Genome-Wide Identification of Aldehyde Oxidase Genes in Moths and Butterflies Suggests New Insights Into Their Function as Odorant-Degrading Enzymes

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INTRODUCTION

The study of gene evolution in insects has provided outstanding advances in the understanding of evolutionary processes, such as expansion or contraction of gene families (Li et al., 2019). Particularly, lepidopterans represent an extraordinary target due to a clear diversification into moth and butterflies lineages (Kawahara et al., 2019). Thus, the impact of gene evolution can be seen
even within moths. For instance, regulation of desaturase genes in two sibling *Helicoverpa* species (i.e., *H. armigera* and *H. assulta*) results in reproductive isolation (Li et al., 2015). Nowadays, the enormous amount of genomic and transcriptomic datasets for insects has provided an opportunity to elucidate novel genes and their evolutionary relationships (Oppenheim et al., 2015), something that can support our understanding of ecological aspects of insects, such as behavior. For many insect species, behavior is mainly driven by olfaction. Olfaction is primarily processed by insect antennae and their small hair-like structures called sensilla, in which a set of proteins work synergistically to maintain an extremely sensitive and dynamic system (Hansson and Stensmyr, 2011; Leal, 2013; He et al., 2019). For instance, odorant-binding proteins (OBPs) and chemosensory proteins (CSPs) function as transporters that carry odorants across the sensillar lymph (Zhou, 2010; Leal, 2013). These odorants reach an heteromeric complex of receptors, such as odorant receptors (ORs), an odorant receptor co-receptor (Orco) and a sensory neuron membrane protein (SNMP), as recently reported (Zhang et al., 2020), to unleash depolarization in olfactory neuron membranes that triggers a behavioral response (Kässel, 2013). Along with these olfactory proteins, odorant-degrading enzymes (ODEs), such as carboxylesterases (CXEs), glutathione-S-transferases (GSTs) and aldehyde oxidases (AOXs), are responsible for resetting the insect olfactory system through the degradation of odorant molecules (Chertemps and Meibèche, 2021; Godoy et al., 2021).

Among ODEs, CXEs and GSTs have received particular attention due to their role in sex pheromone degradation in moths. For example, CXEs have been reported to degrade ester-type molecules (e.g., sex pheromones and plant volatiles) in moths *P*loidia interpunctella, *Spodoptera exigua*, *Grapholita molesta*, *Plutella xylostella*, and *Athetis lepigone* (He et al., 2014a,b,c, 2015; Zhang et al., 2017a; Liu et al., 2019; Wei et al., 2020; Wang et al., 2021a). Likewise, GSTs have been well characterized in terms of function, being the delta class likely related to odorant degrading functions (Durand et al., 2018). This is supported by the reported degrading function of *G. molesta* GST (GmolGSTd1) (Li et al., 2018) and *Cydia pomonella* GST (CpomGSTd2) (Huang et al., 2017). The AOXs, on the other hand, have received less attention so far. However, some species that use aldehydes as semiochemicals (i.e., chemicals that mediate communication between two organisms), have been studied, such as *Manduca sexta*, *Bombyx mori*, *Antheraea polyphemus*, *Amyelois transella*, and *H. armigera*, among others (Riddiford, 1967; Kasang et al., 1978; Coffelt et al., 1979; Zhang et al., 2012). Particularly, AOXs catalyze the oxidation of aldehydes to carboxylic acids (Garattini et al., 2009; Garattini and Terao, 2012). In that sense, a few studies have functionally evaluated that process against aldehyde-based semiochemicals: an early study in *M. sexta* AOX reports that it catalyzes (E/Z)-10,12-hexadecadien-1-ol (bombykal) (Ryczynski et al., 1989), and more recently, *A. transella* AOX2 (AtraAOX2) was reported to hydrolyze plant volatiles (e.g., propanal, hexanal, and heptanal) as well as a sex pheromone component (Z,Z)-11,13-hexadecadienial (Choo et al., 2013). Further evidence in terms of enzymatic activity of AOXs is still lacking. Nevertheless, important aspects of their function and structural features are underpinned by xanthine dehydrogenases (XDHs), an enzyme that catalyzes the oxidation of purines, pterin and aldehydes (Wang et al., 2016).

Among insects, lepidopterans have attracted special attention due to their establishment as crop pests, some with worldwide distribution. It is known that moths rely heavily on the sense of smell (Weiss, 2001), developing long distance attraction based on volatile chemicals (e.g., sex pheromones) (Chemnitz et al., 2015). In fact, hundreds of these volatiles have been identified since the first one reported for *B. mori*, the sex pheromone (E,Z)-10,12-hexadecadien-1-ol (bombykol) (Butenandt et al., 1959). On the contrary, butterflies rely heavily on visual cues and short-range chemical communication, understood as a multisensory integration (Costanzo and Monteiro, 2007), and hence have received less attention in terms of olfaction. Moreover, butterflies represent an interesting group for comparative studies considering their transition from moths approximately 98 Mya (million years ago) (Kawahara et al., 2019). Thus, it is believed that comparing AOXs between moths and butterflies might deepen our understanding of their odorant-degrading function.

Considering the difference in olfactory integration during the life cycle of moths and butterflies, we hypothesize that there is a specific clade of AOXs for both moths and butterflies that could be related to odorant-degrading function as well as both moth- and butterfly-specific gene expansions. Therefore, the objective of this study was to identify novel AOX genes from moths and butterflies using genomic and transcriptomic data and analyze them in terms of gene location, phylogeny, evolutionary processes, and structure.

### MATERIALS AND METHODS

#### Data Collection

Publicly available genomic data were retrieved from NCBI Genome database and InsectBase for major lepidopteran families, such as Bombycidae, Sphingidae, Noctuidae, Pyralidae, Crambidae, and Plutellidae for moths, whereas Nymphalidae, Pieridae and Papilionidae were used for butterflies (Table 1). Each fully represented genome assembly with Reference Sequence (RefSeq) was downloaded from NCBI Assembly database at either contig, scaffold or chromosome level (Supplementary Table 1).

#### Identification of Aldehyde Oxidase Family

Bioinformatics pipeline BITACORA (Vizueta et al., 2020) was used to identify already annotated AOX genes and potentially novel related genes from both moth and butterfly genomes. A database for AOX gene family was built using reported protein sequences for lepidopterans (Ryczynski et al., 1989; Merlin et al., 2005; Pelletier et al., 2007; Choo et al., 2013; Ou et al., 2014; Zhang et al., 2014, 2017b; Yang et al., 2015; Huang et al., 2016; He et al., 2017; Xu and Liao, 2017; Wang et al., 2021b).

1. [https://www.ncbi.nlm.nih.gov/assembly](https://www.ncbi.nlm.nih.gov/assembly)

2. [http://www.insect-genome.com/](http://www.insect-genome.com/)
To identify family and structural domains for AOXs, InterPro server was used. The identified profile was used to search for HMM profile in PFAM database\(^4\) (PF01315; ID Ald_Xan_dh_C). This process increased the likelihood of identifying sequences encoding members of the AOX gene family. Further processing included the trimming of isoforms (98% cutoff) using a provided script in BITACORA pipeline. Subsequently, BLAST searches were run with the identified proteins for manual annotation. Protein domain finder CDvist\(^2\) (Adebali et al., 2015) was used to identify conserved domains of AOXs, namely two (2Fe-2S), one flavin-containing region (FAD-binding domain) and one molybdenum cofactor/substrate-binding domain. All proteins identified in this study are provided in Supplementary Table 1.

**Sequence Analysis and Genome Structure**

The genomic organization of identified AOX genes from both moth and butterfly species that use aldehyde-based semiochemicals was analyzed based on Vogt et al. (2015) and Xu and Liao (2017) including some modifications. Moths *Bombyx mandarina*, *P. xylostella*, and *A. transitella*, and butterflies *Heliconius melpomene* and *Bicyclus anynana*, were selected for this task. Annotated gene features (in GFF3 format) were retrieved from the gene identification protocol based on the BITACORA pipeline and analyzed manually. Thus, species name as well as source, start, end, strand and attributes were used for each AOX gene. Finally, gene localization was prepared in image editor Inkscape 0.48 software.

**Data Preprocessing and Transcriptome Assembly**

To both take advantage of transcriptomic data and include Tortricidae and Eriocrianiidae families, we retrieved antennal RNA-seq data for moth *Lobesia botrana* (data from our laboratory) and non-ditrysian moth *Eriocria semipurpurella* (SRR5328787). FASTQ files for both moths, one containing left-pair reads and other the right-pair reads, were used for assembly. Ribosomal RNA reads were removed by mapping the libraries using Bowtie2 v.2.3.3.1 (Langmead et al., 2009) against a custom rRNA database created from insect ribosomal sequences downloaded from NCBI\(^5\), and keeping non-mapped reads using SAMtools v.1.6 (Li et al., 2009). Low-quality reads were removed based on their q-score composition using NGSQC Toolkit v.2.3 (Patel and Jain, 2012), and high-quality reads were concatenated to build *de novo* transcriptomes using Trinity v.2.6.5 (Grabherr et al., 2011) with a P-value of 0.05 and fold-change value of 2.

**Phylogenetic Analysis**

A phylogeny for the identified AOX genes in moths and butterflies, including XDHS and AOXs from mosquitoes, beetles and bees as outgroups, was built. Full-length amino acid sequences that include conserved domains were aligned using MAFFT server\(^7\) (Katoh et al., 2019). GUIDANCE2 server\(^8\) was used to check consistency of the multiple sequence alignment (Sela et al., 2015). Briefly, the consistency of the alignment was measured with a score less than 0.5, in which sequences were deleted. It is worth noting that confidence scores near 1 and 0, suggest a highly and poorly consistent alignment, respectively. Finally, phylogenetic analysis was performed using maximum-likelihood method with FastTree software (Price et al., 2010). To highlight clades, specific taxa and functional evidence, the phylogenetic tree was edited using FigTree software\(^9\) and image editor Inkscape 0.48 software.

**Molecular Evolution Analysis**

In order to identify putative selective pressures on AOXs, a molecular evolution analysis was performed based on the methodologies reported by Engsontia et al. (2014) and Soffian et al. (2018). Two models were used through EasyCodeML software (Gao et al., 2019) to elucidate selective pressures acting on the evolutionary process of 93 lepidopteran AOX genes, 9 XDHS and 11 AOXs from other insect orders. First, site model was applied to detect positive selection for a set of 113 sequences (Yang et al., 2000). Additionally, a branch-site model

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\(^1\)http://www.ebi.ac.uk/interpro/
\(^2\)http://pfam.xfam.org/
\(^3\)http://cdvist.zhulinlab.org/
\(^4\)https://www.ncbi.nlm.nih.gov/
\(^5\)https://mafft.cbrc.jp/alignment/server/
\(^6\)http://guidance.tau.ac.il/ver2/
\(^7\)http://tree.bio.ed.ac.uk/software/figtree/
\(^8\)http://cdvist.zhulinlab.org/

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**TABLE 1** | Summary of identified aldehyde oxidase genes in moths and butterflies.

| Species  | AOX gene annotation\(^a\)^\(^b\) | Total | Novel | Complete CDS | Average length (aa) | Gene annotation\(^c\) |
|----------|----------------------------------|-------|-------|--------------|--------------------|-------------------|
| **Moths**|                                  |       |       |              |                    |                   |
| *B. mandarina* |                                | 7     | 6     | 7            | 1,272              | 1                 |
| *B. mori*    |                                | 9     | 6     | 9            | 1,266              | 3                 |
| *M. sexta*   |                                | 19    | 16    | 16           | 1,194              | 3                 |
| *H. armigera*|                                | 8     | 2     | 8            | 1,335              | 6                 |
| *S. frugiperda* |                              | 20    | 17    | 19           | 1,259              | 3                 |
| *O. fumacalis* |                              | 8     | 2     | 8            | 1,298              | 6                 |
| *P. xylostella* |                             | 6     | 3     | 5            | 1,271              | 3                 |
| *A. transitella* |                             | 6     | 3     | 6            | 1,349              | 3                 |
| *T. ni*      |                                | 14    | 3     | 11           | 1,209              | 11                |
| **Butterflies**|                              |       |       |              |                    |                   |
| *H. melpomene* |                              | 2     | 2     | 2            | 2,523              | 0                 |
| *P. rapae*   |                                | 6     | 2     | 6            | 1,301              | 4                 |
| *B. anynana* |                                | 11    | 4     | 10           | 1,282              | 7                 |
| *D. plexippus* |                              | 10    | 10    | 7            | 1,215              | 0                 |
| *V. taneamea* |                              | 6     | 5     | 5            | 1,243              | 1                 |
| *P. aegeria* |                                | 9     | 6     | 8            | 1,327              | 6                 |
| *P. polyes*  |                                | 6     | 0     | 6            | 1,231              | 6                 |
| *P. xuthus*  |                                | 7     | 4     | 7            | 1,265              | 4                 |
| *P. machaon* |                                | 7     | 0     | 6            | 1,281              | 7                 |

\(^a\) Complete gene annotation available in Supplementary Table 1.
\(^b\) A complete annotation can be found in Supplementary Table 1.
\(^c\) Based on Interpro, NCBI Gene database and literature searches.

was applied to test the presence of amino acids that evolved under positive selection in a specific clade represented by 8 AOX sequences (most of them functionally studied). All the amino acid sequences were aligned by ClustalW\textsuperscript{10}, and converted to DNA alignment with PAL2NAL server\textsuperscript{11}. A maximum likelihood tree was prepared using the DNA alignment by FastTree software under default parameters. Briefly, the software estimated the ratio of normalized non-synonymous (\(d_N\)) to synonymous (\(d_S\)) (e.g., \(d_S/d_N \) or \(\omega\)) substitution rate via the maximum likelihood method. The \(\omega\) value indicates the mode of evolution, where \(\omega > 1\) suggests evidence of positive selection with amino acid replacement, whereas \(\omega < 1\) refers to purifying selection, and \(\omega = 0\) indicates neutral selection. The specific models (M0, M3, M1a, M2a, M7, M8, and M8a) used under the “site model” method are described in detail in previous reports (Yang et al., 2000; Yang and Nielsen, 2002; Swanson et al., 2003). For the branch-site model, the 8 AOX sequences were labeled in the phylogenetic tree as foreground branch with the remaining clades as background branches. The change in \(\omega\) was evaluated for a set of sites in each foreground branch through an alternative model, whereas neutral evolution was evaluated through a null model. Likelihood ratio tests (LRTs) were used to compare both models and significant results were determined using \(\chi^2\)-tests. Finally, Bayes Empirical Bayes (BEB) analysis was used when LRT was significant to identify positive selected sites (PSSs) within each amino acid sequence (Yang et al., 2005).

Sequence Analysis and Protein Structure Prediction

First, a multiple sequence alignment (MSA) was built with 7 AOX sequences also used in molecular evolution analyses, belonging to A. transistella, B. mori, P. xylostella, H. armigera, Papilio xuthus, Papilio machaon, and B. anynana, in which PSSs were identified and indicated (Supplementary Table 1). Sequences from B. mandarina AOX5 (BmAOX5), Drosophila melanogaster AOX2 (DmelAOX2) and mammal AOXs, such as Mus musculus AOX2 (MmAOX2) and AOX3 (MmAOX3), and Homo sapiens AOX1 (HsAOX1), were also included. MSA was built in Multalin server\textsuperscript{12} and ESPript 3.0\textsuperscript{13} (Corpet, 1988). The amino acid sequence of AtraAOX2 and BanyAOX2 were submitted to BLASTp available on the NCBI website\textsuperscript{14} for template selection. To optimize the structural information available for AOXs, a multiple template-based homology modeling approach was considered as it was reported to increase accuracy in predicted protein models (Sokkar et al., 2011). First, multiple structure alignments were generated by SALIGN command, which is implemented in Modeler 10.1. Five hundred models of each AOX were obtained using Modeler 10.1\textsuperscript{15}. The best models were selected according to the lowest discrete optimized protein energy (DOPE) score provided by the software. The coordinates were analyzed via ProCheck\textsuperscript{16} to check stereochemical quality. Lepidopteran AOXs were visualized through PyMOL software\textsuperscript{17}. The 3D structure of mammal AOXs were retrieved from Protein Data Bank\textsuperscript{18} and DmelAOX2 was downloaded from AlphaFold database\textsuperscript{19}.

RESULTS

Identification and Annotation of Aldehyde Oxidase Genes

Eighteen genome assemblies were retrieved from NCBI assembly database and InsectBase server. From those, 6 were assembled at chromosome level, whereas 11 at scaffold and 1 at contig level (Supplementary Table 1). The use of BITACORA pipeline resulted in a raw amount of 163 putative AOX genes for moths and 100 for butterflies. After homology searches through BLAST followed by conserved domain analyses, 99 AOX genes were left for moths and 65 for butterflies. The average amino acid length of AOXs is 1272 and 1407 for moths and butterflies, respectively. On the other hand, the moth species that showed a higher number of AOX were Spodoptera frugiperda with 20 sequences, M. sexta with 19 sequences and Trichoplasia ni with 14. In butterflies, B. anynana and Danaus plexippus showed 11 and 10 AOX sequences, respectively. The specific number of AOX genes for each species is summarized in Table 1. Overall, 58 novel AOX genes were identified for moths whereas 33 AOX genes were identified for butterflies. BLAST hits for most of the novel AOX genes were either AOXs from other lepidopteran species or XDHs from the same species. In that sense, 6 new AOXs were identified for B. mandarina and B. mori, 16 for M. sexta, 17 for S. frugiperda and 10 for D. plexippus, as the greater numbers found. No novel AOX genes were found for butterflies Papilio polytes and P. machaon. Although most of the lepidopteran species studied here have a few annotated AOX genes, several of them are not fully annotated nor studied in terms of function. It is worth noting that the amount and length of AOX genes might be dependent on genome sequencing and annotation quality, therefore, previous estimates should be taken into account with caution.

Phylogenetic Relationships and Gene Clusters Between Moths and Butterflies

Phylogenetic analysis (Figure 1) suggests the presence of 5 main clades, 2 being related to either antennae specificity or odorant degradation (clades A and B in Figure 1). A clear diversification from insect XDHs and non-lepidopteran AOXs is observable. A clade with putative odorant-degrading function (labeled as A in Figure 1) appears to be a group of AOX genes evolved in ditrysian species, having the non-ditrysian moth E. semipurpurella EsemAOX1 at the base of the clade. Here,
9 moth AOX genes are reported to be enriched in antennae (Figure 1, species indicated with black circles next to their name), from which only *M. sexta* AOX1 (MsexAOX1) has been related to aldehyde-degrading function (Rybczynski et al., 1989). A secondary odorant degrading-related clade (labeled as B in Figure 1) seems to have evolved by gene duplication. This clade includes 5 moth AOX genes enriched in antennae (Figure 1, species indicated with black circles next to their name), and includes *A. transitella* (AtraAOX2), *P. xylostella* (PxylAOX3), and *B. mori* (BmorAOX5) which were functionally studied (Choo et al., 2013; Zhang et al., 2020; Wang et al., 2021b). Furthermore, butterfly- and moth-specific AOX lineages were identified (highlighted in red and gray, respectively, in Figure 1), but no reported odorant-degrading function was found for these. Interestingly, butterflies that have aldehyde-related pheromones have AOXs present in at least one of the odorant-related clades (A or B). There are AOXs of some butterflies that are in these clades, but no aldehyde-related semiochemical
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has been reported yet, such as those present in D. plexippus, Pararge aegeria, Pieris rapae, P. polytes, P. xuthus, P. machaon, and Vanessa tameamea. On the contrary, H. melpomene, that uses aldehyde-based semiochemicals, showed only 1 AOX (HmelAOX2) in clade A. Likewise, B. anynana (with hexadecanal as a pheromone component), has 2 AOXs in clade A, while 5 in clade B.

In terms of gene location, B. mandarina, A. transitella and P. xylostella, that use aldehydes as semiochemicals and have AOX genes related to ODE function, are far from other AOX genes, with the exception of PxylAOX3 (Figure 2). H. melpomene has 2 grouped AOX genes that suggest the same origin for both. Likewise, B. anynana has two big clusters of 4 and 7 AOX genes. Interestingly, from the bigger cluster of B. anynana, the 7 AOX genes were distributed in odorant-degrading clades A and B. Similarly, AOX1, AOX2 and AOX5 of B. mandarina that are grouped in a single cluster, are distributed in clades A and B. Besides HmelAOX2 present in clade A, gene HMEL011718-PA clustered with HmelAOX2, which appeared in the previously mentioned butterfly-specific clade (red clade in Figure 1).

**Selective Pressures on Aldehyde Oxidase Genes**

Positive selection was first evaluated for a set of 113 sequences that included XDHs and AOXs of not only butterflies and moths, but also beetles, mosquitoes, and flies (Table 2). The four models implemented (e.g., M3 vs. M0, M1a vs. M2a, M7 vs. M8 and M8a vs. M8) showed significant differences according to LRT analysis. Interestingly, a purifying selection was suggested as site model (M0) resulted in $\omega = 0.89$.

Additionally, a branch-site model was used to test selective pressures on specific sites (i.e., codons) among 8 closely related AOX sequences, including moths A. transitella AtraAOX2, B. mori BmorAOX5, P. xylostella PxylAOX3, H. armigera HarmAOX2 and Sesamia inferens SinfAOX3, and butterflies B. anynana BanyAOX2, P. xuthus PxutAOX2 and P. machaon PmacAOX2 (Table 3). As expected, most of the enzymes were found to be under positive selection at many sites. For instance, AtraAOX2 resulted in 23.5% of their amino acids as PSSs, from which 105 sites showed either $P < 0.01$ or $P < 0.001$. Similarly, 22.2% of residues in BmorAOX5, 11.9% in HarmAOX, and 11.2% in SinfAOX3 were PSSs, with more than 20 sites identified with $P < 0.001$. In terms of butterflies, BanyAOX2 resulted in PSSs distributed in 40% of the entire sequence. However, less PSSs resulted for PxutAOX2 and PmacAOX2, representing only a 2–3% of the amino acid sequence length.

**Link Between Function, Primary Sequence, and Protein Structure**

To complement our previous methods that included annotation, phylogeny and molecular evolution analyses, a MSA was built followed by AOX structure prediction. The MSA was based on

![Gene location analysis for AOXs identified from lepidopterans that use aldehydes as semiochemicals, such as moths B. mandarina, A. transitella, P. xylostella and butterflies H. melpomene and B. anynana.](image-url)
the same 8 lepidopteran AOX sequences detailed above, as well as *B. mandarina* BmanAOX5, *D. melanogaster* DmelAOX2, and mammal AOXs *M. musculus* MmAOX2 and MmAOX3, and *H. sapiens* HsAOX1, which have been well characterized in terms of structural features and active sites.

As reference, the active site of MmAOX3 comprise Gln772, Glu1266, Lys889, Phe919 and Phe1014 (Terao et al., 2020). From those, Gln772 and Glu1266 at positions equivalent to 739 and 1209 in Figure 3, were found to be conserved between all AOXs (red triangles in Figure 3). Interestingly, Phe919 (in vertebrates) at position 884 (in Lepidoptera) in Figure 3 was conserved among mammal AOXs, but replaced by Pro in all lepidopteran AOXs as well as DmelAOX2 (blue triangle in Figure 3). Lys889 (at position 855 in Figure 3) was also not conserved, with SinfAOX3, HarmAOX2, AtraAOX2, PmacAOX2, PzylAOX3 and PzylAOX3 having Gly, and BmorAOX5, BmanAOX5 and BanyAOX5 having Ser instead (purple triangle in Figure 3).

In terms of structure, we could predict the 3D arrangements for AtraAOX2 and BanyAOX2, which were used to corroborate the identified conserved residues at the active site (Figure 4). The active sites equivalent to MmAOX3 Gln772 and Glu1266 were identified in both Lepidoptera species as well as in DmelAOX2 and vertebrate HsAOX1. Differences in conformation were observed, which are found in large structures that have not been relaxed through molecular dynamics. Nevertheless, our results are consistent with residue locations, supporting, for instance, the role that the insect specific Pro884 plays in the active site.

### DISCUSSION

In this study we identified a total of 164 AOX sequences from both moths and butterflies. In the context of an increasing amount of data from genomic studies, we have taken advantage of publicly available genome assemblies to identify and analyze AOX gene families in Lepidoptera. Particularly, AOXs are metal-containing enzymes that metabolize aldehydes into their corresponding carboxylic acids and other sub products (Krenitsky et al., 1972). Their role in insect chemosensation has been studied since 1989 when *M. sexta* AOX (MsexAOX1) was reported to catalyze (E,Z)-10,12-hexadecadienal (bombykal), the sex pheromone of this species (Rybczynski et al., 1989). However, reports about insect AOXs and their function toward aldehydes took more than 20 years to be published again, when *A. transitella* AOX2 (AtraAOX2) was comprehensively studied (Choo et al., 2013).

Although AOX genes have been related to metabolism of xenobiotics in mammals as well as in *Culex* mosquitoes (Hemingway et al., 2000; Coleman et al., 2002; Terao et al., 2020), recent efforts have been focused on insect AOXs that can act as ODEs in olfactory organs, such as antennae and maxillary palps. Here, we report a profile of sequences related to AOX gene family that provides new data sets for several lepidopterans (Table 1 and Supplementary Table 1). For instance, our analyses revealed 5 full-length and 1 partial AOX sequences for *P. xylostella*, including the only identified AOX so far (PxlAOX3) (Wang et al., 2021b). Similarly, 9 full-length sequences were identified for *B. mori*, including BmorAOX1, BmorAOX2, and BmorAOX5, the only AOXs reported so far (Pelletier et al., 2007; Zhang et al., 2020). Likewise, we report 2 new AOXs for *H. armigera*, in which 6 AOXs had previously been reported, including HarmAOX2, suggested to be a candidate pheromone-degrading enzyme (Xu and Liao, 2017). For butterflies, no AOX-related studies have been published to our knowledge. Hence, this study would be the first to report such enzymes in this group.

In terms of number of AOXs identified in both moths and butterflies, it is interesting to notice that generalist moth species, such as *S. frugiperda*, *M. sexta* and *T. ni* resulted in the highest number of AOXs. However, we could not establish a direct relationship between number of AOXs and the condition of generalist vs. specialist species, something that has been proposed for other chemosensory proteins, such as ORs (Venthur and Zhou, 2018). Thus, we can highlight that, overall, moths resulted in a similar number of AOXs compared with butterflies, excluding *S. frugiperda*, *M. sexta*, and *T. ni*. On the one hand, the amount of identified AOXs could have been determined by the unavailability of well-assembled genomes in both moths and butterflies. On the other hand, this can also be explained because moths are largely dependent on chemosensation (at short and long range) whereas butterflies use pheromones for short range communication and visual cues (Costanzo and Monteiro, 2007). In that sense, it can be suggested that butterflies have some AOXs related to odorant degradation and to a lesser extent for the metabolism of xenobiotics. This assumption is supported by our phylogenetic analysis, where two clades related to odorant-degrading function showed the presence of both moth and butterfly AOXs.
### TABLE 3 | Positive selection analysis using branch-site model on 8 lepidopteran AOX sequences.

| Model          | np  | LnL                  | Estimates of parameters | LRT p-valuea | PSSb |
|----------------|-----|----------------------|-------------------------|--------------|------|
| **Branch-site model** |     |                      |                         |              |      |
| #1 AtraAOX2    | 18  | -19563.928883        | p0:                     | 0.000E + 0.00 |      |
|                |     |                      | 0.34727; p1:           |              |      |
|                |     |                      | 0.47396; p2a:          |              |      |
|                |     |                      | 0.07560; p2b:          |              |      |
|                |     |                      | 0.10317; ω0:           |              |      |
|                |     |                      | 0.06748; ω1: 1.0000000; ω2a: |              |      |
|                |     |                      | 0.06748; ω2b: 1.000000 |              |      |
|                |     |                      | ω0: 0.10317; p2b: 0.07560; p0: 0.06748; ω2b: 1.000000 |              |      |
|                |     |                      | ω0: 0.07560; p2b: 0.10317; p0: 0.06748; ω2b: 1.000000 |              |      |
| #2 PxyAOX3     | 18  | -19576.412306        | p0:                     | 0.000E + 0.00 |      |
|                |     |                      | 0.36546; p1:           |              |      |
|                |     |                      | 0.47336; p2a:          |              |      |
|                |     |                      | 0.07023; p2b:          |              |      |
|                |     |                      | 0.09096; ω0:           |              |      |
|                |     |                      | 0.07760; ω1: 1.0000000; ω2a: |              |      |
|                |     |                      | 0.07760; ω2b: 1.000000 |              |      |
|                |     |                      | ω0: 0.09096; p2b: 0.07760; p0: 0.07760; ω2b: 1.000000 |              |      |
| #3 BmorAOX5    | 18  | -19563.931580        | p0:                     | 0.000E + 0.00 |      |
|                |     |                      | 0.34710; p1:           |              |      |
|                |     |                      | 0.47328; p2a:          |              |      |
|                |     |                      | 0.07599; p2b:          |              |      |
|                |     |                      | 0.10362; ω0:           |              |      |
|                |     |                      | 0.06797; ω1: 1.0000000; ω2a: |              |      |
|                |     |                      | 0.06797; ω2b: 1.000000 |              |      |
|                |     |                      | ω0: 0.10362; p2b: 0.06797; p0: 0.06797; ω2b: 1.000000 |              |      |
| #4 HarmAOX2    | 18  | -19623.819643        | p0:                     | 0.000E + 0.00 |      |
|                |     |                      | 0.39374; p1:           |              |      |
|                |     |                      | 0.51575; p2a:          |              |      |
|                |     |                      | 0.03918; p2b:          |              |      |
|                |     |                      | 0.05133; ω0:           |              |      |
|                |     |                      | 0.08015; ω1: 1.0000000; ω2a: |              |      |
|                |     |                      | 0.08015; ω2b: 1.000000 |              |      |
|                |     |                      | ω0: 0.05133; p2b: 0.08015; p0: 0.08015; ω2b: 1.000000 |              |      |
| #5 SinfAOX3    | 18  | -19633.400356        | p0:                     | 0.000E + 0.00 |      |
|                |     |                      | 0.39394; p1:           |              |      |
|                |     |                      | 0.52825; p2a:          |              |      |
|                |     |                      | 0.03324; p2b:          |              |      |
|                |     |                      | 0.04457; ω0:           |              |      |
|                |     |                      | 0.07706; ω1: 1.0000000; ω2a: |              |      |
|                |     |                      | 0.07706; ω2b: 1.000000 |              |      |
|                |     |                      | ω0: 0.04457; p2b: 0.07706; p0: 0.07706; ω2b: 1.000000 |              |      |
| #6 BanyAOX2    | 18  | -19536.056864        | p0:                     | 0.000E + 0.00 |      |
|                |     |                      | 0.34728; p1:           |              |      |
|                |     |                      | 0.44882; p2a:          |              |      |
|                |     |                      | 0.08922; p2b:          |              |      |
|                |     |                      | 0.11518; ω0:           |              |      |
|                |     |                      | 0.06915; ω1: 1.0000000; ω2a: |              |      |
|                |     |                      | 0.06915; ω2b: 1.000000 |              |      |
|                |     |                      | ω0: 0.11518; p2b: 0.06915; p0: 0.06915; ω2b: 1.000000 |              |      |
| #7 PxytAOX2    | 18  | -19667.749019        | p0:                     | 0.000E + 0.00 |      |
|                |     |                      | 0.33543; p1: 0.44553; p3a: |              |      |
|                |     |                      | 0.09408; p2b: 0.12496; ω0: |              |      |
|                |     |                      | 0.06042; ω1: 1.0000000; ω2a: |              |      |
|                |     |                      | 0.06042; ω2b: 1.000000 |              |      |
| #8 PrmacAOX2   | 18  | -19668.210787        | p0:                     | 0.000E + 0.00 |      |
|                |     |                      | 0.42216; p1:           |              |      |
|                |     |                      | 0.54813; p2a:          |              |      |
|                |     |                      | 0.01293; p2b:          |              |      |
|                |     |                      | 0.01679; ω0:           |              |      |
|                |     |                      | 0.07895; ω1: 1.0000000; ω2a: |              |      |
|                |     |                      | 0.07895; ω2b: 1.000000 |              |      |

*aSignificant difference according to likelihood-ratio test (LRT).

bPositive selected sites included with P > 0.99 according to Bayes Empirical Bayes (BEB) analysis. Other amino acids under positive selection with less than 0.95 of significance are included in Supplementary Material.
To date, two studies have reported a phylogeny for insect AOXs with focus on moths *P. xylostella* and *H. armigera* (He et al., 2017; Xu and Liao, 2017). Both analyses confirm XDHs as common ancestors followed by Dipteran AOXs, and support lepidopteran AOXs as more recently evolved enzymes. In that sense, our phylogenetic analysis is consistent with both. Furthermore, this analysis showed the two ODE-related clades already mentioned as well as a common ancestor in *E. semipurpurella* AOX1 (EsemAOX1), the only AOX identified from its antennal transcriptome. Interestingly, as an old lineage of moths (i.e., non-ditrysia), *E. semipurpurella* represents a model for evolutionary studies. Yuvaraj et al. (2017) showed that moth pheromone receptors could have evolved from plant volatile-related ORs, since two *E. semipurpurella* ORs (EsemOR3 and EsemOR5) that are phylogenetically close to plant volatile-responding ORs, respond to its sex pheromone (2S,6Z-6-nonen-2-ol), which resemble plant volatiles. The lack of more AOXs in *E. semipurpurella* could indicate that gene duplication events in other moths, and likely butterflies, happened in response to the use of more specialized aldehyde-related volatiles, such as sex pheromones. Furthermore, those that are close to EsemAOX1, in ODE-related clade (clade A in Figure 1), could likely be more plant volatile-biased.

It can be argued that moths with functionally studied AOXs, namely BmorAOX5, Pxy1AOX3 and AtraAOX2, are not strictly related to sex pheromone degradation. In fact, AtraAOX2 has not showed specificity for *A. transitella* sex pheromone [(Z,Z)-11,13-hexadecadienal], being also able to catalyze aldehyde-related plant volatiles (Choo et al., 2013). Similarly, Pxy1AOX3 was reported able to degrade sex pheromone [(Z)-11-hexadecenal as well as plant-derived aldehydes, such as phenylacetaldehyde and non-anal (Wang et al., 2021b). Butterflies, *H. melpomene* with [(Z)-9-octadecenal, octadecanal, (Z)-11-icosenal, icosanal and (Z)-13-docosenal as sex pheromone components (Darragh et al., 2017), and *B. anynana* with hexadecanal (Nieberding et al., 2008), represent the only butterflies that use aldehydes as semiochemicals in our data sets. Interestingly, one AOX (i.e., HmelAOX2) was shown by Choo et al. (2013) to degrade both sex pheromone and plant volatiles.

![Figure 3](link-to-figure3)  
**FIGURE 3** | Fragment of a multiple sequence alignment between vertebrate and invertebrate AOXs. Identical residues are highlighted in white letters with red background. Similar residues are highlighted in red and framed in blue. Triangles indicate conserved sites according to Terao et al. (2020). Red triangles show conserved residues across all analyzed species. Blue triangle shows residue conserved only in insect species. Purple triangle shows an active site with different residues within Lepidoptera species. Full alignment can be found in Supplementary Figure 1.
present in an ODE-related clade according to our phylogenetic analysis, while *B. anynana* had seven. It is worth noting that *H. melpomene* as pollinator (Andersson and Dobson, 2003) and *B. anynana* as a fruit-feeding butterfly (Lewis and Wedell, 2007), are both exposed to more aldehydes emitted by plants and fruits. For *B. anynana*, it is noticeable that the seven AOXs (from a total of 12) appear to have emerged independently from the rest. Something similar to what is found in the gene location of moth species, such as *B. mandarina*, *A. transitella* and *P. xylostella*, where their AOX genes potentially related to ODE function, are far from other AOX genes, with the exception of PxylAOX3.

From the two ODE-related clades in our phylogenetic analysis, clade B resulted highly supported by both functional studies and antennal-enriched expression (Figure 1). The fact that AOXs from butterflies were also present in this clade, further suggests that these could use aldehyde-based volatiles as semiochemicals. Although fewer studies have exploited the semiochemistry of butterflies compared with moths, increasing evidence suggests that several species of butterflies, including *H. melpomene* and *B. anynana*, use volatiles as semiochemicals. For example, an early study reported strong antennal responses of *H. melpomene* to several tropical plant-derived volatiles, such as linalool, linalool oxide I and II, oxoisophoroneoxide and phenylacetaldehyde (Andersson and Dobson, 2003). Recently, 55 compounds exclusive of androconia (specialized units where secretory glands are found) in sympatric Pieridae butterflies that would play a role in mating orientation, were reported (Nobre et al., 2021). On the other hand, sequence identity between lepidopteran and mammal AOXs is ∼30%. More specifically, among lepidopteran AOXs, sequence identity starts decreasing at 67%. This divergence within lepidopterans is evidenced in an important amount of PSSs among those phylogenetically close AOXs that were selected for our molecular evolution analyses. Nevertheless, and as expected, residues that are conserved were not PSSs, such as those from the active site.

Our MSA analysis revealed that highly conserved residues in vertebrate AOXs, namely Glu1266, Phe919, Lys889 and Gln772, may or may not be conserved in lepidopteran and *D. melanogaster* AOXs. Thus, Glu1266 (at position 1,209 in our MSA, Figure 3) that is reported to be crucial for catalytic activity resulted highly conserved (Coelho et al., 2012), while Phe919 from vertebrates changes to Pro in insect AOXs (at position 884 in our MSA, Figures 3, 4). It is difficult to predict the effect of
In fact, it is well accepted that Pro restricts protein backbones and subsequent decreased catalytic activity (Boone et al., 2015). Carbonic anhydrase II led to an increased rigidity of the enzyme and Tyr448, were reported to be crucial for ligand selectivity (NS5b polymerase, Pro197 along with Arg200, Cys366, Met414 and Tyr448, were reported to be crucial for ligand selectivity (Li et al., 2010). On the contrary, Pro substitutions in human carbonic anhydrase II led to an increased rigidity of the enzyme and subsequent decreased catalytic activity (Boone et al., 2015). In fact, it is well accepted that Pro restricts protein backbones with the lack of a hydrogen bond donor, disrupting α-helices (Woolfson and Williams, 1990; Van Arnsm et al., 2011). Overall, we believe this study represents the first to group a comprehensive set of AOX genes for several lepidopteran species. We have validated AOX sequences previously described and added 58 more in moths and 33 more in butterflies. We have also uncovered the potential importance of aldehydes as semiochemicals in butterflies, as reflected by the number of AOX present in this group. The information presented herein is a helpful reference for further evolutionary and functional studies in this highly biodiverse order.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

REFERENCES

Adebali, O., Ortega, D., and Zhulin, I. (2015). CDvist: a webserver for identification and visualization of conserved domains in protein sequences. Bioinformatics 31, 1475–1477. doi: 10.1093/bioinformatics/btu836
Andersson, S., and Dobson, H. (2003). Antennal responses to floral scents in the butterfly. J. Chem. Ecol. 29, 2319–2330. doi: 10.1023/A:102627853 1806
Boone, C., Rasi, V., Tu, C., and McKenna, R. (2015). Structural and catalytic effects of proline substitution and surface loop deletion in the extended active site of human carbonic anhydrase II. FEBS J. 282, 1445–1457. doi: 10.1111/febs.13232
Butenandt, A., Beckman, R., Stamm, D., and Hecker, E. (1959). Über den sexuallockstoff des seidenspinners Bombyx mori. Reindarstellung und konstitution. Z Naturforsch B. 14, 283–284.
Chemnitz, J., Jentschke, P. C., Ayasse, M., and Steiger, S. (2015). Beyond species recognition: somatic state affects long-distance sex pheromone communication. Proc. R. Soc. B 282:20150832. doi: 10.1098/rspb.2015.0832
Cheremtseu, T., and Meibèche, M. (2021). “19 - Odor degrading enzymes and signal termination,” in Insect Pheromone Biochemistry and Molecular Biology, 2nd Edn, eds J. Gary, Blomquist, G. Richard, and Vogt (Cambridge, MA: Academic Press), 619–644.
Choo, Y. M., Pelletier, J., Atungulu, E., and Leal, W. S. (2013). Identification and characterization of an antennae-specific aldehyde oxidase from the navel orangeworm, Amylois transitella (Lepidoptera: Pyralidae). J. Chem. Ecol. 5, 955–966.
Coleman, M., Vontas, J. G., and Hemingway, J. (2002). Molecular characterization of the amplified aldehyde oxidase from insecticide resistant Culex quinquefasciatus. Eur. J. Biochem. 269, 768–779. doi: 10.1046/j.0014-2956.2001.02682.x
Corpet, F. (1988). Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res. 16, 10881–10890. doi: 10.1093/nar/16.22.10881
Costanzo, K., and Monteiro, A. (2007). The use of chemical and visual cues in female choice in the butterfly Bicyclus anynana. Proc. R. Soc. B 274, 845–851. doi: 10.1098/rspb.2006.3729
Darragh, K., Vanjari, S., Mann, F., Gonzalez-Rojas, M. F., Morrison, C. R., Salazar, C., et al. (2017). Male sex pheromone components in Heliconius butterflies released by the androconia affect female choice. PeerJ 5:e3953. doi: 10.7717/peerj.3953
Durand, N., Pottier, M. A., Stussart, D., Bozzolan, F., Maibèche, M., and Chertemps, T. (2018). Glutathione-S-transferases in the olfactory organ of the noctuid moth Spodoptera littoralis, diversity and conservation of chemosensory clades. Front. Physiol. 9:1283. doi: 10.3389/fphys.2018.01283
Engsontia, P., Sangket, U., Chotigeat, W., and Satasook, C. (2014). Molecular evolution of the odorant and gustatory receptor genes in lepidopteran insects: implications for their adaptation and speciation. J. Mol. Evol. 79, 21–39. doi: 10.1007/s00239-014-9633-0
Garattini, E., and Terao, M. (2012). The role of aldehyde oxidase in drug metabolism. Expert Opin. 8, 487–503. doi: 10.1517/17425255.2012.663552
Garattini, E., Fratelli, M., and Terao, M. (2009). The mammalian aldehyde oxidase gene family. Hum. Genom. 4, 119–130. doi: 10.1186/1479-7364-4-2-119

AUTHOR CONTRIBUTIONS

RG, AM, and HV conceived the idea. RG and HV performed genome and structural analysis. LCP and AM analyzed phylogenetics and evolution data. HV and LCP wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by Agencia Nacional de Investigación y Desarrollo (ANID) project N° 11200349 and ANID scholarship N° 21190666. LCP was supported by Wellcome Trust Investigator Award 110117/Z/15/Z.

ACKNOWLEDGMENTS

We would like to thank reviewers for the valuable comments that helped to improve this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.823119/full#supplementary-material
He, P., Machuca, J., Venthur, H., Quiroz, A., and Mutis, A. (2019). An overview of antennal esterase in lepidoptera. *J. Insect Behav.* 32, 137–144. doi: 10.1007/s10905-007-9075-2

Lewis, Z., and Wedell, N. (2007). Effect of adult feeding on male mating behaviour in the butterfly, *Bicyclus anynana* (Lepidoptera: Nymphalidae). *J. Insect Behav.* 20, 201–213. doi: 10.1007/s10905-007-9075-2

Li, F., Zhao, X., Li, M., He, K., Huang, C., Zhou, Y., et al. (2019). Insect genomes: progress and challenges. *Insect Mol. Biol.* 28, 739–758. doi: 10.1111/imb.13189

Li, G. W., Chen, X. L., Xu, X. L., and Wu, J. X. (2018). Degradation of sex pheromone and plant volatile components by an antennal glutathione S-transferase in the oriental fruit moth, *Grapholita molesta* Busck (Lepidoptera: Tortricidae). *Arch. Insect Biochem. Physiol.* 99:e21512. doi: 10.1002/arch.21512

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homar, N., et al. (2009). The sequence Alignment/Map format and SAMTools. *Bioinformatics* 25, 2078–2079. doi: 10.1093/bioinformatics/btp352

Li, T., Froeyen, M., and Herdevijn, P. (2010). Insight into ligand selectivity in HCV NS5B polymerase: molecular dynamics simulations, free energy decomposition and docking. *J. Mol. Model.* 16, 49–59. doi: 10.1007/s00894-009-0519-9

Li, Z. Q., Zhang, S. Z., Luo, J. Y., Wang, C. Y., Lv, L. M., Dong, S. L., et al. (2015). Transcriptome comparison of the sex pheromone glands from two sibling *Helicoverpa* species with opposite sex pheromone components. *Sci. Rep.* 5:9324. doi: 10.1038/srep09324

Liénard, M., Wang, H. L., Lassance, J. M., and Lòststedt, C. (2014). Sex pheromone biosynthetic pathways are conserved between moths and the butterfly *Bicyclus anynana*. *Nat. Commun.* 5:3957. doi: 10.1038/ncomms4957

Liu, H., Lei, X., Du, L., Yin, J., Shi, H., Zhang, T., et al. (2019). Antennae-specific carboxylesterase genes from Indian meal moth: Identification, tissue distribution and the response to semiochemicals. *J. Stored Prod. Res.* 84:0101528. doi: 10.1016/j.jspr.2019.101528

Merlin, C., François, M. C., Bozallon, F., Pelletier, J., Jacquin-Joly, E., and Maibeche-Coisne, M. (2005). A new aldehyde oxidase selectivity expressed in chemosensory organs of insects. *Biophys. Biochem. Biol. Res. Commun.* 332, 4–10. doi: 10.1016/j.bbrc.2005.04.084

Nieberding, C. M., de Vos, H., Schneider, M. V., Lassance, J. M., Estramil, N., Andersson, J., et al. (2008). The male sex pheromone of the butterfly *Bicyclus anynana*: towards an evolutionary analysis. *PLoS One* 3:e2751. doi: 10.1371/journal.pone.0002751

Nobre, C. E. B., Lucas, L. A. D., Padilha, R. J. R., Navarro, D. M., Alves, L. C., and Mata, A. C. D. (2021). Specialized androconial scales conceal species-specific semiochemicals of sympatric Sulphur butterflies (Lepidoptera: Pieridae: Coliadinae). *Org. Divers Evol.* 21, 93–105. doi: 10.1007/s13127-020-00475-8

Oppenheim, S., Baker, R., Simon, S., and DeSalle, R. (2015). We can’t all be supermodels: the value of comparative transcriptome to the study of non-model insects. *Insect Mol. Biol.* 24, 139–154. doi: 10.1111/imb.12154

Ou, J., Deng, H. M., Zheng, S. C., Huang, L. H., Feng, Q. L., and Liu, L. (2014). Transcriptomic analysis of developmental features of Bombyx mori wing disc during metamorphosis. *BMC Genomics* 15:820. doi: 10.1186/1471-2164-15-820

Patel, R., and Jain, M. (2012). NGS QC Toolkit: a toolkit for quality control of next generation sequencing data. *PLoS One* 7:e30619. doi: 10.1371/journal.pone.0030619

Pelletier, J., Bozallon, F., Solvar, M., François, M. C., Jacquin-Joly, E., and Maibeche-Coisne, M. (2007). Identification of candidate aldehyde oxidases from the silkworm *Bombyx mori* potentially involved in antennal pheromone degradation. *Gene* 404, 31–40. doi: 10.1016/j.gene.2007.08.022

Price, M., Depaul, P., and Arkin, A. (2010). *FastTree 2* – approximately maximum-likelihood trees for large alignments. *PLoS One* 5:e9490. doi: 10.1371/journal.pone.0009490

Riddiford, L. M. (1967). Trans-2-hexenal: mating stimulant for *Polyphemus moth*. *Science* 158, 139–141. doi: 10.1126/science.158.3797.139

Rybczynski, R., Reagan, J., and Lerner, M. (1989). A pheromone-degrading aldehyde oxidase in the antennae of the moth *Manduca sexta*. *J. Neurosci.* 9, 1341–1353.

Sela, I., Ashkenazy, H., Katoh, K., and Pupko, T. (2015). GUIDANCE2: accurate multiple sequence alignment, interactive sequence choice and visualization. *Nucleic Acids Res.* 43, W7–W14. doi: 10.1093/nar/gkv318

Soffian, A., Subandiyah, S., Makino, H., Watanabe, T., and Horiike, T. (2018). Evolutionary analysis of the highly conserved insect odorant coreceptor (*Oreo*) revealed a positive selection mode, implying functional flexibility. *J. Insect Sci.* 18, 1–8. doi: 10.1093/jisesa/iey120
Sokkar, P., Mohandass, S., and Ramachandran, M. (2011). Multiple templates-based homology modeling enhances structures quality of AT1 receptor: validation by molecular dynamics and antagonist docking. J. Mol. Model. 17, 1565–1577. doi: 10.1007/s00894-010-08660-z

Swanson, W. J., Nielsen, R., and Yang, Q. (2003). Pervasive adaptive evolution in mammalian fertilization proteins. Mol. Biol. Evol. 20, 18–20. doi: 10.1093/molbev/msg023

Terao, M., Garattini, E., Romano, M. J., and Leimkuhler, S. (2020). Evolution, expression, and substrate specificities of aldehyde oxidase enzymes in eukaryotes. J. Biol. Chem. 295, 5377–5389. doi: 10.1074/jbc.REV119.007741

Van Arnim, E. B., Lester, H. A., and Dougherty, D. A. (2011). Dissecting the functions of conserved prolines within transmembrane helices of the D2 dopamine receptor. ACS Chem. Biol. 6, 1063–1068. doi: 10.1021/cb10153g

Venthur, H., and Zhou, J. J. (2018). Odorant receptors and odorant-binding proteins as insect pest control targets: a comparative analysis. Front. Physiol. 9:1163. doi: 10.3389/fphys.2018.01163

Vizuetá, J., Sánchez-Gracia, A., and Rozas, J. (2020). BITACORA: a comprehensive tool for the identification and annotation of gene families in genome assemblies. Mol. Ecol. Resour. 20, 1445–1452. doi: 10.1111/1755-0998.13202

Voigt, R., Grosse-Wilde, E., and Zhou, J. J. (2015). The lepidoptera odorant binding protein gene family: gene gain and loss within the GbBP/PBP complex of moths and butterflies. Insect Biochem. Mol. Biol. 62, 142–153. doi: 10.1016/j.ibmb.2015.03.003

Wang, C. H., Zhang, C., and Hing, X. H. (2016). Xanthine dehydrogenase: an old enzyme with new knowledge and prospects. Bioengineered 7, 395–405. doi: 10.1080/21655979.2016.1260618

Wang, M. M., Long, G. J., Guo, H., Liu, X. Z., Wang, H., Dewer, Y., et al. (2021a). Two carboxylesterase genes in Plutella xylostella associated with sex pheromones and plant volatiles degradation. Pest Manag. Sci. 77, 2737–2746. doi: 10.1002/ps.6302

Wang, M. M., He, M., Wang, H., Ma, Y. F., Dewer, Y., Zhang, F., et al. (2021b). A candidate aldehyde oxidase in the diamondback moth, Plutella xylostella (L.), is potentially involved volatiles and the detoxification of xenobiotics. Pestic. Biochem. Phys. 171,104726. doi: 10.1016/j.pestbp.2020.104726

Wei, H., Tan, S., Li, Z., Li, J., Moural, T. W., Zhu, F., et al. (2020). Odorant degrading carboxylesterases modulate foraging and mating behaviors of Grapholitha molesta. Chemosphere 270:128647. doi: 10.1016/j.chemosphere.2020.128647

Weiss, M. R. (2001). “Vision and learning in some neglected pollinators: beetles, flies, moths and butterflies,” in Cognitive Ecology of Pollination. Animal Behavior and Floral Evolution, eds L. Chittka and J. D. Thomson (Cambridge: Cambridge University Press).

Woolfson, D. N., and Williams, D. H. (1990). The influence of proline residues on α-helical structure. FEBS Lett. 277, 185–188. doi: 10.1016/0014-5793(90)80839-b

Xu, W., and Liao, Y. (2017). Identification and characterization of aldehyde oxidases (AOXs) in the cotton bollworm. Sci. Nat. 104:94. doi: 10.1007/s00114-017-1515-z

Yang, Z., and Nielsen, R. (2002). Codon-substitution models for detecting molecular adaptation at individual sites along specific lineages. Mol. Biol. Evol. 19, 908–917. doi: 10.1093/oxfordjournals.molbev.a004148

Yuvaraj, J. K., Corcoran, J. A., Andersson, M. N., Newcomb, R. D., Anderbrant, O., and Lofstedt, C. (2017). Characterization of odorant receptors from a non-ditryan moth, Eriocrania semiparaperta sheds light on the origin of sex pheromone receptors in lepidoptera. Mol. Biol. Evol. 34, 2733–2746. doi: 10.1093/molbev/msx215

Zhang, J. P., Salcedo, C., Fang, Y. L., Zhang, R. J., and Zhang, Z. N. (2012). An overlooked component: (Z)-9-tetradecenal as a sex pheromone in Helicoverpa armigera. J. Insect Physiol. 58, 1209–1216.

Zhang, Y. N., Xia, Y. H., Zhu, J. Y., Li, S. Y., and Dong, S. L. (2014). Putative pathway of sex pheromone biosynthesis and degradation by expression patterns of genes identified from female pheromone gland and adult antenna of Sesamia inferens (Walker). J. Chem. Ecol. 40, 439–451. doi: 10.1007/s10886-014-0433-1

Zhang, Y. N., Li, Z. Q., Zhu, X. Y., Qian, J. L., Dong, Z. P., and Xu, L. (2017a). Identification and tissue distribution of carboxylesterase (CXE) genes in Athetis lepigone (Lepidoptera: Noctuidae) by RNA-seq. J. Asia-Pac. Entomol. 20, 1150–1155.

Zhang, Y. X., Wang, W. L., Li, M. Y., Li, S. H., and Liu, S. (2017b). Identification of putative carboxylesterase and aldehyde oxidase genes from the antennae of the rice leafroller, Cnaphalocrocis medinalis (Lepidoptera: Pyralidae). J. Asia Pac. Entomol. 20, 907–913. doi: 10.1016/j.aspen.2017.06.001

Zhang, Y., Yang, Y., Shen, G., Mao, X., Jiao, M., and Lin, Y. (2020). Identification and characterization of aldehyde oxidase 5 in the pheromone gland of the silkworm (Lepidoptera: Bombycidae). J. Insect Sci. 20, 1–10. doi: 10.1093/jisesa/ieaa132

Zhou, J. J. (2010). Odorant-Binding Proteins in Insects, 1st Edn. Burlington, VT: Elsevier Inc. doi: 10.1016/S0083-6729(10)80310-9

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