

PROTEIN BAIT, VOLATILE COMPOUNDS AND IRRADIATION INFLUENCE THE EXPRESSION PROFILES OF ODORANT-BINDING PROTEIN GENES IN *BACTROCERA DORSALIS* (DIPTERA: TEPHRITIDAE)

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Abstract. Chemical communication in insects is based on odorant-binding proteins (OBPs), but the molecular mechanisms by which these OBPs allow the perception of volatiles and host chemicals remain unclear. *Bactrocera dorsalis* (Hendel) is one of the most economically important pests of fruits and vegetables. In this study, the OBPs of *B. dorsalis* (BdorOBPs) were evaluated in response to different attractive protein baits, brewer's yeast volatile compounds and irradiation. The expression of 10 genes encoding OBPs in the antennae of *B. dorsalis* during three adult physiological life stages (pre-mating, post-mating and post-oviposition) were analysed. All selected BdorOBP genes were found to contain one conserved pheromone-binding protein/general-odorant-binding protein domain (PBP-GOBP), except OBP8, which contained two of these conserved domains. An unrooted phylogenetic tree was constructed to show the relationships among these 10 BdorOBPs and the OBPs belonging to the same OBP family in other insects. We found significantly different transcript expression profiles in each OBP gene at each stage in response to different treatments; these results revealed that OBP2 expression was significantly increased in response to baits at each adult physiological stage, while OBP2, OBP5 and OBP1 were highly expressed in response to combined-volatiles treatment at all tested physiological stages. OBP3, OBP5 and OBP10 showed high expression in response to irradiation at all tested physiological stages. Thus, we infer that protein baits, brewer's yeast volatiles and irradiation significantly influence the transcript levels of OBP genes, which may act in olfactory perception.

Keywords: *transcript analysis, irradiation, brewer's yeast volatiles, oriental fruit fly, insect olfaction*

Introduction

Bactrocera dorsalis is the most devastating pest in several Asian countries including China. This fruit fly was reported for the first time in Taiwan in 1912 (Hardy, 1973). *B. dorsalis* is a serious quarantine pest throughout the world, resulting in severe financial damage to multiple crops that are among its 270 host plant species (Clarke et al., 2005; Chen et al., 2008; Leblanc et al., 2013; Vargas et al., 2015). The extensive damage caused by this particular pest worldwide raises serious concerns about crop yields due to the high resistance of this pest to chemical insecticides (Hu et al., 2014). A variety of methods such as the sterile insect technique, mass trapping, protein bait spraying and male annihilation have been developed to suppress or eradicate *B. dorsalis* and related species throughout Asia and the Pacific regions (Jessup et al., 2007; Mau et al., 2007; Piñero et al., 2009a; Piñero et al., 2009b; Vargas et al., 2015).

Chemoreception is an important characteristic in the selection of host plants by phytophagous insects. Odorant-binding proteins (OBPs) present in the olfactory system play an essential role in sensitivity to odorants, such as pheromones and host chemicals (Zheng et al., 2013). The olfactory system in insects consists of three central types of proteins: OBPs, odorant receptors (ORs) and odorant-degrading esterases (ODEs) (Justice et al., 2003). Among these proteins, OBPs frequently function in the first step of odour perception. The process of chemical communication in both insects and vertebrates is performed by OBPs (Tegoni et al., 2004; Dahanukar et al., 2005; de Bruyne and Baker, 2008). Insects possess a notably complex and cosmopolitan olfactory system, through which they are potentially able to detect a variety of external volatile compounds including plant volatiles and gender-specific mating pheromones (Hsu et al., 2012; Zheng et al., 2012b; Papadopoulos et al., 2013; Zheng et al., 2013; Liu et al., 2015). Insect OBPs may represent an important molecular target in the application of eco-friendly pest management approaches, as they are proposed to be involved in olfactory cue discrimination due to their intense expression in antennal sensillum lymph (Qiao et al., 2009; He et al., 2011; Sun et al., 2013a, 2014; Pelosi et al., 2014). Insects usually use their antennae as olfactory organs in order to detect the odours of plants, fruits and chemicals in the outside environment (Sun et al., 2016a).

Conversely, many studies have shown that the expression of OBPs is not limited to the olfactory organs; thus, their physiological functions may be broader and more difficult to understand than expected (Park et al., 2000; Foret and Maleszka, 2006; Li et al., 2015; Sun et al., 2012; Yuan et al., 2015). To determine whether the OBPs in the antennae of *Bactrocera* species respond to volatiles, the present study focuses on the expression patterns of 10 OBPs in the antennae of *B. dorsalis* (BdorOBPs) in response to protein baits, volatiles and irradiation. Insect OBPs, which are specifically or primarily expressed in the antennae, have been proposed to play roles in olfactory function in response to different kinds of attractive host plant volatiles (Zhou, 2010; Leal, 2013). Previously, an electroantennogram (EAG) was employed to record the electrophysiological responses of the antennae of *B. dorsalis* to spent brewer's yeast volatiles, alcohols, aldehyde alcohols and aldehydes to determine the olfactory response of the antennae towards chemical odours. The results of that study revealed that volatiles could potentially evoke higher relative EAG values in the antennae of *B. dorsalis* during three adult physiological stages (Jang and Light, 1991; Du et al., 2015). A total of 20 BdorOBP genes have been identified from the *B. dorsalis* transcriptome; however, only 10 BdorOBPs have been characterized (Zheng et al., 2012b, 2013).

B. dorsalis transcriptomes and expression analysis in different tissues have been analysed, but the study of the molecular basis of olfaction in response to different stimuli is still in its initial stages (Shen et al., 2011a; Geib et al., 2014). In the studies mentioned above, scientists have reported the EAG response of *B. dorsalis* to different chemical odours and identified OBP genes that are responsible for the first step in the perception of chemical odours. However, few studies have evaluated the expression profiles of the different OBP genes of an insect pest in response to different volatiles, particularly different baits, spent brewer's yeast volatiles and irradiation. Hence, there is a pressing need to study the influence of different kind of volatiles on the expression levels of OBP genes in the antennae of *B. dorsalis*.

Therefore, the current study was based on the hypothesis that different protein baits, spent brewer's yeast volatiles and irradiation would influence the expression levels of OBP genes at three adult physiological stages in the antennae of *B. dorsalis*. The objective of the study was

to evaluate the effects of different protein baits, spent brewer's yeast volatiles and the application of radiation on the transcript levels of the 10 previously identified BdorOBP genes in the antennae of *B. dorsalis* during the pre-mating, post-mating and post-oviposition stages. The findings of the present study may provide knowledge of the molecular mechanisms of action of the variety of OBPs present in the antennae of *B. dorsalis* and potentially suggest novel tactics for the suppression of *B. dorsalis*. A greater understanding of the mechanisms underlying OBP function may allow the invention of novel control methods to disrupt these internal physical mechanisms, perhaps by influencing the gene expression profiles of OBP genes and thus support novel strategies for long-term effective management of tephritid fruit fly species.

Material and Methods

Insect rearing

The insects were reared at the Institute of Beneficial Insects, Fujian Agriculture and Forestry University, Fuzhou, Fujian Province, China, under controlled conditions (26±1°C; 60-70% RH; 12 hr light/12 hr dark photoperiod) for approximately 60 generations. The eggs of *B. dorsalis* were collected using a homemade ovipositional bottle including artificial juice and then were placed on a single layer of wet tissue paper in a larval diet tray. The adult flies were reared on artificial diet prepared with yeast extract and sugar in accordance with the procedures described by (Spencer and Fujita, 1998).

Experimental materials

Proteins were prepared at the Institute of Beneficial Insects at Fujian Agriculture and Forestry University, Fuzhou, Fujian Province, China, while torula yeast was obtained from Baifude Biotechnology, Wuxi, Jiangsu. Spent brewer's yeast volatile compounds phenyl acetate, ethyl caprylate, and octyl acetate (analytical reagents) were purchased from Solarbio Pvt. Ltd., Shanghai, while paraffin oil (100%) was obtained from Acros Organics (Geel, Belgium).

Experimental methods

Protein baits and volatile regulation for female and irradiation application method for male B. dorsalis

This experiment was planned to evaluate the effects of protein baits and torula yeast on the transcript expression of 10 previously identified full-length cDNAs of OBP genes in the antennae of *B. dorsalis* at different adult life stages: pre-mating (2-3 days), post-mating (7-9 days) and post-oviposition (11-13 days). A total of one hundred (100) mature females were released in a 33 cm × 33 cm × 33 cm cage and starved for 12 hrs overnight prior to feeding using protein bait and torula yeast. The insects were treated with two different protein baits (1:10 dilution with paraffin oil), whereas the control group was treated with an equal volume of normal protein diet (Zheng et al., 2012b). Three spent brewer's yeast volatile compounds and their blend mixtures were prepared: 15% paraffin oil (Control, Ck), 15% (75 µl phenyl acetate + 425 µl paraffin oil), 25% (125 µl ethyl caprylate + 375 µl paraffin oil), 15% (75 µl octyl acetate + 425 µl paraffin oil) and a mixture of all compounds (15% phenyl acetate + 25% ethyl caprylate + 15% octyl acetate) (MIX). Treatments were denoted by Ck: control, PA: phenyl acetate, EC: ethyl caprylate, OA: octyl acetate, MIX: mixture of three volatile compounds (PA + EC

+ OA). *B. dorsalis* was irradiated with ^{137}Cs at 100 Gy for 1, 2 and 3 days at the pupal stage, and kept at 25 ± 1 °C, RH $65\% \pm 5\%$, and L:D = 12:12 after adult emergence for 1 day, 6 days and 8 days. Afterwards, 100 infertile males were collected on each day, and their antennae were dissected.

RNA isolation and qRT-PCR analysis

Adult tissue samples were dissected from 500 individuals. The total RNA for each group was extracted from the antennae of *B. dorsalis* at 2-3 days (pre-mating stage), 7-9 days (post-mating stage) and 11-13 days (post-oviposition stage) by using a Tiangen RNeasy Kit. Two micrograms of total RNA was reverse-transcribed by the PrimeScript 1st strand cDNA Synthesis Kit (TaKaRa, Japan) using the oligo dT primer. qRT-PCR was conducted to detect the expression of genes encoding OBPs by using gene-specific primers (Table 1), Top Green qPCR Super Mix (Transgen, China) and a real-time thermocycler (Bio-Rad, Hercules, CA, USA) following the manufacturer's instructions. For internal standardization, β -Actin was amplified. Prior to starting the gene expression analysis, the PCR efficiency of the genes was validated. The qRT-PCR was performed in a volume of 20 μl containing 10 μl of 2 \times TransStart[®] Top Green qPCR Super Mix, 2 μl cDNA (1:100 diluted), 0.8 μl of each (forward and reverse) primer and 6.4 μl nuclease-free H₂O. The qRT-PCR programme was: 94°C for 30 s followed by 40 cycles at 94°C for 15 s, 60°C for 30 s, and 72°C for 10 s. The melting curve was analysed for the PCR products to confirm the presence of a single amplified fragment at the end of each PCR. The β -actin gene was used as a housekeeping gene to standardize the qRT-PCR results. A relative quantitative computing method ($2^{-\Delta\Delta\text{Ct}}$ method) was used to compute gene expression (Livak and Schmittgen, 2001). Three biological replicates were performed.

Table 1. Primers used for gene expression detection of *BdorOBPs* by qRT-PCR

Primer name	(5'→3') Nucleotide sequence
BdorOBP1RTF	GGCGAGCGTTGTTCCAG
BdorOBP1RTR	ATCGGCACCAGCACTTCC
BdorOBP2RTF	TTCTTCGCTGTTGCTGTTTT
BdorOBP2RTR	AGAAGCATTGACTTTGCCATC
BdorOBP3RTF	TTGACCGAGGAGCAGAAAC
BdorOBP3RTR	TTGACTTTGCCATCGACTT
BdorOBP4RTF	GAAGTCTTCGCTGTTGCTG
BdorOBP4RTR	CTTGACTGTCATCGCCTTTG
BdorOBP5RTF	GGATTTCCACGACTTCATTGAG
BdorOBP5RTR	CTTTAGCCGCATCAGGTTTG
BdorOBP6RTF	ACGAAGCCAAAGTCACGG
BdorOBP6RTR	GCATCAAAGACGCCATCC
BdorOBP7RTF	GTTGCAGGCAAATTCCAGAT
BdorOBP7RTR	AGAGTCCCATTGCTCCACA
BdorOBP8RTF	ACGGCTAAGGCTCTAAAGAACC
BdorOBP8RTR	AAACCACTTGTGACGCTTCG
BdorOBP9RTF	GCGATGCCGACCATGATGAC
BdorOBP9RTR	ACCACCACGATAAGCCCACT
BdorOBP10RTF	GTGTCTTCTGCGAACATGAGG
BdorOBP10RTR	CACTTGTGACCACGATAGGC
β -actinRTF	CTCGTCCAACCGTTCATACC
β -actinRTR	CTGACCTGCCCACTGAAGTT

Statistical analysis

Statistical analysis was performed using the SPSS 17.0 software package (IBM Corporation, Somers, NY, USA) to calculate *Dunnnett's test* for comparing control with other treatments (SPSS for Windows, Version 16.0, 2007).

Results

Identification of PBP-GOBP domains in *BdorOBP* genes

All 10 *BdorOBP* genes were found to contain one conserved PBP-GOBP domain, except for OBP8, which, remarkably, contained two such conserved domains. In the OBP family, the amino acid sequence residues of each *BdorOBP* gene were green when compared with the hidden Markov model (HMM), which indicates a very high-quality hit. *BdorOBP* conserved domains are presented in (Figure 1), while the *BdorOBP* genes along with their accession numbers are presented in (Table 2). The PBP-GOBP conserved domain was identified by using the Pfam database (version 31.0) (Finn et al., 2016).

<*BdorOBP1*|PCI domain|

NADYEEKTEDDFLSAGERCQFRERLAASYQRRFDNFDYPDEEPV
QRYVHCIWTELKLWNDRTGFNVEHIAALYRDKANTEVLVPILSDC
NRNAQNEPTLKWCFYAFKCVLNS



<*BdorOBP2*|PCI domain|

YPPPELLKELQPVHDSCVAKTGVTEEAIKEFSDDGDVHEDELLKCYM
YCVFEETDVLHEDGEVHLEKILDKLPESMHVIALHMGKKCLYPKGD
NKCERAFWLHR



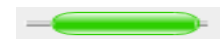
<*BdorOBP3*|PCI domain|

GKLTTEEQKQKVHAAAAECFKETGASEDAVRALLKGDDSQVDGKVK
CFAKCTLGKLDLLQNGKVNEEKVQNILGKLIGEEKAKAAQAKCNGL
KGTDECDTAYQIRQCYAAG



<*BdorOBP4*|PCI domain|

EGMLTPEQIQKVHTLSNECLKETGASEDAIRALIKGDDSQVDGK
VKCYAQCMVLKLG YVENGKVNEEKVQNILGKLIGEEKAKATQ
AKCNGLKGTDECDTAYQIRQCYSAG



<*BdorOBP5*|PCI domain|

PHDPEMRGYIEDCNKEHNVSPKDFHDFIEGKLTTPENMKCSSQC
IMVKQGIMDESGNFKPDAAKAKMKEDKLVA AVDECKDLSGSTPC
DTAFKITSCMLSK



<*BdorOBP6*|PCI domain|

AVCQLPADLEKFHKACMDEAKVTDEQMRQFFQNGMKASDATENI
KCQMKCMMQKQGIWKDGVFDADAKIKELVQNPKFKGKEARTNK



<*BdorOBP7*|PCI domain|

GKFQIRTAQDALDAHEACHEEYRVPEDIYQKFLNYEFP AHKRTNCY
VKCFVERMGLFTEEKGFDEKAIIAQFTAKSSKNLAKVSHGLEKCLDH
NEHSDTCTWANRVFSCWIS



<*BdorOBP8*|PCI domain|

ATTKAAEQDVSDTEILRKCLREVGSKDLVGELQKVARYSKW
TSEEVPCFTRCLASMKHWFDADESKWNKQIADDLGADMYN
YCRYELDRYNEDSCEFAYTGLRCLKQA



TLETYKNIVSCASELNVTMKELQKYAAFPTKEVVPCLFQCLAE
KMNFYTPTYEWNLDNWWVQAFGPMRQDRTASNVCKVSAEHM
KTRDKCEWMYEYNCLER

<*BdorOBP9*|PCI domain|

SAEYVVKNEENLQQYRRECATELKVP AEHIEQFRKWQFPNDA
VTQCYLKCVFEKFLFDAVTGFNVEHIHQQLQGAEVAPPGDA
DHDDVVHDKIAACVDTNEQGSNACEWAYRGGVCFIKE



<*BdorOBP10*|PCI domain|

DEEWVPKNVAQIKAIRQECIKDFPLSEEYIQMKNFYEPDEEPPVR
KYLCTAKKLGVFCEHEGYHADRVAKQFKMDLDEAEVIAIAEG
CADKNVEGSSADVWAYRGHKCVMAS



Figure 1. *BdorOBPs* contain one conserved PBP-GOBP domain, which is a typical characteristic of OBPs. All *BdorOBP* genes contain one conserved PBP-GOBP domain, except *OBP 8*, which contains 2 of these conserved domains. The PBP-GOBP domains were identified by using the Pfam database (version 31.0), and the amino acid sequence residues of each *BdorOBP* gene were green when compared with the hidden Markov model (HMM), which indicates a very high-quality hit.

Table 2. *BdorOBP* genes with their accession numbers and nucleotide lengths

Gene name	Accession number	Length
<i>BdorOBP1</i>	<i>KC559112</i>	159
<i>BdorOBP2</i>	<i>KC559113</i>	138
<i>BdorOBP3</i>	<i>KC559114</i>	146
<i>BdorOBP4</i>	<i>KC559115</i>	136
<i>BdorOBP5</i>	<i>KC559116</i>	130
<i>BdorOBP6</i>	<i>KC559117</i>	104
<i>BdorOBP7</i>	<i>KC559118</i>	142
<i>BdorOBP8</i>	<i>KC559119</i>	274
<i>BdorOBP9</i>	<i>KC559120</i>	148
<i>BdorOBP10</i>	<i>KC559121</i>	149

Phylogenetic analysis of insect OBPs

A phylogenetic tree was generated by obtaining the amino acid sequences of the ten putative OBPs of *B. dorsalis* from the work of (Zheng et al., 2013). The phylogenetic tree was built to demonstrate the relationships of the 10 *BdorOBPs* to 140 OBP sequences from 7 different orders and families of insects that possess the same OBP family, including 20 OBP sequences from *Apis mellifera* (Hymenoptera: Apidae), 7 OBP sequences from *Bombyx mori* (Lepidoptera: Bombycidae), 30 OBP sequences

from *Drosophila pseudoobscura* (Diptera: Drosophilidae), 29 OBP sequences from *Halyomorpha halys* (Hemiptera: Pentatomidae), 15 OBP sequences from *Spodoptera litura* (Lepidoptera: Noctuidae), 10 OBP sequences from *Tenebrio molitor* (Coleoptera: Tenebrionidae) and 44 OBP sequences from *Tribolium castaneum* (Coleoptera: Tenebrionidae), as shown in (Figure 2). This phylogenetic tree clarified the connections between *B. dorsalis* and other insects in the groups listed above. Amino acid sequences were used to build an unrooted phylogenetic tree using MEGA 7.0 with the pairwise deletion option under the JTT empirical amino acid substitution model (Kumar et al., 2016). Branch support was assessed by bootstrap analysis with 1000 replicates.

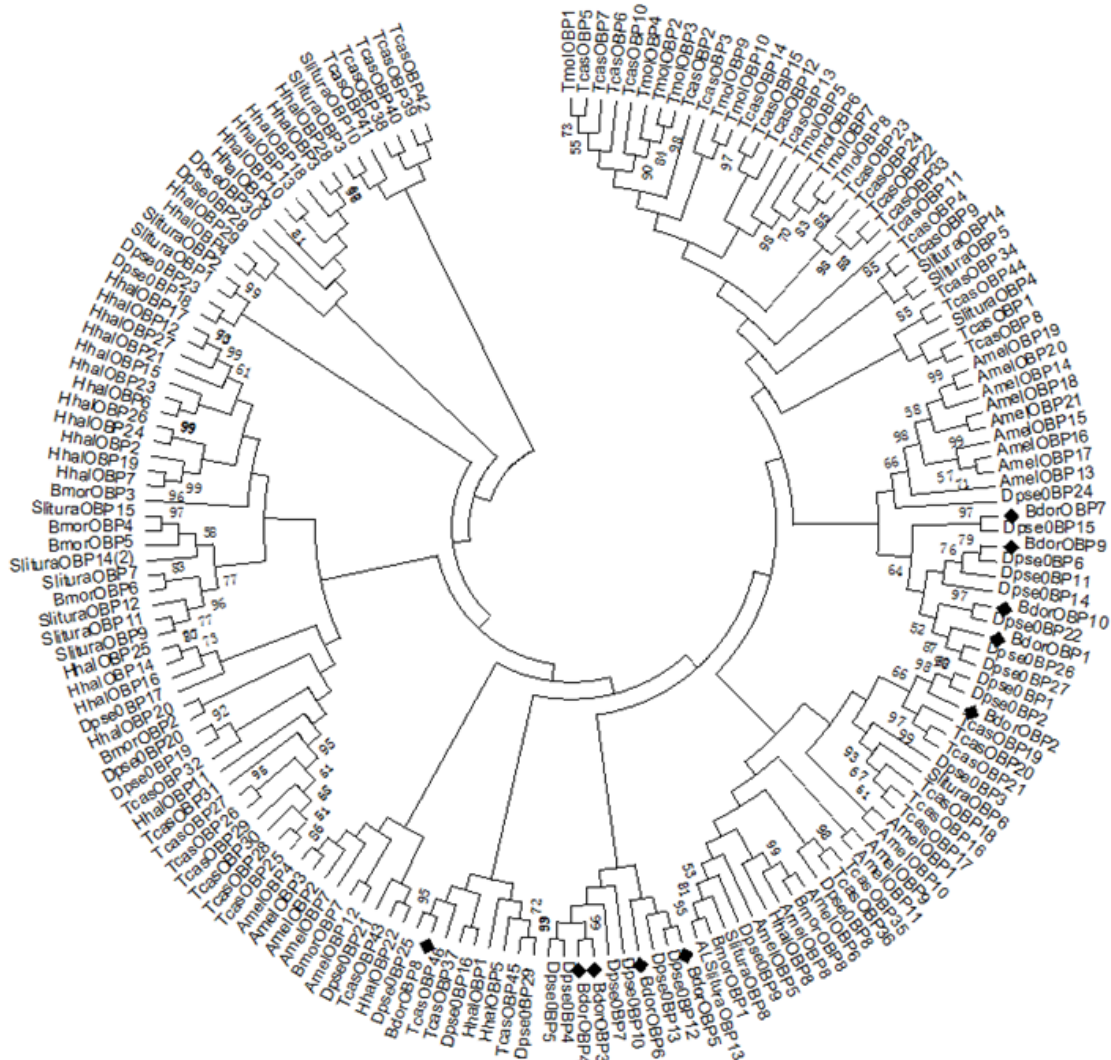


Figure 2. Unrooted phylogenetic tree of candidate OBPs from *Bdor* (*B. dorsalis*) and seven additional insect species with available amino acid sequences (*Amel*: *Apis mellifera*, *Bmor*: *Bombyx mori*, *Dpse*: *Drosophila pseudoobscura*, *Hhal*: *Halyomorpha halys*, *Slitura*: *Spodoptera litura*, *Tmor*: *Tenebrio molitor* and *Tcas*: *Tribolium castaneum*). The highly divergent signal peptides in the N-terminal region were removed, and a neighbour-joining tree was constructed by using MEGA 7 with the pairwise deletion option under the JTT empirical amino acid substitution model. Only bootstrap values above 50% are shown. The black diamonds indicate the 10 *B. dorsalis* OBP genes.

Effects of bait and torula yeast on *Bdor*OBP transcript levels in females

The qRT-PCR results revealed that all *Bdor*OBPs showed significant changes in expression in response to protein bait and torula yeast except OBP4, OBP8 and OBP9, as shown in (Figure 3a). However, *Bdor*OBP3 showed no significant expression in response to starvation (Figure 3a).

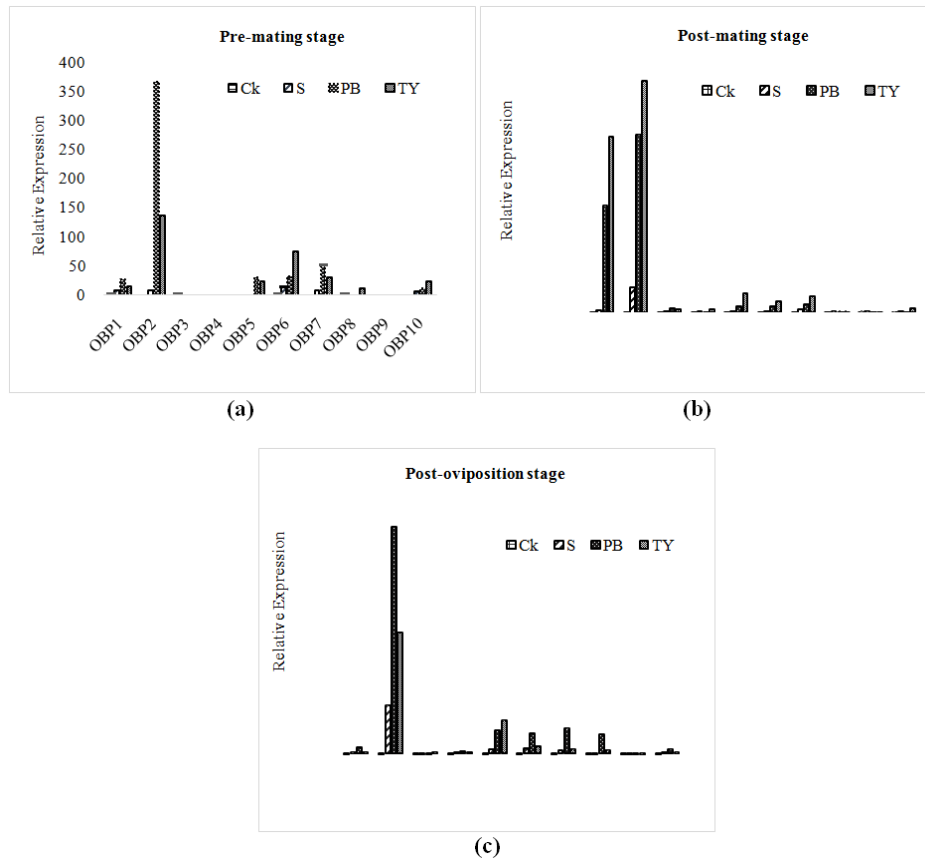


Figure 3. Transcript expression analysis by qRT-PCR of the ten *Bdor*OBP genes in the antennal tissues of mature adult females in response to protein bait and torula yeast. (a) Pre-mating stage; (b) post-mating stage; (c) post-oviposition stage. Treatments were denoted by Ck: control, S: starvation, PB: protein bait, TY: torula yeast. Analysis of variance (ANOVA) indicated a significant difference in the expression levels of OBPs at $p < 0.05$. However, OBP3 was expressed weakly under starvation treatment, and hence, ANOVA indicated that this difference was not significant (Figure 3a). The ANOVA indicated a significant difference among the expression levels of all *Bdor*OBPs ($p < 0.05$, Figure 3b), except those of OBP9 and OBP10. However, OBP6 in response to starvation and OBP8 to starvation and protein bait were not highly expressed (Figure 4b). The ANOVA indicated a significant difference ($p < 0.05$) among the expression levels of all *Bdor*OBPs (Figure 3b), while OBP1 in response to starvation and torula yeast; OBP4, OBP8, OBP9 to starvation; and OBP10 to starvation and torula yeast were not expressed significantly (Figure 3c).

The expression levels of OBP1, OBP2, OBP5, OBP6 and OBP7 were higher in insects given protein bait and torula yeast than those of OBP3 (Figure 3b). OBP6 and OBP8 exhibited no significant expression in response to starvation and protein bait treatments, respectively ($p > 0.05$, Dunnett's test) (Figure 3b). However, the

transcript levels of OBP4, OBP9 and OBP10 were not altered significantly ($p > 0.05$) in response to any of the treatments (Figure 3b). Protein bait and torula yeast increased the transcript level of OBP6, while all of the remaining OBPs showed no response to protein bait and torula yeast, as shown in (Figure 3b). The expression levels of OBP1 and OBP10 were significantly increased in the antennae of *B. dorsalis* in response to starvation and torula yeast, while those of OBP8 and OBP9 showed no response to starvation. OBP4 showed no change in expression in response to the baits at any of the tested stages (Figure 3c). These results suggest that different baits may significantly alter the transcript profiles of some BdorOBPs, which may play an important role in altering the mechanism of insect olfaction.

Effects of volatiles on BdorOBP transcript levels in females

The analysis of the volatile treatments indicated relatively low expression levels in OBP5, OBP8, OBP9 and OBP10, while the remaining BdorOBPs showed significantly higher expression in all treatments, as shown in (Figure 4a).

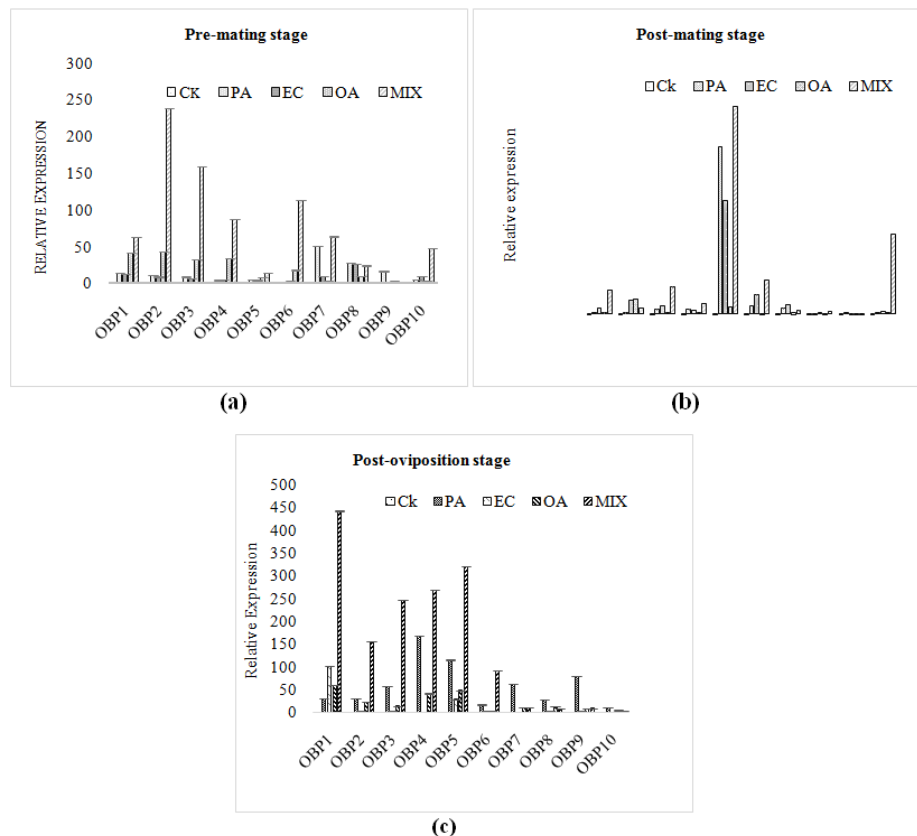


Figure 4. Transcript analysis of the ten BdorOBP genes in the antennal tissues of mature adult females in the (a) pre-mating stage; (b) post-mating stage; and (c) post-oviposition stage. Treatments were denoted by Ck: control, PA: phenyl acetate, EC: ethyl caprylate, OA: octyl acetate, MIX: mixture of three volatile components (PA + EC + OA). Relative transcript levels were calculated using β -actin as the standard. A significant difference in the expression levels of BdorOBP genes was found by Dunnett's test ($p < 0.05$). OBP6 in response to starvation (Figure 4a), OBP8 to PA and EC (Figure 4b), OBP6 to OA, and OBP10 to PA and EC were not expressed significantly (Figure 4c).

Interestingly, higher expression levels were observed in response to MIX and OA at all tested stages, as shown in (Figure 4a, 4b and 4c). Only OBP5 exhibited a significant expression change in response to all treatments, as shown in (Figure 4b). In contrast to the lower expression levels observed in the last five BdorOBPs (OBP6-OBP10), the first five BdorOBPs (OBP1-OBP5) showed significantly increased expression in response to all treatments, particularly MIX and PA, as shown in (Figure 4b). OBP6 showed no change in expression level in response to PA and OA, as shown in (Figure 4a, 4b), while OBP8 and OBP10 also showed no change in response to PA and EC, as shown in (Figure 4b, 4c).

Effects of irradiation on BdorOBP transcript levels in males

The qRT-PCR results showed that the transcript levels of the BdorOBPs changed significantly at all tested stages with a ^{137}Cs irradiation dose of 100 Gy. The expression levels of the BdorOBPs were altered significantly in response to irradiation, except those of OBP3, OBP4 and OBP7. The expression levels of OBP2, OBP5, OBP7 and OBP9 were significantly increased, as shown in (Figure 5a).

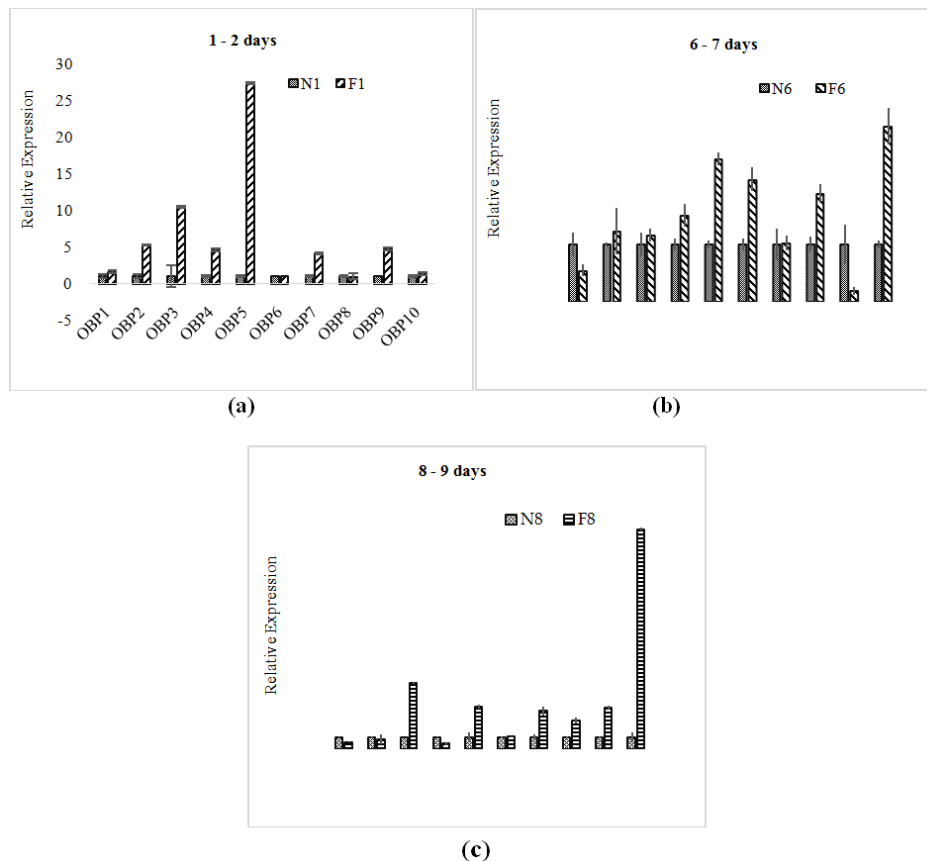


Figure 5. Transcripts of the ten BdorOBP genes in the antennal tissues of adult males at (a) 1-2 days; (b) 6-7 days; (c) 8-9 days. The treatments were denoted by N: normal male and F: irradiated (infertile) male. Relative transcript levels were calculated using β -actin as the standard. A significant difference among the expression levels of all BdorOBP genes was found at $p < 0.05$. Low expression levels were observed in OBP3, OBP4, and OBP7 (Figure 5a), while OBP1 did not show significant expression ($p > 0.05$, Figure 5b, 5c).

However, each OBP was significantly differentially expressed, except OBP1, which showed no change. OBP4, OBP5, OBP6, OBP8, and OBP10 exhibited particularly high expression in response to irradiation, as shown in (Figure 5b). OBP3, OBP5, OBP7, OBP8, OBP9 and OBP10 showed exceptionally abundant expression in contrast to the other OBPs, as shown in (Figure 5c). However, OBP1 showed no response to irradiation, as shown in (Figure 5b, 5c).

Discussion

Insects essentially depend on the antennae to distinguish plant volatiles or sex pheromones in the environment (Steinbrecht, 1997); hence, we selected *B. dorsalis* antennae to evaluate the expression profiles of 10 BdorOBP genes in response to protein baits, brewer's yeast volatiles and irradiation. Previously published papers have reported functional studies of insect OBPs in chemoreception since their discovery in 1981, but the exact functions of insect OBPs in response to different stimuli present in the environment are still unknown (Zhou, 2010; Leal, 2013). Highly developed olfactory systems are present in phytophagous insects, allowing them to use numerous chemical stimuli to identify plant hosts (Pelosi et al., 2006). The OBPs in the olfactory signal reception pathway play an essential role in the transportation of specific odorants to ORs to stimulate insect behaviours (Pelosi et al., 2006). Hence, OBPs are thought to be directly essential for odorant perception and represent potential targets for pest control via insect attraction.

B. dorsalis antennae have a strong EAG response to spent brewer's yeast volatiles (Du et al., 2015), and the results of the present study revealed that OBP2, OBP5 and OBP1, which are present in the antennae of *B. dorsalis*, were highly expressed in response to spent brewer's yeast volatiles at all tested stages. OBPs show significant attraction responses to different volatiles (Cornelius et al., 2000; Hu et al., 2007; Liu et al., 2010; Shen et al., 2011b). Another author has reported that some volatile compounds, such as benzaldehyde, showed very weak attraction effects in all adult physiological stages of female *B. dorsalis* (Ling, 2013), and the results of our study also showed that some OBPs were down-regulated, perhaps due to the weak affinity of volatiles to some OBPs. A similar phenomenon was observed in the case of ester alcohols, the major constituents of aromatic odour in most fruits, which plays an essential role in the attraction behaviour of *B. dorsalis* (Zhang et al., 2007). Tephritidae have shown significant differences in their responses to other esters, such as beer waste yeast, hydrolysates, and volatile compounds of the other 3 kinds of esters (Du et al., 2012).

Previously, thirty-four compounds were selected to investigate the interaction of OBPs with rice plant volatiles (Lou et al., 2005; Sun et al., 2013b). *Cnaphalocrocis medinalis* OBP2 (CmedOBP2) showed a significant response to host volatiles, whereas CmedOBP3 showed relatively little response to these volatiles (Sun et al., 2016b); our study found that all BdorOBP genes showed low or no response to combined volatile treatments at all tested physiological stages, except the OBP2, OBP5 and OBP1 genes, which were highly expressed. Because all insects have different OBP sequences and each OBP family might perform a different function, different OBPs can display different mechanisms in response to different kinds of volatiles present in plants and fruits (Sun et al., 2016b).

A previous report indicated that synthetic volatile compounds targeted OBP3 or OBP7, which were confirmed to be accountable for (*E*)- β -farnesene sensitivity, and provoked significant behavioural responses in aphids (Sun et al., 2012), while the results of our studies revealed that the first five BdorOBP genes (OBP1-OBP5) were significantly expressed in response to all treatments, specifically to MIX and PA, and weak expression was observed in the last five BdorOBP genes (OBP6-OBP10) in response to volatiles.

The prerequisite for the illumination of the molecular mechanism of olfaction is the documentation of the genes accountable for volatile discrimination. Candidate olfaction genes have been recognized in numerous other species through annotation and antennal transcriptome analysis. Although *B. dorsalis* olfaction research has previously lacked transcriptome data for the classical olfactory organs, specifically the antennae, several studies of the *B. dorsalis* transcriptome have recently been conducted (Shen et al., 2011b; Zheng et al., 2012a; Geib et al., 2014). However, limited studies have investigated the effects of the volatile compounds reported for control of *B. dorsalis* on OBP gene expression profiles in *B. dorsalis* and other insect pests. In light of the present findings, the fact that *B. dorsalis* males and females were captured in greater numbers in torula yeast bait traps than in protein bait traps likely reflects a lower availability of proteinaceous food in the habitats sampled by (Siderhurst and Jang, 2010; Royer et al., 2014).

Insect OBPs are responsible for the detection of host plant attractants. A single OBP may participate in the recognition of several chemical odours (Vogt et al., 1991; Pelosi et al., 2014); however, our results reported that only OBP2 was significantly expressed in response to baits at each adult physiological stage among these 10 OBP genes. On the other hand, specific compounds might be recognized by more than one OBP gene, as the results of our study revealed that few OBP genes showed significant responses to spent brewer's yeast volatiles, while others showed no response. To the best of our knowledge, our data exhibit the relationship of these ten OBP genes to spent brewer's yeast volatiles, protein bait and irradiation for the first time.

The combination of different OBPs in insects may form a molecular basis for the ability to identify and distinguish relevant olfactory cues amid the vast spectrum of odorants emitted from fruits or plants (Takken and Knols, 1999; Zwiebel and Takken, 2004). In our study, some OBPs showed the same response to MIX treatment and were highly expressed at all physiological stages. This result further supports the view that OBPs are not just simple, general solubilizers that act as transporters for odorants but also play a vital role in the perception of different kinds of chemicals in the environment (Pelosi et al., 2006; Swarup et al., 2011). An OBP in *Hylamorpha elegans* showed a high binding affinity to β -ionone (Venthur et al., 2016), a volatile compound derived from plant carotenoids (Simkin et al., 2004).

Most of the OBP genes showed significant responses to MIX treatment at all adult life stages (*Figure 4a, 4b and 4c*). Irradiation has been reported to significantly destabilize fly fitness by damaging tissues and interior organs to some degree, inducing metabolic disorders (Lauzon and Potter, 2012; Yao et al., 2017), and our results showed that irradiation significantly altered the transcript levels of OBP genes at all adult life stages (*Figure 5a, 5b and 5c*), with especially abundant expression in the second and third stages (*Figure 5b, 5c*). These significant observations suggest that the effects of applied treatments on the transcript levels of BdorOBP genes may play an essential role in understanding the insect olfaction mechanism in response to different kinds of

protein baits, volatiles released by different kinds of plants in the environment and irradiation applied to insects at the pupal stage.

Our results provide a direct BdorOBP transcript analysis that represents new evidence for the role of olfactory proteins in the reception of protein baits and volatiles. We believe that these findings establish a foundation for understanding the molecular mechanisms by which OBPs contribute to olfactory recognition and have applications for integrated pest management techniques. However, an urgent need exists to identify and characterize the remaining ten OBP genes of *B. dorsalis* in order to understand the transcriptional regulation of all OBPs in response to protein baits, volatiles and irradiation. The functional study of antennae-specific or antennae-enriched OBPs can assist us to better understand the involvement of OBPs in the mechanisms of insect olfaction with respect to different environmental stimuli and to design an odorant-based insect control strategy for the management of *B. dorsalis*.

Conclusions

Since its arrival in mainland China, *B. dorsalis* has become one of the most destructive pests in orchards. Furthermore, this pest is presently spreading to more suitable areas of North China. The use of chemical insecticides remains the foremost way to control *B. dorsalis* in most developed countries, especially in China. In previous decades, various approaches have been established to monitor its detection and dispersal in order to minimize damage, and many studies have been carried out to interpret different aspects of its invasion mechanism. Further studies are still needed to understand its adaptation to new environments, resistance to chemicals, and high procreative ability. For the first time, we have evaluated the effects of different kinds of attractive baits, brewer's yeast volatiles and irradiation on the transcript expression levels of 10 OBP genes in the antennae of *B. dorsalis* during three adult physiological stages. Some BdorOBP genes are significantly up-regulated in response to the applied treatments, and others may play a vital role in mediating the response of males to irradiation, while OBP genes in females showed high transcript levels in response to different baits and brewer's yeast volatiles.

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