Journal of Insect Science (*2017*) 17(4): 80; 1–8
doi: 10.1093/jisesa/iex061
Research article

OXFORD

Characterization of *Spodoptera litura (Lepidoptera: Noctuidae) Takeout* Genes and Their Differential Responses to Insecticides and Sex Pheromone

Xinda Lin,[1,2,*] Ling Zhang,[1,*] and Yanyun Jiang[1]

[1]College of Life Sciences, China Jiliang University, Hangzhou 310018, China (linxinda@cjlu.edu.cn; 312185876@qq.com; 791989761@qq.com), and [2]Corresponding author, e-mail: linxinda@cjlu.edu.cn

*These two authors contributed equally to this work.
Subject Editor: Russell Jurenka

Received 26 April 2017; Editorial decision 12 June 2017

Abstract

Spodoptera litura (*S. litura*) is one of the most serious agricultural insect pests worldwide. Takeout (TO) is involved in a variety of physiological and biochemical pathways and performs various biological functions. We characterized 18 *S. litura TO* genes and investigated their differential responses to insecticides and sex pheromones. All predicted TO proteins have two Cysteines that are unique to the N-terminal of the TO family proteins and contain four highly conserved Prolines, two Glycines, and one Tyrosine. The expression levels of seven *TO* genes in the male antennae were higher than those in the female antennae, although the expression levels of 10 *TO* genes in the female were higher than those in the male. We investigated the effects of the sex pheromone and three insecticides, that is, chlorpyrifos (Ch), emamectin benzoate (EB), and fipronil (Fi), on the expression levels of the *TO* genes in the antennae. The results showed that the insecticides and sex pheromone affect the expression levels of the *TO* genes. One day after the treatment, the expression levels of *SlTO15* and *SlTO4* were significantly induced by the Ch/EB treatment. Two days after the *S. litura* moths were treated with Fi, the expression of *SlTO4* was significantly induced (28.35-fold). The expression of *SlTO10* changed significantly after the Ch and EB treatment, although the expression of *SlTO12* and *SlTO15* was inhibited by the three insecticides after two days of treatment. Our results lay a foundation for studying the role of *TO* genes in the interaction between insecticides and sex pheromone.

Key words: *Spodoptera litura*, sex pheromone, chlorpyrifos, emamectin benzoate, fipronil

The *Takeout* (*TO*) genes encode a family of insect-specific proteins that were first discovered in *Drosophila* (Sarovblat et al. 2000). The TO protein usually contains ~250 amino acids. The TO protein is a type of secretory protein that has an 18-amino acid signal peptide at the N-terminal. Most TO proteins have two highly conserved Cysteine (Cys) residues at the N-terminal (Sarovblat et al. 2000, Du et al. 2003, Dauwalder et al. 2002, Fujikawa et al. 2006, So et al. 2000, Zhu et al. 2008); Wojtasek et al. have demonstrated that the two Cys residues at the N-terminal of Juvenile hormone binding protein (JHBP) are key amino acids for disulphide bond formation (Wojtasek and Prestwich 1995). JHBP shares a high homology with TO, suggesting that the TO family proteins may have characteristics that are similar to those of the ligand (Wojtasek and Prestwich 1995). The crystal structure of *Epiphyas postvittana* TO1 (EpTO1) is a hollow barrel-like structure comprising hydrophobic amino acids and a disulphide bond that is formed by two Cys at the N-terminal (Hamiaux et al. 2009), suggesting that EpTO1 binds the ligand at the top of the protein, carries it to the target cell and then releases it at the bottom of the protein, which indicates that some TO proteins function through binding and transportation (Hamiaux et al. 2009).

The TO protein has been shown to participate in a variety of physiological and biochemical pathways and perform various biological metabolic functions. *Drosophila* TO proteins have been shown to deliver time and food signals for feeding, metabolism, and other activities (Sarovblat et al. 2000). It is also known that the *Drosophila TO* gene is expressed at low levels in rhythmic mutants and is regulated by the CLK-CYC (Clock-Cycle) hetero-dimer (So et al. 2000). It has been reported that the physiological rhythmic transcription factor PARP1 (Pdp1ϵ) regulates the expression of the *TO* gene (Dauwalder et al. 2002). The *TO* gene can be activated by low levels of Pdp1ϵ (Benito et al. 2010). The *TO* gene can also improve the sensitivity of taste nerves to carbohydrates and functions during starvation (Meunier et al. 2007). In *Drosophila melanogaster*, *Manduca sexta*, *Anopheles gambiae*, and *Bombyx mori*, the nutrient level has been shown to regulate the *TO* genes (Meunier

et al. 2007, Du et al. 2003, Justice et al. 2003, Saito et al. 2006). The *TO* genes were expressed in the head, the fat body and other parts of the male fruit-fly but not in the female fruit-fly (Dauwalder et al. 2002). TO affects male courtship behavior by processing sex-biased signals (Dauwalder et al. 2002). The *TO* gene in the termite *Reticulitermes flavipes*, deviate, is expressed in various tissues, such as the thorax and head, and is also involved in trail-following behavior (Schwinghammer et al. 2011). Hagai et al. speculated that juvenile hormone (JH) mediates the regulation of *TO* genes via the development-related factors of the Italian honey bee *Apis mellifera* (Hagai et al. 2007). The expression level of the *TO* gene in *Adenophora glauca* was significantly higher than that in migratory adults (Zhu et al. 2008). The *TO* gene in *Drosophila* is also involved in the regulation of longevity (Bauer et al. 2010). The *TO* gene in *Locusta migratoria* manilensis plays crucial roles in the transformation from mutual exclusion to mutual attraction as the population increases from a low density to a high density (Guo et al. 2011). The receptivity of the chemical receptors in *Helicoverpa armigera* is influenced by the JH (Angioy et al. 1983), suggesting that the *TO* genes may endogenously perform the hormonal functions of lipophilic ligands that act through hydrophobic receptors in lymphocytes and are released into helper cells.

Spodoptera litura (Lepidoptera: Noctuidae, *S. litura*) is a widely distributed crop pest that has a significant impact on the productivity of economic crops (Meagher et al. 2008). The resistance and cross-resistance of *S. litura* to insecticides make it a more difficult pest to control (Rehan and Freed 2014). Identifying the correlation between the *TO* gene and insecticides can lay a foundation for the future development of a strategy to control *S. litura*. Sex pheromones are the key to the courtship behavior of *S. litura* (Lin et al. 2015). It has also been reported that the *TO* gene is associated with courtship behavior (Dauwalder et al. 2002). Therefore, studying the correlation between the *TO* genes and the sex pheromone is important for the understanding of the function of this protein family. The mating ratio of male and female diamondback moths, that is, *Plutella xylostella*, is decreased in an avermectin-resistant strain, indicating that the mating behavior of *P. xylostella* is affected by the insecticide application and the resistance generated (Xu et al. 2010). Volatiles from host plants also contribute to the response of *Spodoptera exigua* to sex pheromones (Deng et al. 2004). The electroantennogram (EAG) responses of *Choristoneura occidentalis* and *Orgyia pseudotsugata* to sex pheromones and a mixture of sex pheromones and insecticides were significantly different. Following a treatment of sex pheromones, the EAG response is high, although the EAG response to the sex pheromone/insecticide mixture was significantly lower than that in the control group, indicating that the insecticides affected the response to the sex pheromone (Sower and Shorb 1985). To control the pest both short term and long term, the pheromone and the insecticide are sometimes used together (Trimble et al. 2001). Insecticides have been shown to affect the courtship and sex pheromone communication systems in insects (Dallaire et al. 2004, Wei and Du 2004, Clark and Haynes 1992, Yang and Du 2003). Changes in the sex pheromone communication system are correlated with insecticide resistance in moths (Xu et al. 2010, Elsayed et al. 2001). Sub-lethal doses of the insecticide malathion decrease the localization of females by sex pheromone (Zhou et al. 2005, Elsayed et al. 2001). In addition, it has been reported that insecticides could affect olfactory function even in vertebrate, such as in salmon (Moore and Waring 1996).

It has been reported that the *TO* gene is highly expressed in the antennae of *Aedes aegypti* (Bohbot and Vogt 2005), and previous studies have also shown that the *TO* gene is highly expressed in *D. melanogaster* and *Phormia regina* (Vanaphan et al. 2012, Fujikawa et al. 2006). Our recent work revealed that the *TO* genes in *Nilaparvata lugens* (*N. lugens*) are male-biased and regulated by JH signaling (Lin et al. 2017). However, the role of the *TO* family genes in the cross interaction between insecticides and the sex pheromone remains unclear. Here, we studied the potential role of the *TO* family genes by measuring their expression levels in the antennae of male and female *S. litura* adults and the changes in the expression of the *TO* genes in response to insecticides and the sex pheromone.

Materials and Methods

Sequence Analysis

Eighteen *S. litura TO* gene sequences were obtained from previously reported transcriptome data (Feng et al. 2015), which were named *TO1-18*. All sequences were deposited in GenBank. The accession numbers are: *SlituTO1* (MF196295), *SlituTO2* (MF196296), *SlituTO3* (MF196297), *SlituTO4* (MF196298), *SlituTO5* (MF196299), *SlituTO6* (MF196300), *SlituTO7* (MF196301), *SlituTO8* (MF196302), *SlituTO9* (MF196303), *SlituTO10* (MF196304), *SlituTO11* (MF196305), *SlituTO12* (MF196306), *SlituTO13* (MF196307), *SlituTO14* (MF196308), *SlituTO15* (MF196309), *SlituTO16* (MF196310), *SlituTO17* (MF196311), *SlituTO18* (MF196312). A Blast search was performed in NCBI. Maximum Likelihood (ML) was used to construct a phylogenetic tree using previously identified 92 *TO* genes from eight species along with 18 *SlituTO* genes using MEGA6 (Hall 2013). The *SlituTO* genes were translated into amino acid sequences, and the sequences were aligned using ClustalW2 (Larkin et al. 2007). A WebLogo sequence alignment map was generated at http://weblogo.berkeley.edu/logo.cgi.

Biological Materials and Experimental Treatment

Male and female *S. litura* pupae were purchased from Jiyuan Baiyun Industrial Co., Ltd. (Henan, China). The male *S. litura* used in the insecticide treatment experiment and the sex pheromone treatment experiment were trapped using traps (SL02-SP, main components: Z9, Z11-14: Oac, Z9, Z12-14: OAc), which were a gift from Ningbo Newcomb Biotechnology Company (Ningbo, China). The trapped male *S. litura* adults were incubated with 10% sucrose for 24 h. The moths were divided into the control group, chlorpyrifos group, emamectin Benzoate group, fipronil group, and sex pheromone group. The control group was treated with 10% sucrose water; the insecticide treatment was performed by adding 1 ppm (Haynes 1988) of the insecticide in 10% sucrose water; and the sex pheromone group was treated with 10% sucrose water, the moths were placed in a plastic basket and PVC lure was placed outside the basket. The antennae of the surviving insects were cut 1 day and 2 days after the treatment. Three sets of biological replicates were established in each group.

RNA Extraction, cDNA Synthesis and qRT-PCR

The antennae were cut and ground in TRIzol (Takara, Dalian, China), extracted using chloroform, precipitated using isopropanol, washed with ethanol, and finally dissolved in DEPC. The PrimeScript RT-PCR Kit (Roche, Shanghai) was used for the reverse transcription with a total of 20 μL in the following three steps: 1) 1 μg of total RNA, 1 μL of OligdT, and RNA free Water to 13 μL were added in a 65 °C water bath for 10 min; 2) 4 μL of Buffer, 2 μL of dNTP, 0.5 μL of the inhibitor, and 0.5 μL of Transcriptase were added in a 55 °C water bath for 30 min; and 3) the solution was

placed in an 85 °C water bath for 5 min. The SYBR FAST qPCR Master Mix2 (KAPA) was used for the qRT-PCR with a total volume of 20 μL as follows: 10 μL of 2 × KAPA SYBR FAST qPCR Master Mix 2 Universal, 0.4 μL each (10 μM), ≤ 20 ng of cDNA, 0.4 μL of 50 × ROX High, and PCR-grade water to 20 μL. The qRT-PCR reaction conditions were as follows: 95°C for 3 min, 95°C for 3 s, and 55°C for 20 s (40×). The primers are listed in Table 1. RPL10 was used as the reference gene (Lu et al. 2013).

Data Analysis

The qRT-PCR data of the *TO* gene expression levels in the antennae of male and female *S. litura* adults were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). The *TO* gene expression in the *S. litura* female antennae was analyzed by SPSS20.0 (IBM-SPSS, Somers, NY) using Student's *t*-test. The effects of the insecticides and sex pheromones on the expression of the *TO* gene in male *S. litura* were analyzed using a single factor ANOVA analysis, and the LSD method was used for multiple comparisons. The figures were generated using OriginPro 9.1.

Heat Map

The heat map was generated with HemI 1.0 using the data of the effects of the insecticide and sex pheromone on the expression levels of the *TO* genes in male moths, and the hierarchical average linkage method was used as the clustering method.

Results

Sequence Analysis of the *TO* Family Genes in *S. litura*

Eighteen *TO* family genes were identified by analyzing previously reported *S. litura* transcriptome data (Feng et al. 2015) and named *SlituTO1–SlituTO18*. We translated the 18 *S. litura TO* gene sequences into amino acids, and a phylogenetic tree was generated by comparing 92 *TO* genes from other eight species with the *TO* genes from *S. litura* (Fig. 1). SlituTO8, SlituTO11, SlituTO6, SlituTO17, SlituTO12, and SlituTO16 clustered together, and they are highly homologous intraspecies (Fig. 1). SlituTO1, SlituTO4, SlituTO8, SlituTO10, SlituTO11, Slitu12, and SlituTO18 have the highest homology with the TO genes from *B. mori* (*Bm*), which indicates that these TO genes are conserved, at least in Lepidoptera.

We also compared the TO genes using WebLogo (Fig. 2). There are two highly conserved Cys (100%) in the N-terminus; three highly conserved amino acids, that is, Proline (100%), Glycine (100%), Tyrosine (100%), in the middle; and a highly conserved Glycine (100%) in the C-terminus (Fig. 2).

Differential Expression Levels of the *TO* Genes Between Male and Female *S. litura*

The expression levels of the *TO* genes in the antenna of the female and male adults are shown in Figure 3. The expression level of the genes in the females is normalized to that in the males. There was no significant difference in the expression of *SlTO2* between the male and the female (Fig. 3). There were seven *SlTO* genes that were more highly expressed in the male than in the female, and three of these genes were the most highly expressed genes, that is, *SlTO6* (94.2-fold), *SlTO10* (115.3-fold), and *SlTO12* (167.2-fold). Ten *TO* genes were more highly expressed in the female than in the male, and five of the *TO* genes, that is, *SlTO4*, *SlTO7*, *SlTO11*, *SlTO17*, and *SlTO18*, were more highly expressed by more than threefold (Fig. 3).

Effects of the Insecticides and the Sex Pheromones on the Expression Levels of the *TO* Genes

We assessed the expression levels of 18 *TO* genes in male and female *S. litura* 1 day and 2 days after the insecticide treatment and the sex pheromone treatment.

Most of the genes (11 of 18) are not changed or only have minor changes after the insecticide or sex pheromone treatment (≤ fourfold, Supp Table 2 [online only], Supp Fig. 1 [online only]). However, the remaining seven genes have relatively higher fold changes after the insecticide treatment or the sex pheromone treatment (> fourfold, Supp Table 2 [online only], Supp Fig. 1 [online only]). The expression level of *SlTO6* was significantly decreased 1 day after the chlorpyrifos treatment, although the expression level of *SlTO12* and

SlTO15 significantly increased. The expression levels of *SlTO6*, *SlTO10*, and *SlTO12* were significantly decreased after a 1-day

Table 1. Primers used in the qRT-PCR

Gene	5′ primer	3′ primer
SlituTO1	TGCCATCCTACATATCCTCA	GTCCTTGAAAGTGACCTTGA
SlituTO2	GATGGAGAGAAACACTGGAG	GCTAATGATGGCCTTGACTA
SlituTO3	GACTACATCATGAGAGGCAG	GTTGAAGAGGTTGGAGAAGT
SlituTO4	ACAAGATCAACCCAGACAAG	CTATCCAATGTCCGTCCTTT
SlituTO5	CGAGTACATGAAAGGAGGAG	GAACTGATGCGTTTCTAGGT
SlituTO6	TATCGCTTCGTCATCCAAG	CCACTTCTTTCCAGTTTTCG
SlituTO7	GGATGCTCAAGTCCTCATAG	AAGTCCTGTCTCCATTGAAC
SlituTO8	CAACTGGCATTTCACTCATC	GTGCTTCTACATCTTCCACA
SlituTO9	CAGAGCCAGCATGTCTAATA	TCTCTAAGTATGCCTCGACA
SlituTO10	TGGTGGACTTCAAGTTGAG	ATGGGGATTTTAGAGAAGGC
SlituTO11	GAACGGACCTGGATCTAAAG	CAATCTTTCACCACCCAGTA
SlituTO12	GTGACGAAGCACCTACTTAT	CGATCTTCCAGTGTTTCTCT
SlituTO13	ATACACCTTTGACTACGGTG	GCACTGCAATGAAGAACTTT
SlituTO14	GCTACGGCCTAAGATGATAG	ACCTGTTCTTGTCTAGGTCT
SlituTO15	TAAAACTCGACGGACAGTAC	AACACTAGGTCGGACTTTTC
SlituTO16	CTGTCGATCTGGAGTTGAAA	ATCTGTACTTCATGAGCGAC
SlituTO17	GTAGACGCTAGTTCACCAAA	AGAATATAGACCACCGTTGC
SlituTO18	TCTCGTAATCACCCTGACTA	GAAATCGCCTTCCAGTTTTC
SlituRPL10	GACTTGGGTAAGAAGAAG	GATGACATGGAATGGATG

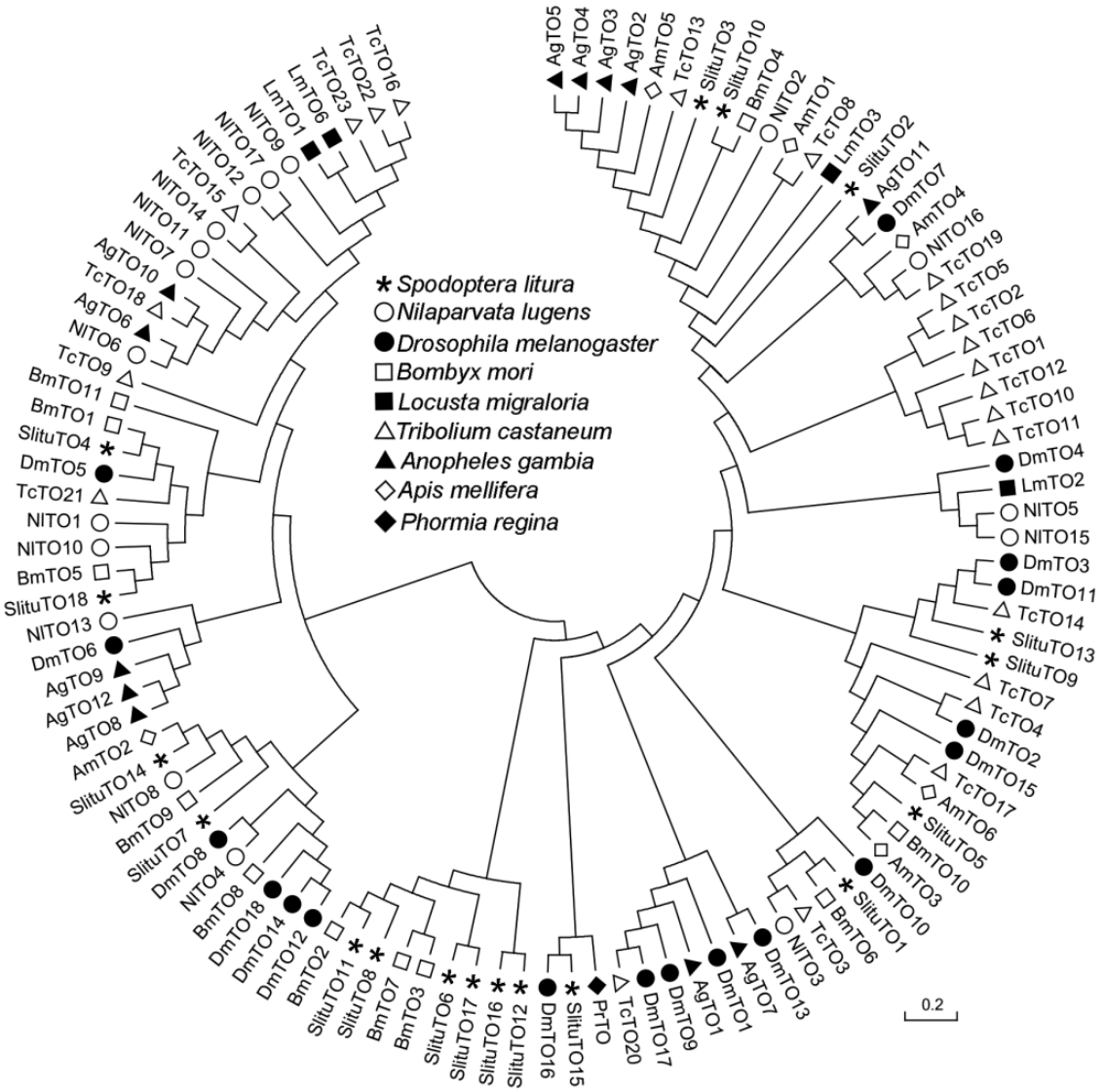

Fig. 1. Phylogenetic analysis of the Takeout family proteins from *S. litura* and other species. Sl: *Spodoptera litura*; Nl: *Nilaparvata lugens*; Dm: *Drosophila melanogaster*; Bm: *Bombyx mori*; Lm: *Locusta migraloria*; Tc: *Tribolium castaneum*; Ag: *Anopheles gambia*; Am: *Apis mellifera*; Pr: *Phormia regina*.

treatment with emamectin benzoate (Fig. 4, Supp Table 2 [online only], Supp Fig. 1 [online only]), although the expression levels of *SlTO4*, *SlTO9*, and *SlTO15* significantly increased (Fig. 4, Supp Table 2 [online only], Supp Fig. 1 [online only]). The expression levels of *SlTO2*, *SlTO6*, and *SlTO10* were significantly decreased 1 day after the treatment with fipronil, although the expression of *SlTO15* significantly increased (Fig. 4, Supp Table 2 [online only], Supp Fig. 1 [online only]). The expression levels of *SlTO2*, *SlTO7*, *SlTO10*, *SlTO12*, and *SlTO17* were significantly decreased 1 day after the sex pheromone treatment, although those of *SlTO1*, *SlTO8*, *SlTO9*, and *SlTO13* significantly increased (Fig. 4, Supp Table 2 [online only], Supp Fig. 1 [online only]).

The expression levels of *SlTO2*, *SlTO11*, *SlTO12*, *SlTO15*, and *SlTO18* were significantly decreased after the 2-day treatment with chlorpyrifos (Fig. 4, Supp Table 2 [online only], Supp Fig. 1 [online only]), although the expression level of *SlTO10* significantly increased (Fig. 4, Supp Table 2 [online only], Supp Fig. 1 [online only]). The expression levels of *SlTO3*, *SlTO12*, *SlTO15*, and *SlTO18* significantly decreased after the 2-day treatment with emamectin benzoate (Fig. 4, Supp Table 2 [online only], Supp Fig. 1 [online only]), although the expression levels of *SlTO1*, *SlTO4*, *SlTO5*, and *SlTO10* significantly increased (Fig. 4, Supp Table 2 [online only], Supp Fig. 1 [online only]). The expression of *SlTO2*, *SlTO3*, and *SlTO12* significantly decreased after the 2-day treatment with fipronil, although the expression levels of *SlTO4* (28.5-fold), *SlTO5*, and *SlTO7* significantly increased (Fig. 4, Supp Table 2 [online only], Supp Fig. 1 [online only]). The expression levels of *SlTO3*, *SlTO6*, *SlTO11*, and *SlTO13* significantly increased after the 2-day treatment with the sex pheromone, although the expression levels of *SlTO1*, *SlTO7*, *SlTO9* significantly decreased (Fig. 4, Supp Table 2 [online only], Supp Fig. 1 [online only]).

Discussion

The TO proteins are highly conserved and contain N-terminal Cys that influence the disulphide bond formation (Dauwalder et al. 2002, Du et al. 2003, Fujikawa et al. 2006, Sarovblat et al. 2000, So et al. 2000, Larkin et al. 2007). Moreover, the TO proteins contain four highly conserved amino acids, two Glycines, one Proline, and

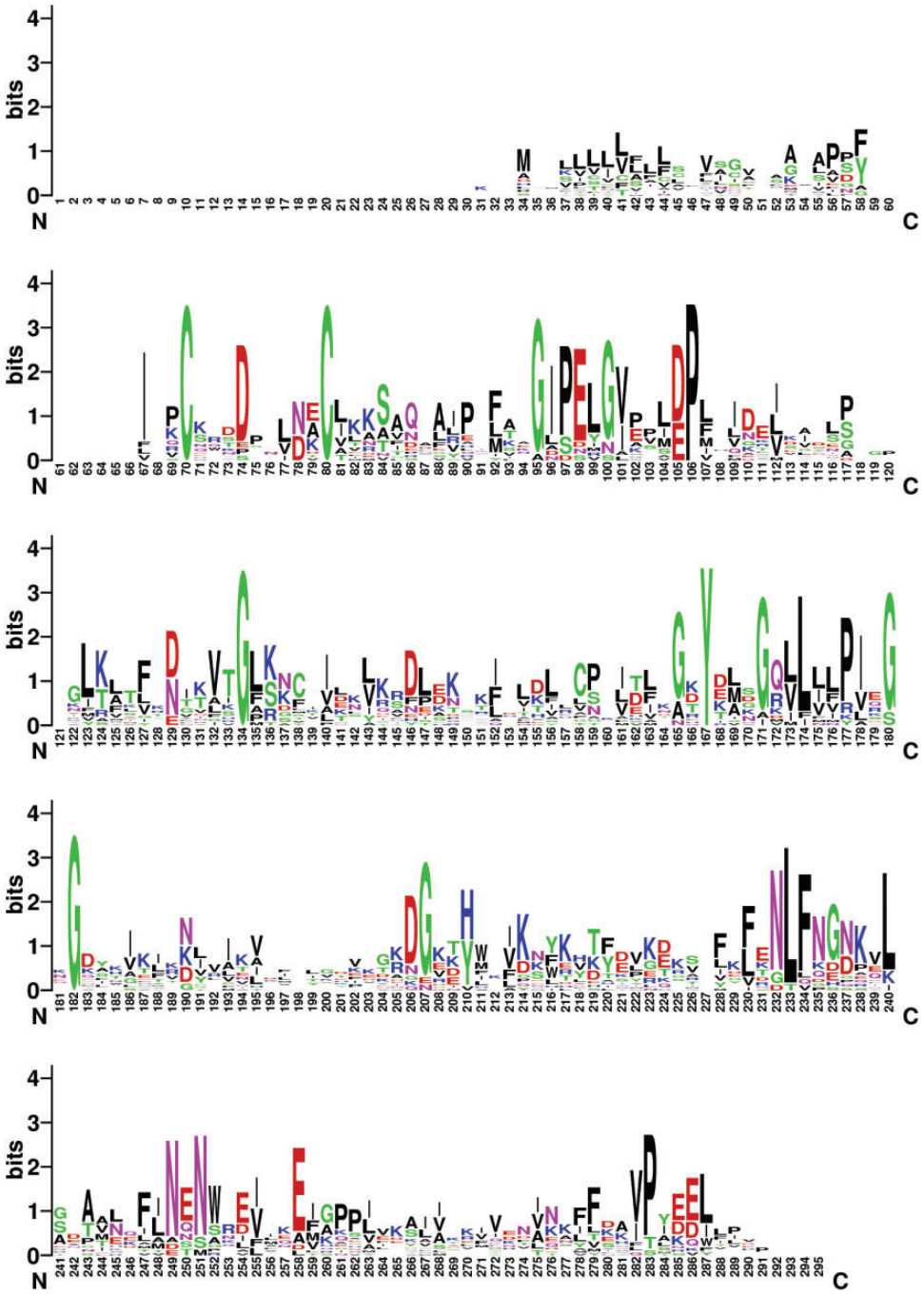

Fig. 2. WebLogo alignment of Takeout protein sequences. Alignment of 18 Takeout proteins from *S. litura*. The x-axis represents the amino acid position. The y-axis (bits) represents the relative proportion of the amino acid at one position. The height of the logo varied inversely with the variability at the position.

one Tyrosine (Fig. 2). The role of the TO protein family remains unknown (Chamseddin et al. 2012, Saito et al. 2006, Fujikawa et al. 2006, So et al. 2000). The *TO* family genes in *S. litura* are closely related to those in *B. mori*, which indicates that the *TO* gene in the two Lepidoptera species had a high homology.

By searching previously reported studies, we analyzed the bias of the *TO* genes expressed in the male and female of species, including *D. melanogaster*, *N. lugens*, *B. mori*, and *P. regina* (Supp Table 1 [online only]). There are three types of *TO* genes as follows: male biased, female biased, and not sex biased. In *S. litura*, 10 *TO* genes are female biased, 7 *TO* genes are male biased, and 1 *TO* gene has no bias. However, in the other three species, most of the *TO* genes are actually male biased (Supp Table 1 [online only]). The expression profiles of the *TO* genes in *D. melanogaster* and *N. lugens* are male biased (Sarovblat et al. 2000, Lin et al. 2017, Dauwalder et al. 2002), whereas here, we found that the expression levels of the *TO* family genes in *S. litura* are different from those in *D. melanogaster* and *N. lugens* (Supp Table 1 [online only], Fig. 3). The expression levels of the *TO* family genes in female *S. litura* adults are higher than those in the male, suggesting that the sex bias of the *TO* family genes is different in different species. As shown in Supp Table 1 [online only], the sex bias of the *TO* family genes is similar in *S. litura*

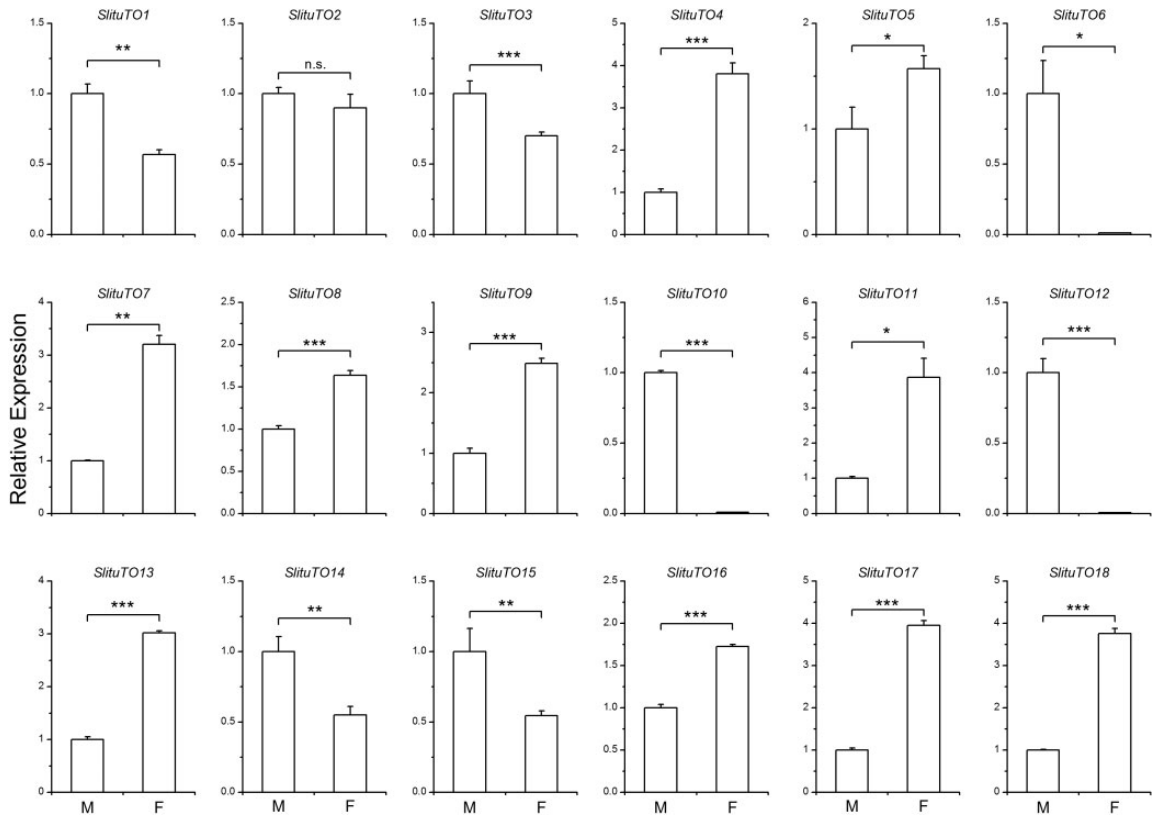

Fig. 3. Difference in the expression levels of the *Takeout* genes between *S. litura* male and female adults. M: Male; F: Female; Student's *t*-test, *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

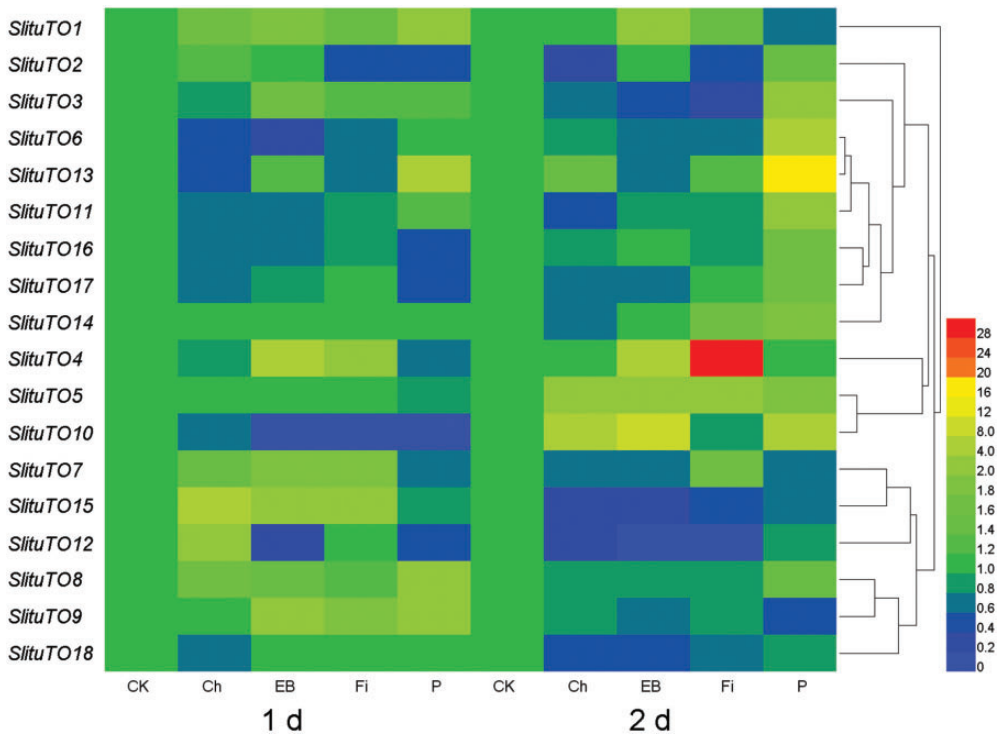

Fig. 4. Heatmap showing the influence of three insecticides and the sex pheromone on the expression levels of 18 *Takeout* genes in *S. litura* male adults after a 1 d and 2 d treatment. 1 d: expression changes in *TO* genes in male *S. litura* after 1 d of insecticide or sex pheromone treatment; 2 d: expression changes in *TO* genes in male *S. litura* after two days of insecticide or sex pheromone treatment; CK: control; Ch: chlorpyrifos; EB: emamectin benzoate; Fi: fipronil; P: sex pheromone. Hierarchical average linkage was used as the clustering method.

and *B. mori*, which is likely because these two species belong to Lepidoptera and is consistent with the phylogenetic analysis (Fig. 3). Interestingly, we found that three *S. litura TO* genes, that is, *SlTO6*, *SlTO10*, and *SlTO12*, are expressed only in the male; however, we have not identified *TO* genes that are expressed specifically in the female (Fig. 3). In fact, we found that the fold-change of all *TO* genes that were highly expressed in the female are less than fourfold.

The insecticide treatments showed that certain *S. litura TO* genes, such as *SlTO4*, *SlTO10*, and *SlTO15*, are highly induced by at least one insecticide with a fold-change > 4. *SlTO*3, *SlTO*4, *SlTO*6, *SlTO*10, *SlTO*12, *SlTO*13, and *SlTO*15 have a high degree of fold-change (Supp Table 2 [online only]). Altogether, *SlTO6* and *SlTO13* are mainly responsive to the sex pheromone treatment; *SlTO3*, *SlTO4*, *SlTO12*, and *SlTO15* are mainly responsive to the insecticide treatment, and *SlTO10* is responsive to both the insecticide and sex pheromone treatment with a high degree of fold-change (Supp Table 2 [online only]). Moreover, the expression changes in *SlTO10* after the treatment with the insecticides were similar to those observed after the sex pheromone treatment.

We recently showed that the *N. lugens TO* genes are regulated by the JH signaling pathway (Lin et al. 2017), but the mechanism by which the *TO* family genes regulate physiological functions remains unclear. Moreover, an understanding of the role of the *TO* genes in the insecticide-induced gene expression and inhibition of the *TO* genes could help us understand the role of the *TO* family genes in vivo. Future works investigating the function of the *TO* genes in vivo are required.

Insecticides and sex pheromones are directly and indirectly linked via physiological and biochemical pathways. The application of sex pheromones decreases the pest population and achieves a long-term pest population decrease; it is also an important pest management tool for controlling several notorious insect pests (Witzgall et al. 2010, Hirano 1980). In addition, the application of insecticides can modify sex pheromone communication in insects, including natural enemies (Delpuech et al. 1999). Understanding the effect of insecticides on the application of sex pheromones is crucial for the efficient control of insect pests in the field because the interaction between insecticides and trapping males might be correlated; if the application of insecticides lowers the trapping efficiency of the sex pheromone lure, additional measures should be taken to increase the pest control efficiency, such as adjusting the recipe of the sex pheromone lure. In addition, understanding the potential interaction between insecticides and sex pheromones at the molecular level could also help us understand the molecular mechanisms underlying the sex pheromone signaling pathway and insecticide toxicology. Our work provided a basis for exploring this important issue in the future.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

Authors' Contributions

X.L. and L.Z. designed the research study, analyzed the data, and wrote the paper. X.L., L.Z., and Y.J. performed the experiments.

Acknowledgments

We would like to thank Bo Wang for the collection of the insects. This work was supported by the Special Fund for Agro-scientific Research in the Public Interest in China (Grant 201203036).

References Cited

Angioy, A. M., A. Liscia, R. Crnjar, A. Porcu, A. Cancedda, and P. Pietra. 1983. Mechanism of JH influence on the function of labellar chemosensilla in Phormia: experimental suggestions. Bollet. Della Soc. Ital. Biol. Speriment. 59(10): 1453–1456.

Bauer, J., M. Antosh, C. Chang, C. Schorl, S. Kolli, N. Neretti, and S. L. Helfand. 2010. Comparative transcriptional profiling identifies takeout as a gene that regulates life span. Aging. 2: 298–310.

Benito, J., V. Hoxha, C. Lama, A. A. Lazareva, J. F. Ferveur, P. E. Hardin, and B. Dauwalder. 2010. The circadian output gene takeout is regulated by Pdp1epsilon. Proc. Natl. Acad. Sci. U. S. A. 107: 2544–2549.

Bohbot, J., and R. G. Vogt. 2005. Antennal expressed genes of the yellow fever mosquito (*Aedes aegypti* L.); characterization of odorant-binding protein 10 and takeout. Insect Biochem. Molec. Biol. 35: 961–979.

Chamseddin, K. H., S. Q. Khan, M. L. Nguyen, M. Antosh, S. N. Morris, S. Kolli, N. Neretti, S. L. Helfand, and J. H. Bauer. 2012. Takeout-dependent longevity is associated with altered Juvenile Hormone signaling. Mech. Ageing Dev. 133: 637–646.

Clark, D. C., and K. F. Haynes. 1992. Sublethal effects of cypermethrin on chemical communication, courtship, and oviposition in the cabbage looper (Lepidoptera: Noctuidae). J. Econ. Entomol. 85: 1771–1778.

Dallaire, R., A. Labrecque, M. Marcotte, E. Bauce, and J. Delisle. 2004. The sublethal effects of tebufenozide on the precopulatory and copulatory activities of *Choristoneura fumiferana* and *C. rosaceana*. Entomol. Exp. Appl. 112: 169–181.

Dauwalder, B., S. Tsujimoto, J. Moss, and W. Mattox. 2002. The Drosophila takeout gene is regulated by the somatic sex-determination pathway and affects male courtship behavior. Genes Dev. 16: 2879–2892.

Delpuech, J., B. Legallet, O. Terrier, and P. Fouillet. 1999. Modifications of the sex pheromonal communication of Trichogramma brassicae by a sublethal dose of deltamethrin. Chemosphere. 38: 729–739.

Deng, J. Y., H. Y. Wei, Y. P. Huang, and J. W. Du. 2004. Enhancement of attraction to sex pheromones of *Spodoptera exigua* by volatile compounds produced by host plants. J. Chem. Ecol. 30: 2037–2045.

Du, J., K. Hiruma, and L. M. Riddiford. 2003. A novel gene in the takeout gene family is regulated by hormones and nutrients in Manduca larval epidermis. Insect Biochem. Molec. Biol. 33: 803–814.

Elsayed, A. M., H. M. Fraser, and R. M. Trimble. 2001. Modification of the sex-pheromone communication system associated with organophosphorus-insecticide resistance in the obliquebanded leafroller (Lepidoptera: Tortricidae). Can. Entomol. 133, 15.

Feng, B., X. Lin, K. Zheng, K. Qian, Y. Chang, and Y. Du. 2015. Transcriptome and expression profiling analysis link patterns of gene expression to antennal responses in *Spodoptera litura*. BMC Genomics. 16: 269.

Fujikawa, K., K. Seno, and M. Ozaki. 2006. A novel Takeout-like protein expressed in the taste and olfactory organs of the blowfly, *Phormia regina*. FEBS J. 273: 4311–4321.

Guo, W., X. Wang, Z. Ma, L. Xue, J. Han, D. Yu, and L. Kang. 2011. CSP and takeout genes modulate the switch between attraction and repulsion during behavioral phase change in the migratory locust. PLoS Genet. 7: 199–212.

Hagai, T., M. Cohen, and G. Bloch. 2007. Genes encoding putative Takeout/juvenile hormone binding proteins in the honeybee (*Apis mellifera*) and modulation by age and juvenile hormone of the takeout-like gene GB19811. Insect Biochem. Molec. Biol. 37: 689–701.

Hall, B. G. 2013. Building phylogenetic trees from molecular data with MEGA. Molec. Biol. Evol. 30: 1229.

Hamiaux, C., D. Stanley, D. R. Greenwood, E. N. Baker, and R. D. Newcomb. 2009. Crystal structure of *Epiphyas postvittana* takeout 1 with bound ubiquinone supports a role as ligand carriers for takeout proteins in insects. J. Biol. Chem. 284: 3496–3503.

Haynes, K. F. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Ann. Rev. Entomol. 33: 149.

Hirano, C. 1980. Capture efficiency of sex pheromone traps for males of *Spodoptera litura*. Jpn. J. Appl. Entomol. Zool. 24: 217–220.

Justice, R. W., S. Dimitratos, M. F. Walter, D. F. Woods, and H. Biessmann. 2003. Sexual dimorphic expression of putative antennal carrier protein

genes in the malaria vector *Anopheles gambiae*. Insect Molec. Biol. 12: 581–594.

Larkin, M. A., G. Blackshields, N. P. Brown, R. M. Chenna, P. A. Mcgettigan, H. Mcwilliam, F. Valentin, I.M.W. Wallace, A. Wilm, and R. Lopez. 2007. Clustal W. Clustal X version. Bioinformatics. 23: 2947–2948.

Lin, X., L. Zhang, and Y. Jiang. 2017. Distinct roles of Met and interacting proteins on the expressions of takeout family genes in brown planthopper. Front. Physiol. 8: 100.

Lin, X., Q. Zhang, Z. Wu, and Y. Du. 2015. Identification and differential expression of a candidate sex pheromone receptor in natural populations of *Spodoptera litura*. PLoS ONE. 10(6): e0131407.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 25: 402–408.

Lu, Y., M. Yuan, X. Gao, T. Kang, S. Zhan, W. Hu, and J. Li. 2013. Identification and validation of reference genes for gene expression analysis using quantitative PCR in *Spodoptera litura* (Lepidoptera: Noctuidae). PLoS ONE. 8: e68059.

Meagher, R. L., J. Brambila, and E. Hung. 2008. Monitoring for exotic spodoptera species (Lepidoptera: Noctuidae) in Florida. Florida Entomol. 91: 517–522.

Meunier, N., Y. H. Belgacem, and J. R. Martin. 2007. Regulation of feeding behaviour and locomotor activity by takeout in Drosophila. J. Exp. Biol. 210: 1424–1434.

Moore, A., and C. P. Waring. 1996. Sublethal effects of the pesticide Diazinon on olfactory function in mature male Atlantic salmon parr. J. Fish Biol. 48: 758–775.

Rehan, A., and S. Freed. 2014. Selection, mechanism, cross resistance and stability of spinosad resistance in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Crop Protect. 56: 10–15.

Saito, K., Z. H. Su, A. Emi, K. Mita, M. Takeda, and Y. Fujiwara. 2006. Cloning and expression analysis of takeout/JHBP family genes of silkworm, *Bombyx mori*. Insect Mol. Biol. 15: 245–251.

Sarovblat, L., W. V. So, L. Liu, and M. Rosbash. 2000. The Drosophila takeout gene is a novel molecular link between circadian rhythms and feeding behavior. Cell. 101: 647–656.

Schwinghammer, M. A., X. Zhou, S. Kambhampati, G. W. Bennett, and M. E. Scharf. 2011. A novel gene from the takeout family involved in termite trail-following behavior. Gene. 474: 12–21.

So, W. V., L. Sarov-Blat, C. K. Kotarski, M. J. Mcdonald, R. Allada, and M. Rosbash. 2000. Takeout, a novel Drosophila gene under circadian clock transcriptional regulation. Mol. Cell. Biol. 20: 6935–6944.

Sower, L. L., and M. D. Shorb. 1985. Pesticides have little impact on attraction of three species of male moths to sex pheromone. J. Econ. Entomol. 78: 908–912.

Trimble, R. M., D. J. Pree, and N. J. Carter. 2001. Integrated control of oriental fruit moth (Lepidoptera: Tortricidae) in peach orchards using insecticide and mating disruption. J. Econ. Entomol. 94: 476–485.

Vanaphan, N., B. Dauwalder, and R. A. Zufall. 2012. Diversification of takeout, a male-biased gene family in Drosophila. Gene. 491: 142–148.

Wei, H., and J. Du. 2004. Sublethal effects of larval treatment with deltamethrin on moth sex pheromone communication system of the Asian corn borer, *Ostrinia furnacalis*. Pest. Biochem. Physiol. 80: 12–20.

Witzgall, P., P. Kirsch, and A. Cork. 2010. Sex pheromones and their impact on pest management. J. Chem. Ecol. 36: 80–100.

Wojtasek, H., and G. D. Prestwich. 1995. Key disulfide bonds in an insect hormone binding protein: cDNA cloning of a juvenile hormone binding protein of *Heliothis virescens* and ligand binding by native and mutant forms. Biochemistry. 34: 5234–5241.

Xu, Z., G. C. Cao, and S. L. Dong. 2010. Changes of sex pheromone communication systems associated with Tebufenozide and Abamectin resistance in Diamondback Moth, *Plutella xylostella* (L.). J. Chem. Ecol. 36: 526–534.

Yang, Z. H., and J. Du. 2003. Effects of sublethal deltamethrin on the chemical communication system and PBAN activity of Asian corn borer, *Ostrinia furnacalis* (Güenee). J. Chem. Ecol. 29: 1611–1619.

Zhou, H., J. Du, and Y. Huang. 2005. Effects of sublethal doses of malathion on responses to sex pheromones by male Asian corn borer moths, *Ostrinia furnacalis* (Guenée). J. Chem. Ecol. 31: 1645–1656.

Zhu, H., A. Casselman, and S. M. Reppert. 2008. Chasing migration genes: a brain expressed sequence tag resource for summer and migratory monarch butterflies (*Danaus plexippus*). PLoS ONE. 3: e1345.