shanghai.

qPCR analysis

Ovaries from 20 experimental or control adults were dissected into Eppendorf tubes on ice. After dissection, the samples were frozen immediately in liquid nitrogen and stored at -80°C for RNA extraction. Total RNA was isolated with TRIzol (Invitrogen, USA) and treated with DNase I (Promega, USA) according to the manufacturer's protocol. The cDNA was synthesized with a RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas, Lithuania), and qPCR was performed using an ABI 7900 HT (ABI, USA) with a volume of 25 µl containing 1 µl of 1:10 cDNA diluted with ddH₂O, 12.5 µl 2 x SYBR Green Master Mix (ABI, USA) and 100 nM of each primers. The qPCR program was 94°C for 3 min, and then 40 cycles of 94°C for 30s for denaturation, 55°C for 30s for annealing and 72°C for 30s for extension. The experiment was repeated three times, and expression levels of each gene were normalized to 18S ribosomal RNA (18S rRNA, GenBank accession number: X13457, primers: 5'-AGATGCCCTTAGATGTCCGG-3', 5'-AAGGGCAGGGACGTAATCAA-3'). The primers specific to selected genes were designed using Primer 3.0 [34].

Statistical analysis

The data from ELISA, fecundity, Vt stability and qPCR detection were analyzed using one-way analysis of variance (ANOVA) with SPSS 17.0 software. For all tests, α was set at

0.05. All data shown in figures and tables were expressed as mean $\pm$ SD.

Results

Amount of Cry1Ab protein in *P. pseudoannulata*

The amount of Cry1Ab in *Bt*-treated spiders increased with feeding time, ranging from 0.09 $\pm$ 0.008 ng/g at day 3 to 0.34 $\pm$ 0.020 ng/g at days 9, and declined to a lower level of 0.27 $\pm$ 0.017 ng/g at day 12 ($F=134.894$; $df=3, 8$; $p<0.001$). Cry1Ab was not detected in the control spiders (Fig. 1). Result suggested the amounts of Cry1Ab changed with time in the spiders, which may be related to Cry protein degradation within the spiders.

Influence of *Bt*-containing prey on spider fecundity

Control and experimental adults were assigned to breeding pairs and maintained on their rice plants. Control and treatment spiders deposited similar numbers of eggs in the first sac ($F=0.034$; $df=1, 16$; $p=0.857$). Egg hatch rates for both groups also were similar (Table1). The pre-oviposition period and the post-parturition periods were similar for the experimental and control groups.

Influence of *Bt*-containing prey on sugar, protein or lipid contents or Vt amino acid composition within eggs

There were no significant differences between eggs from the experimental and control spiders in the content of sugar ($F=0.200$; $df=1,4$; $p=0.678$), protein ($F=0.099$; $df=1,4$; $p=0.657$) or

lipid ($F=0.017$; $df=1,4$; $p=0.902$) (Table 2). Similarly, the amino acid composition of egg proteins did not differ between the experimental and control groups (Fig. 2).

***De novo* assembly of all unigenes**

We obtained 81,160,826 clean DNA sequences with a mean of 40,580,413 reads per transcriptome after removing the low-quality reads. These reads were assembled into 88,034 unigenes with an average length of 496 bp. All unigenes (>200 bp) were used as a library for subsequent bioinformatics analysis (Table 3). All raw reads were uploaded to the NCBI SRA database (accession numbers: SRR 1972512, SRR 1972511).

Functional annotation

Among all unigenes, 26,903 (30.6% of all unigenes) were matched in the Nr database, 22,810 (25.9%) to the Swiss-prot database, 20,952 (23.8%) to the COG, 23,875 (27.1%) to the GO database, and 5,071 (5.8%) to the KEGG database. We elaborate here on two classes of these sets of matches.

The unigenes annotated in the GO database were divided into three gene ontology classes: biological processes, cellular components and molecular functions. Among them, biological processes made up the majority (65.5%), followed by molecular functions (24.6%) and cell components (9.8%). The three major subcategories were “cellular processes”, “single-organism process” and “metabolic processes” within biological processes (Fig. 3).

There were 20,952 unigenes with significant matches in 25 categories within the COG

database. Among these, the “General function prediction only” cluster contributed the highest proportion, followed by “Signal transduction mechanisms” and “Post-translation modification, protein turnover, chaperones”. The smallest groups were “Cell motility”, “Nuclear structure” and “Coenzyme transport and metabolism” (Fig. 4).

Analysis of genes involved in Vt production

Because we were interested in the possible influence of the Cry toxin on fecundity, we focused upon expression of genes relating to egg production. We obtained eight Vt-related genes by GO classification of all unigenes. Three act in regulating vitellogenesis, four in controlling vitelline membrane formation and chorion-containing eggshell formation, and one in affecting vitellogenesis and vitellogenin receptor activity (Table 4). The DNA sequences of eight Vt-related genes are listed in supplemental Table S1.

We did not record significant differences in expression of Vt-related genes between the experimental and control groups (Fig. 5).

Functional enrichment analysis of DEGs

In total, the expression of 81 unigenes was changed in Cry1Ab-treated spiders, compared to controls (Table S2). The fold-change of all DEGs was more than ten, implying a potential negative effect of Cry1Ab on expression of these genes in experimental spiders.

To better understand the underlying biological processes of DEGs, we conducted GO term enrichment analysis based on GO classification. DEGs were significantly enriched in 26

GO categories (Table 5). Of these, most were categorized in “Biological processes” (17) followed by “Molecular function” (7) and “Cellular component” (2). Some GO categories act in developmental processes of larval spiders, such as “embryo development”, “somatic muscle development”, and “structural constituent of eye lens”. There were some biological processes or enzymes indirectly regulating larval development, including “regulation of autophagy”, “phosphatidylinositol-3-phosphatase activity”, and “phosphatidylinositol biosynthetic process”. Two GO categories involved with hormone biosynthesis were annotated, including “juvenile hormone (JH) biosynthetic process” and “JH acid methyltransferase activity”.

Discussion

Herbivorous insects are directly exposed to *Bt* proteins when feeding on transgenic rice tissue, and predators obtain the toxins by consuming *Bt* toxin-contaminated herbivores [12,35]. Shao et al. [36] indicated the final protein processing product of Cry1Ab toxin is an approximately 60kDa protein fragment in the guts of non-target organisms, such as BPHs. Because there are no antibodies to the protein fragments, we remain unable to confirm our ELISA results with Western blots. We acknowledge the shortcoming, which we intend to address in future work. The insecticidal activity of the Cry1Ab activated by BPH gut proteases had similar toxicity compared to the toxin in a target organism, the diamond back moth, *Plutella xylostella*. The molecular weight of the detected-Cry1Ab fragments is similar to the activated Cry toxin in the target organism [36], indicating a potential adverse status of

non-target species in *Bt* rice cropping systems.

In this report, we quantified several fecundity-related parameters, including the pre-oviposition period, number of eggs in the first egg sac, and egg hatching rates. None of these parameters was influenced by dietary Cry proteins. This result is similar to reports on other non-target organisms. The development and reproduction of the spider mite *Tetranychus urticae* Koch and its predator, the predatory mite, *Phytoseiulus macropilis* Banks, over three successive generations were not altered by dietary *Bt* toxins in cotton fields [37]. Similar findings were recorded for another spider, *Ummeliata insecticeps* [38].

Sufficient nutrients are needed for maintaining normal embryonic development and fecundity [39]. We found that total protein, sugar and lipid contents of eggs were similar in the test and control groups, indicating that dietary Cry1Ab did not quantitatively alter the major nutritional profile of females during ovarian maturation. In line with this finding, *Bt* toxin accumulation did not alter the amino acid composition of the spider Vt. We inferred the dietary Cry 1Ab toxin did not influence the reproductive biology of the spiders at the organismal level.

We identified eight genes associated with Vt synthesis, including vitellogenesis, vitellogenin receptor activity and vitelline membrane formation. Our analysis of 81 DEGs indicates no Vt-associated genes were differentially expressed. We inferred that dietary Cry1Ab protein does not influence expression of Vt-related genes in wolf spider ovaries within one generation. We confirmed this by qPCR. Nonetheless, the effects of altered expression of 81 genes on *P. pseudoannulata* multigenerational fitness need further

investigation.

We identified five enriched GO categories connected to developmental processes, including “embryo development”, “somatic muscle development”, “structural constituent of eye lens”, “musculoskeletal movement” and “peripheral nervous system neuron axonogenesis”. These findings indicate to us the possibility of alterations in developmental processes such as formation of organs and tissues during embryogenesis, although this possibility remains to be tested.

Autophagy acts in many cellular processes, including recycling of cellular components, for example, in cell death during arthropod midgut development [40], and in phosphatidylinositol-3-phosphatase activity [41]. The enriched GO categories “regulation of autophagy”, “phosphatidylinositol-3-phosphatase activity” and “phosphatidylinositol biosynthetic process” draw attention to the roles of phosphatidylinositol 3-phosphate in membrane trafficking and maturation of autophagosomes. JHs regulate many aspects of insect physiology, such as development, reproduction, diapause, and polyphenisms [42-43]. Our analysis indicated that two DEGs acted in regulating JH concentrations. These included “JH biosynthetic process” and “JH acid methyltransferase activity”. Altered expression of these genes could influence diverse processes due to the large array of arthropod physiological functions that require very tightly controlled circulating JH concentrations. Our data on several reproductive parameters indicate that Cry1Ab accumulation did not translate into phenotypic differences between experimental and control spiders. It would appear that changes in gene transcription did not result in measurable phenotypic changes. Recognizing

that this study is focused on a single generation of spiders, our findings suggest to us altered expression of a number of genes, which did not result in changes in reproductive fitness. The potential multigenerational effects of these changes remain to be examined.

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Figure Captions

Fig.1 **Bioaccumulation of Cry1Ab protein in *P. pseudoannulata*.** The histogram bars indicate amounts of Cry1Ab protein (mean $\pm$ SD) detected in *P. pseudoannulata* adults fed on *Bt* rice-fed BPHs for the indicated time periods, determined by ELISA. Bars annotated with the same lowercase letters are not significantly different (one-way ANOVA, $p < 0.05$).

Fig. 2. **Dietary Cry1Ab protein did not influence the amino acid composition of vitellin prepared from *P. pseudoannulata*.** The histogram bars indicate mean percentages of the indicated amino acids as proportions of total amino acids. Statistical analysis revealed no significant differences in amino acid composition between experimental and control spiders.

Fig. 3. **GO classification of all unigenes prepared from ovaries of *P. pseudoannulata*,** analyzed using Blast2GO (level 2).

Fig. 4. **COG classification of all unigenes prepared from ovaries of *P. pseudoannulata*.**

Fig. 5. **Dietary Cry1Ab protein did not influence expression of genes encoding vitellin-related proteins.** The histogram bars indicate relative expression of eight genes prepared from experimental and control spiders and the error bars represent one SEM. qPCR analysis was performed as described in M&Ms. The histogram bars indicate mean accumulation of cognate mRNA for each gene, $n = 3$ independent biological experiments $\pm$ SD. RQ = mean relative quantitation.

Tables

Table 1. Influence of Cry1Ab-expressing rice on fecundity of *P. pseudoannulata*.

	Pre-oviposition period (d)	Post-oviposition period (d)	Egg number	Hatch rate (%)
Control group	8.0 ± 1.225	4.3 ± 0.707	125.9 ± 9.200	78.7 ± 4.180
Test group	8.4 ± 0.882	3.9 ± 1.054	126.6 ± 5.810	78.5 ± 2.970

Table 2. Effect of Cry1Ab accumulation on Vt characteristics.

	Protein (µg/mL)	Sugar (%)	Lipid (%)
Control group	642.2±10.127	2.2±0.017	57.1±1.150
Test group	639.0±14.988	2.20±0.029	57.00±0.880

Table 3. Summary statistics of de novo transcriptome assembly.

	All (≥200bp)	≥500 (bp)	≥1000 (bp)	N50	Total Length (bp)	Average Length (bp)
Unigene	88,034	24,921	8,937	604	43,696,650	496.36

Table 4. Vt-related genes identified by GO classification.

	Unigene number	Gene function	Primers
1	comp64295_c0_seq1	vitellogenesis	F:TCTTCCGATCTCTGAGGGTGA P:TCCTAGCTCTGAATGGAGCCT
2	comp48808_c0_seq1	Vitellogenesis and vitellogenin receptor activity	F: GGAATGGATGGATCTGGCGT P: CCGAAGCGACAGGATGATGA
3	comp88717_c0_seq1	vitellogenesis	F: CTCCAAGGGCTGCTACGAAA P: TGCTCCACACCATCTTG CAT
4	comp86327_c0_seq1	vitellogenesis	F: TGCAGTTTGCTCCACACCAT P:CTCCAAGGGCTGCTACGAAA
5	comp54173_c0_seq1	Defined in table footnote	F: TGGACGGACTGCACATATCG P:TCCACTTCAAGCGAGCCTTC
6	comp53145_c0_seq1	Defined in table footnote	F: GGCAGTTACTGGACGAACCA P: GCCCCTACAGTTGCAGGAAT
7	comp47318_c0_seq1	Defined in table footnote	F: CTCCGAACGACGAGAGCTAC P: CCGGTCATTTGTACTCCGCT
8	comp38723_c0_seq1	Defined in table footnote	F: CGTGATTGTCCAATCGTGCC P: TTCAGTACGTGCAAGGCGAT

Genes 5 – 8 mediate vitelline membrane formation involved in chorion-containing eggshell formation.

Table 5. GO enrichment analysis of DEGs. Categories are defined as P = Biological Process; F = Molecular Function; C = Cellular Component.

GO id	GO Term	category	FDR
GO:0050881	musculoskeletal movement	P	4.61E-06
GO:0009790	embryo development	P	4.61E-06
GO:0007525	somatic muscle development	P	8.01E-06
GO:0006661	phosphatidylinositol biosynthetic process	P	5.39E-05
GO:0048936	peripheral nervous system neuron axonogenesis	P	6.39E-05
GO:0010506	regulation of autophagy	P	3.27E-08
GO:0006718	juvenile hormone biosynthetic process	P	1.48E-05
GO:0046716	muscle cell cellular homeostasis	P	6.09E-05
GO:0045110	intermediate filament bundle assembly	P	8.01E-06
GO:0006497	protein lipidation	P	1.45E-05
GO:0006950	response to stress	P	1.48E-05
GO:0051320	S phase	P	1.48E-05
GO:0038083	peptidyl-tyrosine autophosphorylation	P	6.39E-05
GO:0010998	regulation of translational initiation by eIF2 alpha phosphorylation	P	4.61E-06
GO:0060052	neurofilament cytoskeleton organization	P	6.88E-05
GO:0060968	regulation of gene silencing	P	6.88E-05
GO:0008295	spermidine biosynthetic process	P	6.88E-05
GO:0035049	juvenile hormone acid methyltransferase activity	F	1.48E-05
GO:0005212	structural constituent of eye lens	F	2.34E-05
GO:0004438	phosphatidylinositol-3-phosphatase activity	F	4.61E-06
GO:0004766	spermidine synthase activity	F	2.40E-05
GO:0019010	farnesoic acid O-methyltransferase activity	F	2.40E-05
GO:0005021	vascular endothelial growth factor-activated receptor activity	F	6.39E-05
GO:0016936	galactoside binding	F	6.88E-05
GO:0005883	neurofilament	C	6.88E-05
GO:0031467	Cul7-RING ubiquitin ligase complex	C	8.01E-06