Compared to Odorant-binding Proteins in the Reproductive System and Antennae of Athetis Dissimilis using Transcriptome Analysis

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Abstract

Odorant-binding proteins (OBPs) are prevalent in the antennal transcriptomes of different orders of insects. Studies on OBPs have focused on their role in the insect chemosensory system, but knowledge of their functions in the insect testis is limited. We sequenced the transcriptomes of the *Athetis dissimilis* reproductive organs and analyzed the expression of OBPs in different tissues. We identified 23 OBPs in the testis and ovaries and 31 OBPs in antennal transcriptomes. The results of real-time quantitative PCR revealed that 23 of the 54 OBP genes were highly expressed in both female and male antennae, including three that exhibited male-biased expression and 15 that exhibited female-biased expression. A total of 24 OBPs were highly expressed in the testis of *A. dissimilis*, while expression of OBPs in the ovaries was very low. These findings highlight the functional diversity of OBPs in insects and can facilitate further studies on the OBPs in *A. dissimilis* and lepidopteran species.

Background

The olfactory system in insects regulates their intersex communication, host-plant interactions, oviposition, foraging, escape from predators and reproduction. Insects have a complex chemosensory system in which pheromones and plant odors are initially recognized by odorant-binding proteins (OBPs) expressed in the antennal sensilla lymph that transfer the odorants to membrane-bound olfactory receptors (ORs) to activate olfactory receptor neurons (ORNs) and stimulate behavioral responses.

OBPs are small water soluble proteins that have six positionally conserved cysteines to form three interlocking disulphide bridges that stabilize the protein's three-dimensional structure. OBPs were first discovered in the antenna of *Antheraea polyphemus* that distinguish and bind to lipophilic odorant compounds. However, emerging data suggests that OBPs are not restricted to the sensory organs of insect and show expression in non-sensory organs including reproductive organs. Li *et al.* showed that OBP22 was highly expressed in the male reproductive organs of *Aedes aegypti* and transfers to females during mating as a carrier for the urine and saliva of vertebrate. Sun *et al.* also found that OBP10 is highly abundant in seminal fluid of *Helicoverpa armigera* and *H. assulta* and transfers to female during mating. OBP10 also binds 1-dodecene, a known insect repellent.

*Athetis dissimilis* Hampson (Lepidoptera: Noctuidae) is an important agricultural pest. Li *et al.* distinguished *A. dissimilis* from *A. lepigon* that mainly distributes to Asian countries including China, Japan, Philippines, Korea, Indonesia and India causing serious damages to maize, wheat, peanut, soybean and sweet potato. Because of the fact that larvae of *A. dissimilis* live under plant residues, it is difficult to control the spread of the pest with chemical pesticides. Therefore, novel control managements are urgently needed to mitigate crop damage. We first sequenced the antennal transcriptomes of *A. dissimilis* and characterized 5 OBPs that showed tissue-specific expression patterns. Of note, *Adis*OBP6 was highly expressed in the testes of *A. dissimilis*. We reasoned that the testis of insects possess a defined set of OBPs in a manner comparable to the antenna. In this study, we reanalyzed the previous antennal transcriptome data and identified 31 OBP genes. We also sequenced the transcriptomes of the *A. dissimilis* reproductive organs, and studied the expression of OBPs in the antennae, testis and ovaries. Our study provides a new reference for studying the function of OBP genes.

Results

Illumina sequencing and assembly

A total of 34,565,866, 32,154,799, and 26,952,526 clean reads containing 10.35, 9.63, and 8.07 giga base (Gb) pairs of clean nucleotides respectively, were obtained from the three replicates of the *A. dissimilis* ovaries. A total of 27,752,168, 28,900,040, and 30,838,686 clean reads containing 8.29, 8.65 and 9.23 giga base (Gb) pairs of clean nucleotides respectively, were obtained from the three replicates of *A. dissimilis* testes. The quality of the transcriptome sequences was high, with Q30 percentages of 94.03%, 94.36%, 94.21%, 94.42%, 94.27% and 94.01% for the three replicates of *A. dissimilis* ovaries and testes, with a GC content were ~ 50% (Table 1). Then 221,074 transcripts and 82,016 unigenes with N50 length of 1,350 and 1,243 were obtained from assembled using Trinity (Table 2).

| Sample name | Clean reads | Clean bases | GC Content (%) | Q30 (%) |
|-------------|-------------|-------------|----------------|---------|
| Ovaries     | Repeat 1    | 34,565,866  | 10.35 G        | 48.00   | 94.03 |
|             | Repeat 2    | 32,154,799  | 9.63 G         | 48.35   | 94.36 |
|             | Repeat 3    | 26,952,526  | 8.07 G         | 48.27   | 94.21 |
| Testis      | Repeat 1    | 27,752,168  | 8.29 G         | 48.85   | 94.42 |
|             | Repeat 2    | 28,900,040  | 8.65 G         | 47.20   | 94.27 |
|             | Repeat 3    | 30,838,686  | 9.23 G         | 46.65   | 94.01 |

Table 1: Summary of the sequence assemblies according to the RNA-seq data of the *A. dissimilis*. 


Table 2

Summary of de novo assembly of the *A. dissimilis* transcriptomes.

| Length Range | Transcript | Rate% | Unigene | Rate% |
|--------------|------------|-------|---------|-------|
| < 300        | 0          | 0     | 0       | 0     |
| 300–500      | 83,670     | 37.85 | 37104   | 45.24 |
| 500–1000     | 70,088     | 31.70 | 24792   | 30.23 |
| 1000–2000    | 44,935     | 20.33 | 12864   | 15.68 |
| > 2000       | 22,381     | 10.12 | 7256    | 8.85  |
| **Total Number** | **221,074** |       | **82,016** |       |
| **Total Length** | **216,261,287** |       | **73,549,396** |       |
| **N50 Length** | **1,350** |       | **1,243** |       |
| **Mean Length** | **978.23** |       | **896.77** |       |

Functional annotation

Significant matches of 33,587 unigenes (96.91%) in the NR; 29,936 (86.38%) in the eggnog; 20,134 (58.09%) in the Pfam; 15,174 (43.78%) in the Swissprot database; 14,775 (42.63%) in the KEGG; 7,797 (22.50%) in the GO; and 6,712 (19.37%) in the COG were observed. As a result, up to 34,658 putative coding sequences were identified (Table 3). NR database queries revealed a high percentage of *A. dissimilis* sequences that closely matched to sequences of *Helicoverpa armigera* (19072, 56.87%), *Amyelois transitella* (1936, 5.77%), *Bombyx mori* (1543, 4.60%), *Papilio machaon* (1155, 3.44%), *Papilio xuthus* (868, 2.59%), *Plutella xylostella* (844, 2.52%), *Danaus plexippus* (634, 1.89%), *Branchiostoma belcheri* (473, 1.41%), and *Papilio polytes* (368, 1.10%) (Fig. 1).

Table 3

Functional annotation of the *A. dissimilis* transcriptomes.

| Database | Number | Rate (%) | 300 ≤ Length < 1000 | Length ≥ 1000 |
|----------|--------|----------|---------------------|--------------|
| COG      | 6,712  | 19.37    | 2,638               | 4,074        |
| GO       | 7,797  | 22.50    | 4,453               | 3,344        |
| KEGG     | 14,775 | 42.63    | 8,205               | 6,570        |
| Pfam     | 20,134 | 58.09    | 8,577               | 11,557       |
| Swissprot| 15,174 | 43.78    | 6,987               | 8,187        |
| eggNOG  | 29,936 | 86.38    | 16,283              | 13,653       |
| NR       | 33,587 | 96.91    | 18,939              | 14,648       |
| All      | 34,658 | 99.97    | 19,914              | 14,744       |

For GO analysis, 7,797 unigenes (22.50%) could be assigned to three GO terms including: cellular components (886 unigenes, 11.36%), molecular functions (5,683 unigenes, 72.89%) and biological process (1,228 unigenes, 15.75%) (Fig. 2). The “molecular functions” were highest represented (72.89% transcripts). For the “molecular functions” ontology, catalytic activity and binding were most prevalent.

**Identification of putative odorant-binding proteins**

In the *A. dissimilis* antennal and reproductive organ transcriptome, we identified 54 candidate OBPs (Genbank accession number: KR780027-KR780030, MH900289-MH900338), 31 of which were from the antennae (through the analysis of previous *A. dissimilis* antennal transcriptomes) and 23 from the testis and ovaries transcriptomes of *A. dissimilis* (Table 4). A total of 44 *Adis*OBP sequences had full-length ORFs. Their cDNAs encoded protein of 131–293 amino acids with molecular weights of 11.6–33.2 kDa and isoelectric points of 4.44–9.74. Excluding the 7 *Adis*OBPs (*Adis*OBP28, 30, 31, 35, 36, 41, 42, 52, 53 and 54) signal peptides were predicted at the N-terminus. *Adis*OBPs had 39–99% sequence homology with previously identified OBPs from other insect species, displaying a high level of sequence similarity. For example, *Adis*OBP13 has a 95% identity with *Spodoptera exigua* OBP9 (Table 1). *A. dissimilis* OBPs had only 11.87% identity.
| Order   | Gene name   | GenBank accession no. | ORF (aa) | Molecular weight(kD) | Isoelectric point | Signal peptide | Full length | Homology search with the known proteins | Gene annotation | Species          | Protein ID       |
|---------|-------------|-----------------------|----------|----------------------|-------------------|---------------|-------------|--------------------------------------|----------------|------------------|-----------------|
| c69042  | AdisPBP1    | KR780029              | 166      | 17.32                | 5.19              | Yes           | yes         | PBP1                                | Mamestra brassicae | AAC05702         |
| c65047  | AdisPBP2    | KR780030              | 162      | 18.08                | 5.30              | Yes           | yes         | PBP2                                | Mamestra brassicae | AAC05701         |
| c65143  | AdisPBP3    | MH900289              | 164      | 18.71                | 5.25              | Yes           | yes         | PBP3                                | Agrotis ipsilon   | AFM36758         |
| c47645  | AdisGOBP1   | KR780027              | 163      | 18.89                | 5.19              | Yes           | yes         | GOBP1                               | Sesamia inferens  | AGS36742         |
| c60029  | AdisGOBP2   | KR780028              | 161      | 18.09                | 5.09              | Yes           | yes         | GOBP2                               | Agrotis ipsilon   | AFM36760         |
| c68783  | AdisOBP1    | MH900290              | 293      | 33.20                | 5.76              | Yes           | yes         | OBP                                 | Bombyx mori       | NP_001153663     |
| c69959  | AdisOBP2    | MH900291              | 246      | 27.36                | 5.40              | Yes           | yes         | OBP10                               | Ostrinia fumacalis| BAV56797         |
| c60098  | AdisOBP3    | MH900292              | 145      | 16.22                | 8.37              | Yes           | yes         | OBP                                 | Spodoptera exigua | ADY17886         |
| c65852  | AdisOBP5    | MH900293              | 242      | 26.78                | 6.33              | Yes           | yes         | OBP35                               | Dendrolimus punctatus | ARO70194         |
| c72710  | AdisOBP8    | MH900294              | 240      | 27.01                | 6.53              | Yes           | yes         | OBP25                               | Spodoptera exigua | AKT26502         |
| c61153  | AdisOBP9    | MH900295              | 167      | 18.50                | 4.51              | Yes           | yes         | OBP10                               | Sesamia inferens  | AGS36751         |
| c60049  | AdisOBP11   | MH900296              | 141      | 16.38                | 4.47              | Yes           | yes         | OBP8                                | Spodoptera exigua | AGH70104         |
| c65401  | AdisOBP13   | MH900297              | 133      | 15.14                | 9.01              | Yes           | yes         | OBP9                                | Spodoptera exigua | AGH70105         |
| c58306  | AdisOBP14   | MH900298              | 185      | 20.13                | 6.04              | Yes           | yes         | OBP1                               | Agrotis ipsilon   | AGR39564         |
| c64058  | AdisOBP15   | MH900299              | 146      | 16.43                | 6.29              | Yes           | yes         | OBP6                                | Agrotis ipsilon   | AGR39569         |
| c53621  | AdisOBP16   | MH900300              | 118      | -                    | -                 | internal      | OBP18       | Spodoptera exigua                    | AKT26496         |
| c68160  | AdisOBP17   | MH900301              | 252      | 28.95                | 6.19              | Yes           | yes         | OBP23                               | Spodoptera exigua | AKT26500         |
| c67912  | AdisOBP18   | MH900302              | 203      | 22.50                | 5.69              | Yes           | yes         | OBP19                               | Helicoverpa assulta | AGC92793         |
| c60881  | AdisOBP19   | MH900303              | 139      | 14.55                | 8.58              | Yes           | yes         | OBP5                                | Agrotis ipsilon   | AGR39568         |
| c71719  | AdisOBP20   | MH900304              | 139      | 15.69                | 7.52              | Yes           | yes         | OBP8                                | Spodoptera litura | AKI87969         |
| c65033  | AdisOBP21   | MH900305              | 147      | 15.65                | 4.90              | Yes           | yes         | OBP5                                | Helicoverpa armigera | AEB54581         |
| c63129  | AdisOBP22   | MH900306              | 146      | 15.92                | 7.53              | Yes           | yes         | OBP23                               | Spodoptera litura | XP_022826767     |
| c57331  | AdisOBP23   | MH900307              | 149      | 15.96                | 5.03              | Yes           | yes         | OBP26                               | Spodoptera exigua | AKT26503         |
| c64709  | AdisOBP24   | MH900308              | 148      | 16.77                | 5.45              | Yes           | yes         | OBP7                                | Helicoverpa armigera | AEB54591         |
| c81048  | AdisOBP25   | MH900309              | 71       | -                    | -                 | Internal      | OBP22       | Spodoptera exigua                    | Helicoverpa armigera | AKT26499         |
| c53707  | AdisOBP26   | MH900310              | 134      | 14.28                | 4.51              | Yes           | yes         | OBP34                               | Helicoverpa assulta | ASA40070         |

Note: Genes beginning with the lowercase letter “c” came from the identification of antenna transcriptome, and genes beginning with “Gene” came from testis identification.
| Order | Gene name | GenBank accession no. | ORF (aa) | Molecular weight(kD) | Isoelectric point | Signal peptide | Full length | Homology search with the known proteins | Gene annotation | Species | Protein ID |
|-------|-----------|-----------------------|----------|---------------------|------------------|---------------|-------------|----------------------------------------|----------------|---------|------------|
|       |           |                       |          |                     |                  |               |             |                                        |                 |         |            |
| c28876 | AdisOBP27 | MH900311              | 124      | -                   | -                | -             | internal    | OBP11                    | Spodoptera exigua | AGP033457.1 |
| c67118 | AdisOBP28 | MH900312              | 236      | 27.80               | 4.90             | No            | yes         | OBP9                     | Spodoptera litura | ALD65883  |
| c57589 | AdisOBP29 | MH900313              | 129      | -                   | -                | -             | 5' lose     | OBP33                    | Helicoverpa assulta | ASA40072  |
| c62521 | AdisOBP30 | MH900314              | 180      | 20.26               | 4.84             | No            | yes         | OBP9                     | Helicoverpa armigera | AEB54592  |
| c63839 | AdisOBP31 | MH900315              | 116      | 12.77               | 6.12             | No            | yes         | OBP14                    | Spodoptera exigua | AGP03460  |
| Gene.53346 | AdisOBP32 | MH900316              | 184      | 20.65               | 6.32             | Yes          | Yes         | GOBP70                   | Helicoverpa armigera | XP_021188671 |
| Gene.77161 | AdisOBP33 | MH900317              | 207      | 23.94               | 9.19             | Yes          | Yes         | OBP19                   | Helicoverpa assulta | AGC92793  |
| Gene.60926 | AdisOBP34 | MH900318              | 193      | 22.42               | 5.48             | Yes          | Yes         | OBP9                     | Cnaphalocrocis medinalis | ALT31639 |
| Gene.32069 | AdisOBP35 | MH900319              | 137      | 15.34               | 8.85             | No           | Yes         | OBP                      | Helicoverpa armigera | AEX07279  |
| Gene.44893 | AdisOBP36 | MH900320              | 143      | 15.92               | 5.57             | No           | Yes         | OBP19                   | Helicoverpa assulta | AGC92793  |
| Gene.35132 | AdisOBP37 | MH900321              | 102      | -                   | -                | -            | 5' lose     | OBP24                   | Cnaphalocrocis medinalis | ALT31654 |
| Gene.54044 | AdisOBP38 | MH900322              | 141      | 15.05               | 8.77             | Yes          | Yes         | OBP5                     | Agrotis ipsilon | AGR39568 |
| Gene.7082  | AdisOBP39 | MH900323              | 156      | 17.94               | 4.86             | Yes          | Yes         | PBP1                     | Helicoverpa armigera | XP_021192649 |
| Gene.113597 | AdisOBP40 | MH900324              | 166      | 19.09               | 8.61             | Yes          | Yes         | OBP38                   | Dendrolimus punctatus | ARO70197 |
| Gene.77158 | AdisOBP41 | MH900325              | 141      | 16.29               | 9.12             | No           | Yes         | OBP19                   | Helicoverpa armigera | AGC92793  |
| Gene.14505 | AdisOBP42 | MH900326              | 102      | 11.15               | 5.44             | No           | Yes         | OBP23                   | Spodoptera litura | ALD65897  |
| Gene.54039 | AdisOBP43 | MH900327              | 76       | -                   | -                | -            | 5' lose     | OBP                      | Helicoverpa armigera | AEX07280  |
| Gene.58201 | AdisOBP44 | MH900328              | 76       | -                   | -                | -            | 5' lose     | OBP23                   | Spodoptera litura | ALD65897  |
| Gene.32531 | AdisOBP45 | MH900329              | 150      | 16.43               | 4.77             | Yes          | Yes         | OBP2                     | Agrotis ipsilon | AGR39565 |
| Gene.5319  | AdisOBP46 | MH900330              | 70       | -                   | -                | -            | 5' lose     | OBP14                   | Spodoptera exigua | AGP03460  |
| Gene.86678 | AdisOBP47 | MH900331              | 120      | -                   | -                | -            | 5' lose     | OBP13                   | Sesamia inferens | AGS36753  |
| Gene.141496 | AdisOBP48 | MH900332              | 106      | 12.10               | 6.95             | No           | Yes         | OBP39                   | Dendrolimus punctatus | ARO70198 |
| Gene.142856 | AdisOBP49 | MH900333              | 157      | 17.96               | 9.74             | Yes          | Yes         | OBP18                   | Dendrolimus punctatus | ARO70177 |
| Gene.17592 | AdisOBP50 | MH900334              | 144      | 16.21               | 4.44             | Yes          | Yes         | OBP9                     | Helicoverpa armigera | AEB54592  |
| Gene.54647 | AdisOBP51 | MH900335              | 84       | -                   | -                | -            | 5' lose     | OBP39                   | Dendrolimus punctatus | ARO70198 |
| Gene.76032 | AdisOBP52 | MH900336              | 105      | 11.60               | 4.71             | No           | Yes         | OBP                     | Spodoptera litura | ALD65897  |

Note: Genes beginning with the lowercase letter “c” came from the identification of antenna transcriptome, and genes beginning with “Gene” came from testis identification.
Table 1: Information about the OBPs from four insects

| Order | Gene name | GenBank accession no. | ORF (aa) | Molecular weight(kD) | Isoelectric point | Signal peptide | Full length | Homology search with the known proteins |
|-------|-----------|-----------------------|----------|----------------------|-------------------|---------------|------------|--------------------------------------|
|       | Gene.111996 | MH900337              | 105      | 12.28                | 8.21              | No            | yes        | OBP Operophtera brumhata KOB73304     |
|       | Gene.158529 | MH900338              | 131      | 14.34                | 4.86              | No            | yes        | OBP11 Spodoptera exigua AGP03457       |

Note: Genes beginning with the lowercase letter "c" came from the identification of antenna transcriptome, and genes beginning with "Gene" came from testis identification.

Multiple sequence alignments of the A. dissimilis OBPs revealed the presence of expected conserved cysteines (Fig. 3). The phylogenetic tree of A. dissimilis and other lepidopteran OBPs constructed using the neighbor-joining method, indicated five clades that contained four possible subclass OBPs (Fig. 4). In addition, the tree showed low levels of clustering highlighting the diversity of the lepidopteran OBPs. Five AdisOBPs (AdisPBP1-3, GOBP1-2) belonged to PBP/GOBP. A total of 35 OBPs (AdisOBP1, 3–5, 6–10, 12–17, 19–22) were ‘Classic’ OBPs that contained six positionally-conserved cysteine residues. Seven OBPs (AdisOBP14-16, 18, 33, 36 and 41) belonged to ‘Plus-C’ subclass OBP genes. Nine OBPs belonged to ‘Minus-C’ subclass OBP genes. Interestingly, AdisOBP1, AdisOBP17 and AdisOBP40 did not belong to any of the four subclass OBPs (Fig. 4). However, BLAST results showed that these three genes were homologous with OBP genes of Bombyx mori, Spodoptera exigua and Dendrolimus punctatus. The transcription abundance of A. dissimilis OBPs in antennae of female and males, ovary and testis are profiled in Fig. 5.

Expression of the OBPs in the antennae, ovaries and testis of A. dissimilis

To understand the functions of the identified OBPs in A. dissimilis, we measured the relative expression levels of OBPs in different tissues of A. dissimilis via fluorescence qRT-PCR (Fig. 6). A total of 24 OBPs (AdisGOBP1-2, PBP1-3, OBP1-2, 8–9, 11, 17, 20–22, 24, 26–31, 50 and 54) were highly expressed in the antennae compared to the reproductive organs, including three OBPs (AdisPBP1, OBP17 and OBP26) that exhibited male-biased expression, 15 OBPs (AdisGOBP2, PBP2-3, OBP1-2, 11, 20–22, 27–28, 30–31, 50 and 54) that exhibited female-biased expression, and five OBPs (Adis GOBP1, OBP8-9, 24 and 29) showed comparable expression in the male and female antennae of A. dissimilis.

A total of 24 OBPs (AdisOBP3, 5, 15, 18–19, 23, 25, 33–41, 44–45, 47–49 and 51–53) were highly expressed in the testis of A. dissimilis compared to other tissues. The expressive of the OBPs were low in the ovaries of A. dissimilis.

Discussion

Insects rely on peripheral sensilla on the antennae to distinguish plant odorants and pheromones, a knowledge of the molecular mechanisms of olfaction is essential for better using olfactory-based pest management strategies and the development of novel strategies. OBPs are more accessible targets for research, considering they are small, soluble, stable and easier to manipulate and modify. About exact functions of the OBPs are unclear, but it is widely believed that their function is to capture and transfer outside odorants to ORs located on the membranes of ORNs.

In this study, we identified 31 novel OBPs through the analysis of A. dissimilis antennal transcriptomes, expressing five previously reported AdisOBPs. The number of OBPs in A. dissimilis antennae were similar to the antennal transcriptomes of S. litura (33) and S. littoralis (36) but more abundant than S. exigua (11), M. sexta (18) and H. armigera (26). We additionally sequenced the transcriptomes of A. dissimilis ovaries and testis. The alignments against the Nr database showed that 56.87% of the A. dissimilis unigenes were comparable to Helicoverpa armigera sequences. A total of 24 OBPs were identified in the transcriptomes of A. dissimilis reproduction organs.

Based on the cluster analysis of the phylogenetic trees, five AdisOBPs belonged to PBP/GOBP; 35 AdisOBPs belonged to ‘Classic’ OBPs; 7 AdisOBPs belonged to ‘Plus-C’ OBPs; and 9 AdisOBPs belonged to ‘Minus-C’ OBPs. These results were similar to the classifications of most insect OBPs. Interestingly, AdisOBP1, AdisOBP17 and AdisOBP40 did not cluster into these 4 subclass OBPs, but multiple sequence alignments of the A. dissimilis OBPs revealed that 3 of the OBPs contain no conserved cysteines. Their construction requires further to verification.

Insect OBPs are expressed in the sensory organs. Our result showed that 23 AdisOBPs were significantly expressed in both female and male antennae compared to other tissues. Only the expression of 3 AdisOBPs were significantly higher in the antennae of males compared to females, suggesting that females require more abundant OBPs for spawning. OBPs are also expressed in the non-olfactory organs, such as those required for reproduction. In this study, 24 AdisOBPs showed significant expression in the testis of A. dissimilis compared to other tissues, but the expression of AdisOBPs in the ovaries was low. It was previously speculated that OBPs expressed in the testis deliver compounds to the females during mating. Hence, it is understandable to presume that such stable proteins could be used in the testis of insect where there is need for transportation of hydrophobic molecules in aqueous media or protection of chemicals from degradation, as well as to assure a gradual release of semiochemicals in the environment. So these proteins have been named for "encapsulins", to imply the common role of encapsulating small ligands.

Like antennae, insect testes contain a large number of OBP genes. These genes may also be involved in the development of testis or the movement of sperm and so on. The functions of these genes need us to further study. Our results provide a reference for the study of these genes.

Materials And Methods
Insect rearing and sample preparation

The *A. dissimilis* strain was collected from Luoyang (province of Henan, China) corn fields (112°26´ E, 34°43´ N) in 2014 and maintained at the Henan Science and Technology University. Colonies were reared on an artificial diet at 25 ± 1°C, 80 ± 5% relative humidity and a 16-h/8-h light/dark cycle.

Based on preliminary data, we found that the *A. dissimilis* sperm and eggs began to mature 3 days after emergence. We respectively collected the ovaries and testes of 3-day old virgin females and male adults (*n* = 40 per treatment) from three biological replications. Dissections were performed in sterile PBS-DEPC and immediately frozen in liquid nitrogen until RNA isolation.

cDNA library preparation and sequencing

Total RNA from the *A. dissimilis* ovaries and testis tissues were extracted using RNAiso Plus kit (TaKaRa, Dalian, China) and treated with DNase I (TaKaRa, Dalian, China) as per the manufacturer's protocols. RNA was assessed through 1% agarose gel electrophoresis and Nanodrop 2000 (Thermo Scientific, Waltham, MA, USA). Qubit 2.0 (Life Technologies, Carlsbad, CA, USA) and Agilent 2100 (Agilent, Santa Clara, CA, USA) analysis.

Following the TruSeq RNA Sample Preparation Guide v2 (Illumina, San Diego, CA, USA), mRNA was enriched using magnetic beads crosslinked with Oligo (dT). Enriched RNA was then fragmented using fragmentation buffer and first-strand cDNA synthesis was used to produce small mRNA fragments, random primers, reverse transcriptase, and second-strand cDNA synthesis through the addition of dNTPs, DNA polymerase I, and RNase H. Double-stranded cDNA was purified with AMPure XP beads (Beckman Coulter, Brea, CA, USA) and treated to repair ends, remove poly(A) tails, and link sequencing adapters. Fragment sizes were selected using AMPure XP beads and cDNA libraries were constructed through PCR amplification (Veriti® 96-Well Thermal Cycle, Applied Biosystems, Foster City, USA). The concentration and insert size of the cDNA libraries were detected using Qubit 2.0 and Agilent 2100 and quantified via q-PCR (CFX-96, Bio-Rad, Hercules, CA, USA).

Finally, sequencing was performed using the Illumina HiSeq™ 4000 platform to generate 150-bp paired-end reads. Sequencing analyses were performed by the Genomics Services of the Beijing Biomarker Technologies Co., Ltd. (Beijing, China). Raw data processing and base calling were performed using Illumina software.

Assembly and Functional annotation

Raw data (raw reads) in the FASTQ format were first modified into clean data (clean reads) through Perl scripts. This was performed through the removal of reads containing adapter sequences, >10% unknown nucleotides and quality values ≤ 20. The Q20, Q30, and GC content were then calculated using high-quality data.

Transcriptomes were assembled using Trinity (version trinityrnaseq_r20131110) with default settings, except for min_kmer_cov set to 246. Unigene functions were annotated based on NCBI non-redundant protein sequences (NR, NCBI blast 2.2.28+, e-value = 1e-5), NCBI nucleotide sequences (NT, NCBI blast 2.2.28+, e-value = 1e-5), Protein family (Pfam, HMMER 3.0 package, hmmscan, e-value = 0.01), eukaryotic Ortholog Groups (KOG, NCBI blast 2.2.28+, e-value = 1e-3), SwissProt (NCBI blast 2.2.28+, e-value = 1e-5), the Kyoto Encyclopedia of Genes and Genomes (KEGG; KEGG Automatic Annotation Server [KASS], e-value = 1e-10) and Gene Ontology (GO, Blast2GO v2.5, e-value = 1e-6). Coding sequences (CDS) were predicted through aligning transcriptome sequences to the NR and Swiss-Prot database or using estscan 3.0.3. FPKM values are used to represent the expression abundance of the corresponding Unigenes.

Sequence and phylogenetic analysis

Sequence similarities were assessed using the NCBI-Blast network server (http://blast.ncbi.nlm.nih.gov/). The signal peptides of OBPs were predicted using SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/)49. Multiple sequence alignments were assessed using DNAMAN 6.0. Sequence alignments of the candidate OBPs were performed using ClustalX 2.150 and used to construct phylogenetic trees with PhyML in Seaview v.4 based on the Jones–Taylor–Thornton (JTT) model with nearest-neighbor interchanges. Trees were viewed and edited using FigTree v.1.3.1.

Expression analysis through quantitative real-time polymerase chain reaction

Male antennae, female antennae, ovaries and testes tissue from adults at 3 post-eclosion were excised and frozen in liquid nitrogen. Total RNA was extracted using RNAiso Plus kits (TaKaRa, Dalian, China) and isolated RNA was transcribed to rst-strand cDNA using PrimeScript™ RT reagent with gDNA Eraser (TaKaRa, Dalian, China) following the manufacturer's protocols. Real-time quantitative PCR (RT-qPCR) was performed with SYBR® Premix Ex Taq™ II (TaKaRa). The *A. dissimilis* GADPH gene was used as an endogenous control to correct for sample-to-sample variations. A 200 ng/mL cDNA sample was used for per tissue. Primers were designed using Primer Premier 5.0 software and are listed in supportment Table 1. RT-qPCR reactions contained: 10 μL of SYBR Premix Ex Taq II, 20 ng of cDNA template, 0.2 μM of each primer and nuclease-free water. The cycling conditions were 1 cycle of 95°C for 5 min, followed by 40 cycles of 95°C for 5 s and 55°C for 30 s. Melt curve conditions were 95°C for 10 s and 65°C for 30 s. No-template controls (NTC) were included to detect possible contamination. Three biological replicates were analyzed and the relative expression of the OBP genes was measured using the 2^\(-\Delta\Delta^{CT}\) method. Expression was calculated relative to levels in the female antennae, which were arbitrarily set to 1. Differences in the expression of *AdisOBP* genes
between the different tissues were compared using a one-way nested analysis of variance (ANOVA), followed by a Tukey's honestly significance difference (HSD) test using SPSS (SPSS Institute 17.0, SPSS Inc, Chicago, IL, USA).

**Declarations**

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**Author Contributions**

H.Z.S and Y.Q.S conceived this project and analyzed the data. Z.Y.S, Q.H.L, Q.X.C and J.F.D assisted the preparation of samples and experimental operation. Y.Q.S wrote the main manuscript text. H.Z.S edited the manuscript. All the authors commented on and agreed the manuscript.

**Additional Information**

**Competing Interests:**

The authors declare no competing interests.

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