Identification of odorant-binding proteins in the reproductive system 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 expressive of OBPs in different tissues. We identified a total of 54 OBPs including 23 OBPs in the transcriptomes of testis and ovaries, and 31 OBPs in antennal transcriptomes. Through fluorescence qPCR, the 23 identified OBPs were found to be highly expressed in both female and male antennae compared to the reproductive organs. Of the identified OBPs, 5/23 showed comparable expression in female and male antennae; 3/23 were more highly expressed in males compared to females; and 15/23 OBPs were more highly expressed in females compared to males. A total of 24 OBPs were highly expressed in the testis of A. dissimilis whilst expression in the ovaries was 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 [1–5]. 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 [6–11]. 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 [12-17]. OBPs were first discovered in the antenna of Antheraea polyphemus that distinguish [12] and bind to lipophilic odorant compounds [18-23]. 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 OBPs22 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 [24]. 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 [25].

Athetis dissimilis Hampson (Lepidoptera: Noctuidae) is an important agricultural pest. Li et al. distinguished A. dissimilis from A. lepigone [26] 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 [27–28]. 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 [29] and characterized 5 OBPs that showed tissue-specific expression patterns [30]. Of note, AdisOBP6 was highly expressed in the testes of A. dissimilis [30]. We reasoned that the testis of insects possess a defined set of OBPs in a manner comparable to the antenna. In this study, we sequenced the transcriptomes of the A. dissimilis reproductive organs and studied the expression of the OBPs in the antenna of female and males and testis and ovaries, and provide new targets for pest management in the future.

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   | 94.42 |
| Testis      | Repeat 1    | 27,752,168  | 8.29 G         | 48.85   | 94.27 |
|             | 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*.

| 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|         |       |

Table 2

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

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

| 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   |          | 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 (Table 4). A total of 44 AdisOBP 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 AdisOBPs (AdisOBP28, 30, 31, 35, 36, 41, 42, 52, 53 and 54) signal peptides were predicted at the N-terminus. AdisOBPs had 39–99% sequence homology with previously identified OBPs from other insect species, displaying a high level of sequence similarity. For example, AdisOBP13 has a 95% identity with Spodoptera exigua OBP9.
(Table 1). *A. dissimilis* OBPs had only 11.87% identity.

### Table 4

The characteristic of candidate OBP genes in the antennae and reproductive organs of *A. dissimilis*.

| Order | Gene name | GenBanRF access no. | Molecular weight (kDa) | Full length (aa) | Gene annotation | Species ID | Protein ID | Score | E-value | Identity (%) |
|-------|-----------|---------------------|------------------------|------------------|----------------|------------|------------|-------|----------|---------------|
| c6904 | AdisPB P1 | KR780 029           | 166                    | 17.32            | 5.19           | Yes        | Yes        | PBP1  | AAC05 702 | 79            |
| c6504 | AdisPB P2 | KR780 030           | 162                    | 18.08            | 5.30           | Yes        | Yes        | PBP2  | AAC05 701 | 81            |
| c6514 | AdisPB P3 | MH900 289           | 164                    | 18.71            | 5.25           | Yes        | Yes        | PBP3  | AFM36 758 | 82            |
| c4764 | AdisG OBP1| KR780 027           | 163                    | 18.89            | 5.19           | Yes        | Yes        | GOBP1 | AGS36 742 | 99            |
| c6002 | AdisG OBP2| KR780 028           | 161                    | 18.09            | 5.09           | Yes        | Yes        | GOBP2 | AFM36 760 | 88            |
| c6878 | AdisOB P1 | MH900 290           | 293                    | 33.20            | 5.76           | Yes        | Yes        | OBP   | NP 11145 | 63            |
| c6995 | AdisOB P2 | MH900 291           | 246                    | 27.36            | 5.40           | Yes        | Yes        | OBP10 | BAV56 797 | 66            |
| c6009 | AdisOB P3 | MH900 292           | 145                    | 16.22            | 8.37           | Yes        | Yes        | OBP   | ADY17 886 | 51            |
| c6585 | AdisOB P5 | MH900 293           | 242                    | 26.78            | 6.33           | Yes        | Yes        | OBP35 | ARO70 194 | 46            |
| c7271 | AdisOB P8 | MH900 294           | 240                    | 27.01            | 6.53           | Yes        | Yes        | OBP25 | AKT26 502 | 63            |
| c6115 | AdisOB P9 | MH900 295           | 167                    | 18.50            | 4.51           | Yes        | Yes        | OBP10 | AGS36 751 | 79            |
| c6004 | AdisOB P10| MH900 296           | 141                    | 16.38            | 4.47           | Yes        | Yes        | OBP8  | AGH70 104 | 86            |
| c6540 | AdisOB P11| MH900 297           | 133                    | 15.14            | 9.01           | Yes        | Yes        | OBP9  | AGH70 105 | 95            |
| c5830 | AdisOB P12| MH900 298           | 185                    | 20.13            | 6.04           | Yes        | Yes        | OBP1  | AGR39 564 | 74            |
| c6405 | AdisOB P13| MH900 299           | 146                    | 16.43            | 6.29           | Yes        | Yes        | OBP6  | AGR39 569 | 88            |
| c5362 | AdisOB P14| MH900 300           | 118                    | -                | -              | Yes        | Yes        | OBP18 | AGR39 568 | 48            |
| c6816 | AdisOB P15| MH900 301           | 252                    | 28.95            | 6.19           | Yes        | Yes        | OBP23 | AKT26 496 | 81            |
| c6791 | AdisOB P16| MH900 302           | 203                    | 22.50            | 5.69           | Yes        | Yes        | OBP19 | AGR92 793 | 81            |
| c6088 | AdisOB P17| MH900 303           | 139                    | 14.55            | 8.58           | Yes        | Yes        | OBP5  | AGR39 568 | 62            |
| c7171 | AdisOB P18| MH900 304           | 139                    | 15.69            | 7.52           | Yes        | Yes        | OBP8  | AKI879 69 | 87            |
| Accession | Organism | Species | Length | E values | Expression | Protein | Length | E values | Expression | Protein |
|-----------|----------|---------|--------|----------|------------|---------|--------|----------|------------|---------|
| c6503     | AdisOB  | MH9000  | 305    |          | Yes        | OBP5    |        |          |            |         |
| c6312     | AdisOB  | MH9000  | 306    |          | Yes, yes   | OBP23   | 238    | 2e-78    |            |         |
| c5733     | AdisOB  | MH9000  | 307    |          | Yes, yes   | OBP26   | 233    | 1e-76    |            |         |
| c6470     | AdisOB  | MH9000  | 308    |          | Yes, yes   | OBP7    | 187    | 5e-57    |            |         |
| c8104     | AdisOB  | MH9000  | 309    |          |            |         |        |          |            |         |
| c5370     | AdisOB  | MH9000  | 310    |          |            |         |        |          |            |         |
| c2887     | AdisOB  | MH9000  | 311    |          |            |         |        |          |            |         |
| c6711     | AdisOB  | MH9000  | 312    |          |            |         |        |          |            |         |
| c5758     | AdisOB  | MH9000  | 313    |          |            |         |        |          |            |         |
| c6252     | AdisOB  | MH9000  | 314    |          |            |         |        |          |            |         |
| c6383     | AdisOB  | MH9000  | 315    |          |            |         |        |          |            |         |
| Gene.5    | AdisOB  | MH9000  | 316    |          |            |         |        |          |            |         |
| Gene.7    | AdisOB  | MH9000  | 317    |          |            |         |        |          |            |         |
| Gene.6    | AdisOB  | MH9000  | 318    |          |            |         |        |          |            |         |
| Gene.3    | AdisOB  | MH9000  | 319    |          |            |         |        |          |            |         |
| Gene.4    | AdisOB  | MH9000  | 320    |          |            |         |        |          |            |         |
| Gene.3    | AdisOB  | MH9000  | 321    |          |            |         |        |          |            |         |
| Gene.5    | AdisOB  | MH9000  | 322    |          |            |         |        |          |            |         |
| Gene.7    | AdisOB  | MH9000  | 323    |          |            |         |        |          |            |         |
| Gene.1    | AdisOB  | MH9000  | 324    |          |            |         |        |          |            |         |
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 protein subfamilies (Fig. 4). In addition, the tree showed low levels of clustering highlighting the diversity of the lepidopteran OBPs. Five (AdisPBP1-3, GOBP1-2) AdisOBPs 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

| Gene. | AdisOB | MH900 | 141 | 16.29 | 9.12 | No | Yes | OBP19 | Helicoverpa assulta | AGC92 793 | 115 | 2e-29 | 44 |
|-------|--------|-------|-----|-------|------|----|-----|------|---------------------|-----------|-----|--------|-----|
| 7158  | P41    | 325   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 102 | 11.15 | 5.44 | No | Yes | OBP23 | Spodoptera litura | ALD65 897 | 98.6 | 3e-24 | 49 |
| 4505  | P42    | 326   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 76  | -     | -    | -  | 5’ lose | OBP23 | Spodoptera litura | AEX07 280 | 87.8 | 1e-20 | 59 |
| 4039  | P43    | 327   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 76  | -     | -    | -  | 5’ lose | OBP19 | Helicoverpa armiger a | AGC92 793 | 71.6 | 6e-14 | 48 |
| 8201  | P44    | 328   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 150 | 16.43 | 4.77 | Yes | Yes | OBP2 | Agrotis ipsilon | AGR39 565 | 119 | 1e-31 | 42 |
| 2531  | P45    | 329   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 70  | -     | -    | -  | 5’ lose | OBP14 | Spodoptera exigua | AGP03 460 | 117 | 2e-32 | 81 |
| 319   | P46    | 330   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 120 | -     | -    | -  | 5’ lose | OBP13 | Sesamia inferens | AGS36 753 | 137 | 8e-39 | 53 |
| 6678  | P47    | 331   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 106 | 12.10 | 6.95 | No | Yes | OBP39 | Dendrolimus punctatus | ARO70 198 | 183 | 4e-57 | 82 |
| 41496 | P48    | 332   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 157 | 17.96 | 9.74 | Yes | Yes | OBP18 | Dendrolimus punctatus | ARO70 177 | 119 | 3e-31 | 51 |
| 42856 | P49    | 333   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 144 | 16.21 | 4.44 | Yes | Yes | OBP9  | Helicoverpa armiger a | AEB54 592 | 163 | 5e-49 | 54 |
| 7592  | P50    | 334   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 84  | -     | -    | -  | 5’ lose | OBP39 | Dendrolimus punctatus | ARO70 198 | 140 | 1e-40 | 86 |
| 4647  | P51    | 335   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 105 | 11.60 | 4.71 | No | Yes | OBP  | Spodoptera litura | ALD65 897 | 111 | 4e-29 | 52 |
| 6032  | P52    | 336   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 105 | 12.28 | 8.21 | No | yes | OBP  | Operophtera brumata | KOB73 304 | 194 | 1e-61 | 88 |
| 11996 | P53    | 337   |     |       |      |    |     |      |                     |           |     |        |     |
| Gene. | AdisOB | MH900 | 131 | 14.34 | 4.86 | No | yes | OBP11 | Spodoptera exigua | AGP03 457 | 226 | 3e-74 | 79 |
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 protein subfamilies (Fig. 4). 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 23 OBPs (AdisGOBP1-2, PBP1-3, OBP1-2, 8–9, 11, 17, 20–22, 24, 26–31, 50 and 54) were highly expressed in both female and male antennae compared to the reproductive organs of females and males. A total of 5/23 OBPs (Adis GOBP1, OBP8-9, 24 and 29) showed comparable expression in the male and female antennae of A. dissimilis; 3/23 (AdisPBP1, OBP17 and OBP26) were higher in males; and 15/23 were higher in females.

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 [31], 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 [8, 11, 32]. Insect OBPs are present on the antennae where they execute odorant functions [11, 33–36]. In this study, we identified 31 novel OBPs through the analysis of A. dissimilis antennal transcriptomes, expressing five previously reported AdisOBPs [30]. The number of OBPs in A. dissimilis antennae were similar to the antennal transcriptomes of S. littura (33) [37] and S. littoralis (36) [38] but more abundant than S. exigua (11) [39], M. sexta (18) [40] and H. armigera (26) [41]. 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 [25, 37, 42]. Interestingly, AdisOBP1, AdisOBP17 and AdisOBP40 did not cluster into these 4 protein subfamilies, 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 [22, 25, 37, 43–45]. 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’s 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 [24-25, 46-48]. 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 were low. It was previously speculated that OBPs expressed in the testis deliver compounds to the females during mating [24–25]. 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 [49].

Conclusions

In summary, we demonstrate that the A. dissimilis chemosensory genes show functional diversity. These findings enhance our knowledge of the roles of OBPs in A. dissimilis and lepidopteran species and provide a base for studying OBPs novel targets of pest management strategies.

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 2 [50]. 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 [51].

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/) [52]. Multiple sequence alignments were assessed using DNAMAN 6.0. Sequence alignments of the candidate OBPs were performed using ClustalX 2.1 [53] 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.

Transcript expression profiling

Sequencing Reads were compared to the Unigene libraries using Bowtie, and expression
levels were estimated by combining RSEM. FPKM values are used to represent the expression abundance of the corresponding Unigenes [54]. Averages of three biological replicates as the actual expression values for each transcript were obtained. Based on the RNA-seq data, tissue-specific expression of the OBPs were profiled.

**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 first-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/µL 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 across the samples were measured using the $2^{-\Delta\Delta CT}$ method [55]. 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).

Abbreviations

CO: Carbon dioxide; FPKM: Fragments per kb per million fragments; GO: Gene ontology;
GR: Glutamate receptor; iGluR: Ionotropic glutamate receptor; IR: Ionotropic receptor;
JTT: Jones-Taylor-Thornton amino acid substitution model; OR: Odorant receptor; ORF: Open reading frame; TMD: Transmembrane domain.

Declarations

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Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Authors’ contributions

All authors contributed to research design and manuscript preparation. Conceived and designed the experiments: YS, HS, JZ. Performed the experiments: YS, HS, JZ. Analyzed the data: YS, HS, JZ. Contributed reagents/materials/analysis tools: YS, JZ. Wrote the paper: YS, HS. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.
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Figures

The Blastx results of A. dissimilis reproductive organs unigenes in NR database.
Gene Ontology (GO) classifications of A. dissimilis reproductive organs unigenes according to their involvement in biological processes, cellular component and molecular function.
Figure 3
Sequence alignments of A. dissimilis OBPs.
Figure 4

Phylogenetic relationships of candidate OBP proteins from A. dissimilis and Lepidoptera species.
Figure 5

Heat map showing the abundance of unigenes encoding OBPs in the A. dissimilis different tissues transcriptomes presented as normalized reads in reads per kilobase per million mapped reads (RPKM). In the figure each column represents 1 samples; each line represents 1 OBP gene. The color depth represents the number of reads contained in OBPs; red means more; blue means less. FA: female antennae; MA: male antennae; Ov: ovaries; Te: testis.
Figure 6

Expression profiles of the candidate OBPs in different tissues of *A. dissimilis*. FA: female antennae; MA: male antennae; Ov: ovaries; Te: testis. The standard errors are represented by the error bars; different lowercase letters (a, b, c) above the bars denote significant differences at \( p < 0.05 \).

Supplementary Files

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