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CYPs in different families are involved in the divergent regio-specific epoxidation of alkenyl sex pheromone precursors in moths

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ABSTRACT

In moth species that utilize alkenyl sex pheromones, the epoxidation of alkenes confers further diversity on the chemical structures of pheromone components. Hc_epo1 (CYP341B14), the first pheromone gland (PG)-specific epoxidase identified from the fall webworm *Hyphantria cunea* (Erebidae), specifically epoxidizes the Z9 double bond in the triene precursor, (3Z,6Z,9Z)-3,6,9-henicosatriene (Z3,Z6,Z9-21:H). In the present study, we identified a novel PG-specific epoxidase, As_epo1, from the Japanese giant looper *Ascotis selenaria* (Geometridae), which secretes cis-3,4-epoxy-(6Z,9Z)-6,9-nonadecadiene (epo3,Z6,Z9-19:H) as the main sex pheromone component. A functional assay using the Sf9 insect cell line–baculovirus expression system showed that As_epo1 specifically epoxidizes the Z3 double bond in the pheromone precursor triene, (3Z,6Z,9Z)-3,6,9-nonadecatriene (Z3,Z6,Z9-19:H). As_epo1 also Z3-specifically epoxidized a triene with a longer carbon chain, Z3,Z6,Z9-21:H, which does not occur in this species. A phylogenetic analysis indicated that As_epo1 belonged to the CYP340 family, not the CYP341 family to which Hc_epo1 belongs. These results suggest that moth PG-specific epoxidases with divergent regio-specificities have evolved independently.

1. Introduction

The chemical structures of female sex pheromones in moths are markedly diverse, which contributes to premating reproductive isolation between a large number of moth species (Ando and Yamamoto, 2018; El-Sayed, 2014). Moth sex pheromones have been classified into type I, type II, and others based on their chemical structures (Ando et al., 2004; Ando and Yamakawa, 2011; Millar, 2000). Type-II pheromones, utilized by moth species in Geometridae and Erebidae, are predominantly composed of C17–C23 hydrocarbons with 2–3 *cis* double bonds at the 3, 6, or 9 position, and/or their corresponding epoxides (Arn et al., 1997). Most, but not all, type-II sex pheromones are biosynthesized from dietary linoleic acid or α-linolenic acid ingested during the larval stage, typically via chain elongation, decarboxylation, and subsequent epoxidation (Millar, 2000; Rule and Roelofs, 1989; Wei et al., 2003). Thus, the double bonds characteristic of most type-II compounds originate from dietary polyunsaturated fatty acids.

The optional epoxidation of alkenes, which occurs in the sex pheromone gland (PG) of female moths, confers further diversity on the chemical structures of sex pheromones (Fujii et al., 2007, 2015; Matsuoka et al., 2006; Millar, 2000; Miyamoto et al., 1999; Wei et al., 2003). We previously functionally characterized two PG-specific epoxidases, Hc_epo1 and Li_epo1, from two arctiid moths *Hyphantria cunea* and *Lemyra imparilis*, respectively. These two epoxidases specifically catalyze the epoxidation of the Z9 double bond in triene precursors in *H. cunea* and *L. imparilis* (Rong et al., 2014, 2019). Hc_epo1 and Li_epo1 are orthologs in the same cytochrome P450 (CYP) subfamily, CYP341B (Rong et al., 2014, 2019). The finding that CYPs in CYP341B specifically epoxidize the Z9 double bond stimulated our interest in the CYP family(ies) to which epoxidases specific for Z3 and Z6 double bonds belong.

The Japanese giant looper *Ascotis selenaria* (Geometridae) utilizes cis-3,4-epoxy-(6Z,9Z)-6,9-nonadecadiene (epo3,Z6,Z9-19:H) as its main sex pheromone component (Ando et al., 1997; Witjaksono et al., 1999). Tracing experiments using the deuterium-labeled pheromone precursor, (3Z,6Z,9Z)-3,6,9-nonadecatriene (Z3,Z6,Z9-19:H), suggested that an epoxidase specific for the Z3 double bond is expressed in the rod-shaped PG of *A. selenaria* (Miyamoto et al., 1999). To identify PG-specific epoxidase in *A. selenaria*, we initially investigated whether Z3-specific epoxidase in *A. selenaria* also belongs to CYP341B. However, no band of the expected size was obtained by PCR using the degenerate primers designed based on the amino acid sequences of the CYP341B family in the public database. Accordingly, in the present study, we used RNA sequencing (RNA-seq) of the PG of *A. selenaria* to identify the PG-specific CYP, and subsequently performed a functional assay to establish whether this CYP exhibits Z3-specific epoxidase activity for the triene

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precursor, Z3,Z6,Z9-19:H. We also examined the substrate specificity of this CYP using an alkene with a longer carbon chain length, (3Z,6Z,9Z)-3,6,9-henicosatriene (Z3,Z6,Z9-21:H), which does not occur in *A. selenaria*.

2. Materials and methods

2.1. Insect and cell line

The eggs of *A. selenaria* were sourced from the culture maintained in the Ando Laboratory, Tokyo University of Agriculture and Technology (TUAT), and reared on an artificial diet (Insecta LF, Nosan Corp.), at 24 °C under a 16-h light and 8-h dark photoperiod. The Sf9 insect cell culture was maintained in TC-100 medium (AppliChem) with 10% fetal bovine serum as described previously (Katsuma et al., 2006).

2.2. Chemicals

Authentic Z3,Z6,Z9-19:H and Z3,Z6,Z9-21:H were provided by Prof. Tetsu Ando (TUAT). The epoxides of these compounds were obtained as follows. Z3,Z6,Z9-19:H or Z3,Z6,Z9-21:H (10 mg, 0.03 mmol) and m-chloroperoxybenzoic acid (mCPBA; > 70%, 10 mg, 0.06 mmol) were dissolved in dry CH_2Cl_2 (1 mL) and stirred on ice for 2 h and then at room temperature for 1 h. After the neutralization and subsequent removal of residual mCPBA by a silica gel column, a mixture of positional isomers of alkene epoxides was eluted by 10% diethyl ether in hexane.

2.3. Total RNA preparation from the PG

Rod-shaped PGs were dissected from 1- to 3-day-old virgin female moths in PBS (2.5 mM KCl, 141 mM NaCl, 8.1 mM Na_2HPO_4, and 2.5 mM KH_2PO_4, pH = 7.0) during the late scotophase (D4–D8), when the pheromone titer was maximal (Fujii et al., 2007). Total RNA was extracted from each tissue using the RNAiso reagent (Takara Bio) according to the manufacturer's protocol.

2.4. RNA sequencing (RNA-seq)

Sequencing library construction and RNA-seq on an Illumina HiSeq 2000 platform were performed by Macrogen Japan Corp. (Kyoto) using 4 μg of total RNA prepared from the PG of *A. selenaria* in our laboratory. Trimming of raw paired-end reads and subsequent *de novo* assembly by Trinity (version 2.4.0) were performed using Galaxy/NAAC, a web-based platform for a bioinformatics analysis (https://galaxy.dna.affrc.go.jp/nias/static/register_en.html). The expression levels of contigs assembled by Trinity were estimated using Salmon (Patro et al., 2017). TPM (Transcripts per kilobase million) was used as the index of expression levels in the present study (Wagner et al., 2012). The top 2000 strongly expressed contigs were then subjected to blastX in the blast2go platform (https://www.blast2go.com/) against a local non-redundant protein sequence database (nr) downloaded from NCBI (ftp://ftp.ncbi.nlm.nih.gov/blast/db/).

2.5. Tissue distribution analysis of candidate genes

Tissues were dissected from 1- to 3-day-old virgin female moths in PBS during the late scotophase (D4–D8). Total RNA (*ca.* 1 μg) obtained from the respective tissue was reverse-transcribed using the PrimeScript™ II 1st strand cDNA Synthesis Kit (Takara Bio) and the oligo-dT primer supplied in the kit. RT-PCR was performed with gene-specific primer pairs for the candidate genes (Table S2) and Ex Taq DNA polymerase (Takara Bio) under the following conditions: 94 °C for 1 min, 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 68 °C for 1 min, and finally 72 °C for 7 min.

2.6. Recombinant expression system

The coding region of *As_epo1* with or without the His-tag at the C terminus was amplified by PCR with a pair of gene-specific primers, which contained restriction enzyme sites (Table S2). Recombinant *Autographa californica* nuclear polyhedrosis viruses (AcNPVs) expressing As_epo1 with or without the His-tag, As_epo1-His-AcNPV and As_epo1-AcNPV, respectively, were constructed using the Bac-to-Bac Baculovirus Expression System (Invitrogen), as described previously (Fujii et al., 2011). Two micrograms of As_epo1-His-AcNPV, As_epo1-AcNPV, or Bac1-AcNPV (negative control) was transfected into Sf9 cells with Cellfectin® II reagent (Invitrogen). The expression of the As_epo1 protein in Sf9 cells was checked by a Western blotting analysis. Sf9 cells infected by As_epo1-His-AcNPV, As_epo1-AcNPV, or Bac1-AcNPV, as well as medium, were collected every 12 h post-infection by centrifugation at 1000 × *g* for 10 min. Cells were rinsed three times with PBS and homogenized in PBS containing a protease inhibitor cocktail (4% v/v of the final volume; Complete Mini, Roche Applied Science). Samples prepared from cells and medium were separated on a 10% gel by SDS-PAGE, and transferred to an Immobilon-P membrane (Millipore). The membrane was incubated with a mouse anti-His antibody (MBL, 1:5000 dilution) for 1 h and subsequently with a goat anti-mouse IgG-HRP conjugate (1:10,000 dilution) for 1 h. Chemiluminescence emitted by Immobilon Western Chemiluminescent HRP Substrate (Millipore) was detected using ImageQuant 400 (GE Healthcare Bioscience). The same samples were also analyzed by SDS/PAGE and stained with Coomassie Brilliant Blue (CBB) as loading controls.

2.7. Functional assay of recombinants

TC-100 medium containing an alkene substrate was prepared as follows. Forty micrograms of Z3,Z6,Z9-19:H (or Z3,Z6,Z9-21:H) and 1.5 mg of NADPH (Oriental Yeast Co. ltd.) were dissolved in 20 μL of dimethyl sulfoxide and added to 4 mL of serum-free TC-100 medium. The medium was sonicated for 1 h before use. Sf9 cells seeded on a 60-mm cell culture dish containing 4 mL of serum-free TC-100 medium were infected with As_epo1-His-AcNPV, As_epo1-AcNPV, or Bac1-AcNPV. Medium was replaced 48 h post-infection with that containing Z3,Z6,Z9-19:H or Z3,Z6,Z9-21:H, and then incubated for a further 12 h. Cells were subsequently rinsed with PBS three times, and resuspended in 100 μL of distilled water. The products from the alkene substrate were then extracted from cells with 500 μL of hexane at room temperature for 1 h and analyzed by GC–MS.

2.8. GC–MS analyses

Analyses of epoxyalkenes were conducted using QP2010 SE GC–MS (Shimadzu), as described previously (Rong et al., 2014). The epoxydiene (epo3,Z6,Z9-19:H or epo3,Z6,Z9-21:H) was detected at *m/z* 79 (Ando and Yamakawa, 2011). The study by Ando and Yamakawa (2011) was referred to for the mass fragment pattern of these compounds.

2.9. Phylogenetic analysis

We used an online service provided by the DNA Data Bank of Japan (DDBJ; http://clustalw.ddbj.nig.ac.jp/index.php?lang=en) for the phylogenetic analysis. CYP15C1 (*Bombyx mori*, AB124839) was used as an outgroup.

2.10. Data deposition

The nucleotide sequence of *As_epo1* (*CYP340BD2*) has been submitted to DDBJ (http://www.ddbj.nig.ac.jp/) with Accession No. LC424537.

Table 1
Contigs strongly expressed in pheromone glands of *A. selenaria*, which were annotated as CYP by a blastX search of the NCBI non-redundant protein sequence database.

Rank of expression level	Contig	Length (bp)	ORF status	Length of deduced amino acid sequence	Expression level (TPM[a])	Annotation
1	**AsPG17297_c0_seq1**	1769	complete	484aa	7593.7	CYP340
2	**AsPG4083_c0_seq1**	2412	complete	531aa	904.0	CYP9A
3	**AsPG16213_c0_seq1**	1236	3prime_partial	399aa	423.3	CYP340
4	**AsPG14868_c0_seq1**	1803	complete	492aa	415.9	CYP340
5	**AsPG14198_c0_seq1**	1711	complete	487aa	194.3	CYP4AU
6	**AsPG17427_c0_seq1**	2141	complete	556aa	147.6	CYP4G
7	**AsPG17348_c0_seq1**	2945	complete	494aa	139.9	CYP4L
8	**AsPG17691_c1_seq1**	3456	complete	485aa	53.2	CYP340

[a] Transcripts per kilobase million.

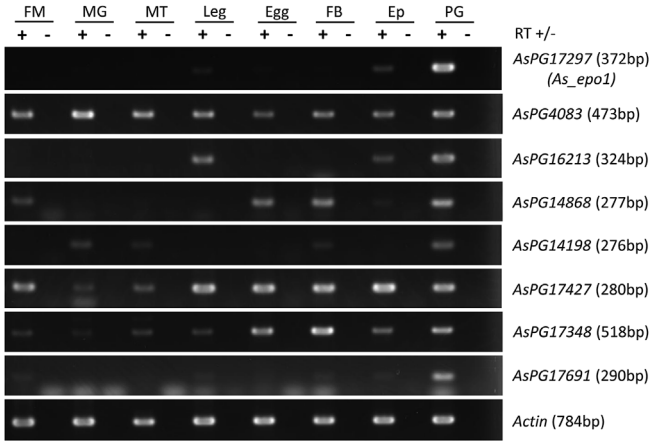

Fig. 1. Expression of epoxidase candidate genes in various tissues of *A. selenaria*. FM: flight muscles, MG: midgut, MT: Malpighian tubule, Leg: legs, Egg: eggs, FB: fat bodies, Ep: epidermis, and PG: pheromone glands.

3. Results

3.1. Genes strongly expressed in the PG

A total of 24,363 contigs were assembled by Trinity from the 56, 615, 028 reads obtained by RNA-seq. Since the enzyme genes involved in sex pheromone biosynthesis identified to date were unexceptionally strongly and specifically expressed in the PG, we selected contigs that fulfilled these requirements. Among the contigs assembled by Trinity, the top 2000 strongly expressed contigs were extracted based on TPM. These contigs were subjected to a blastX search of the NCBI nr database and eight contigs annotated as CYP were consequently selected as candidates of epoxidase involved in sex pheromone biosynthesis in *A. selenaria* (Table 1). Four contigs (AsPG17297, AsPG16213, AsPG14868, and AsPG17691) were annotated as CYP340 and the remaining 4 contigs (AsPG4083, AsPG14198, AsPG17427, and AsPG17348) were annotated as CYP9A, CYP4AU, CYP4G, and CYP4L, respectively (Table 1).

3.2. Tissue distribution of candidate gene transcripts

The tissue distribution patterns of 8 contigs selected as candidates were investigated by RT–PCR using candidate-specific primers (Table S2). Among the 8 contigs, AsPG17297, which was the most strongly

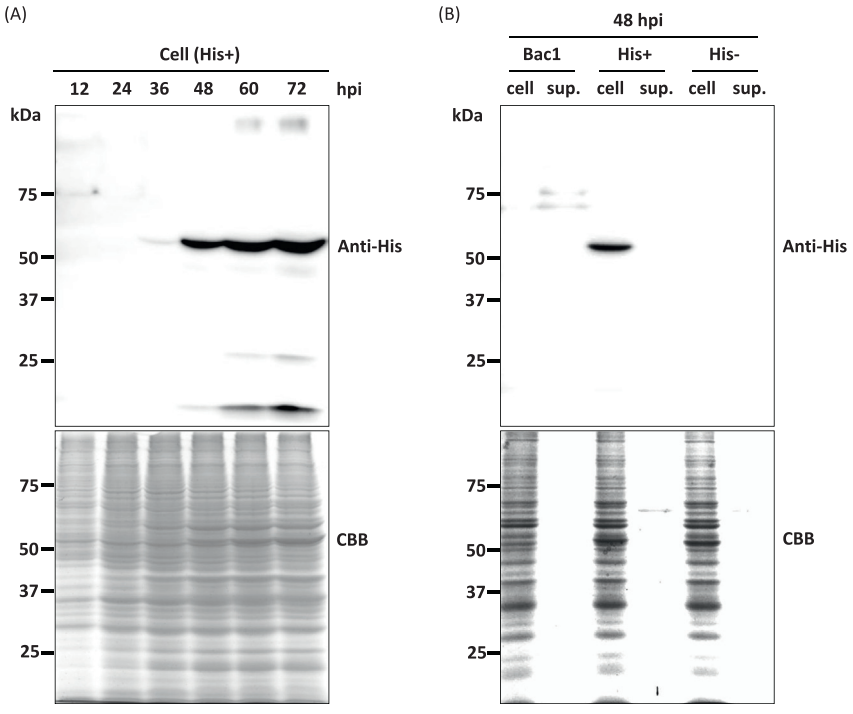

Fig. 2. Expression of the recombinant As_epo1 protein using a baculovirus expression system. (A) Time course of the expression of the As_epo1 protein in Sf9 cells infected with As_epo1-His-AcNPV. (B) The expression of the As_epo1 protein in Sf9 cells 48 hpi with Bac1-AcNPV, As_epo1-His-AcNPV, and As_epo1-AcNPV. Bac1-AcNPV: negative control. As_epo1-His-AcNPV and As_epo1-AcNPV: AcNPV constructs expressing the As_epo1 protein with and without the His-tag at the C terminus, respectively. The molecular weight of As_epo1 with a histidine tag was estimated to be *ca.* 57 kDa. The same samples were also analyzed by SDS/PAGE and stained with CBB. hpi: hours post-infection.

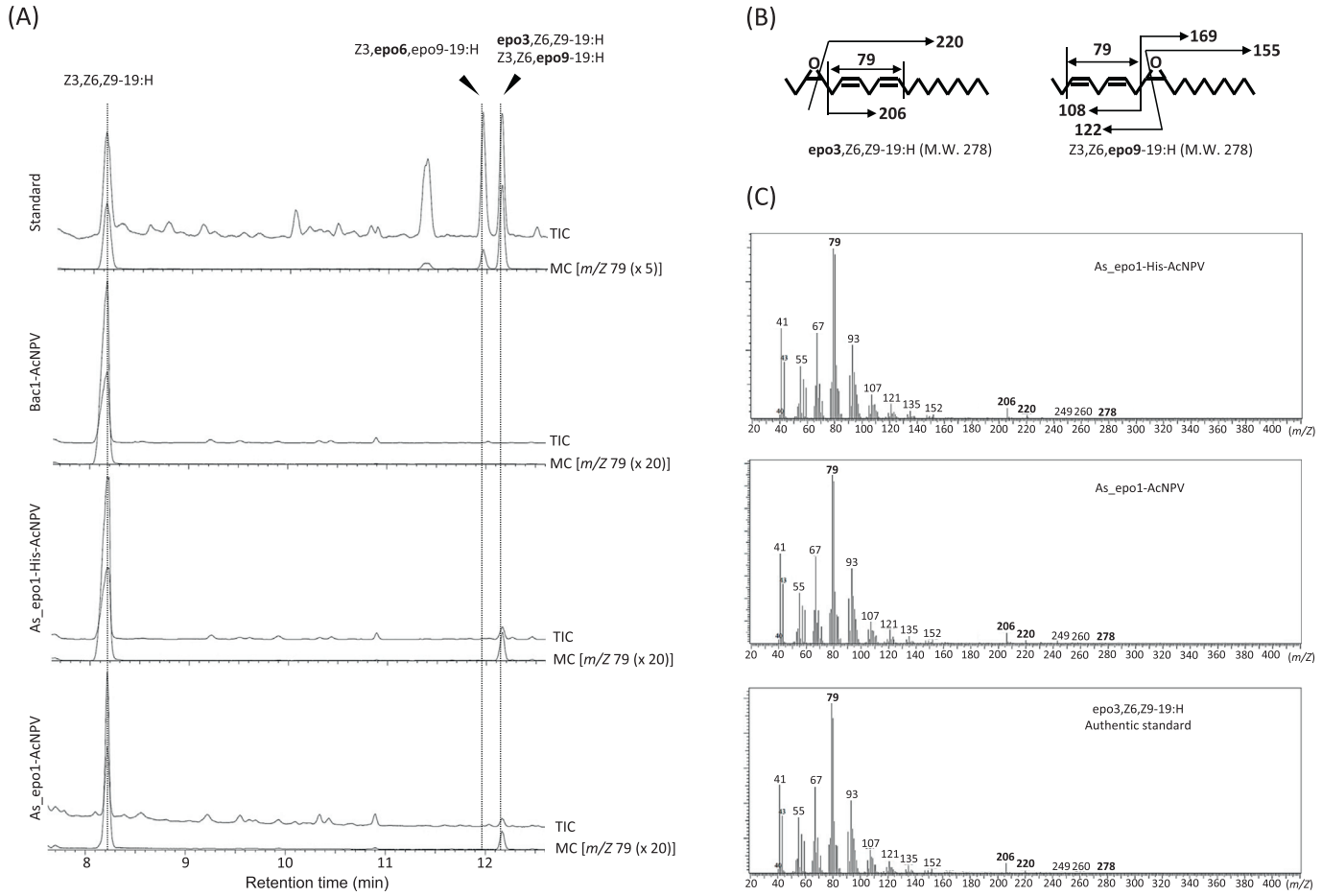

Fig. 3. Results of the functional assay on As_epo1 using Z3,Z6,Z9-19:H as the substrate. (A) Total ion chromatograms (TIC) and mass chromatograms (*m/z* 79; base ion peak of the substrate and product in this study) of a mixture of Z3,Z6,Z9-19:H and its epoxide isomers epo3,Z6,Z9-19:H, Z3,epo6,Z9-19:H, and Z3,Z6,epo9-19:H standards (upper traces), an extract from Sf9 cells infected with Bac1-AcNPV (middle traces; negative control), and an extract from Sf9 cells infected with As_epo1-His-AcNPV or As_epo1-AcNPV (lower traces). (B) Diagnostic fragment ions of the two epoxy ring positional isomers of Z3,Z6,Z9-19:H (Ando and Yamakawa, 2011). Two isomers have the diagnostic ion, *m/z* 79, in common. (C) Mass spectra of the products of As_epo1-His (upper) and As_epo1 (middle), and synthetic epo3,Z6,Z9-19:H (lower). Bold figures indicate diagnostic fragment ions of epo3,Z6,Z9-19:H.

expressed, exhibited PG-specific expression (Fig. 1). We named this contig *As_epo1* (*A. selenaria* epoxidase candidate 1) and hereafter focused on it. Although AsPG17691, in addition to AsPG17297, was PG-specifically expressed (Fig. 1), it was disregarded in the present study because its expression level was as low as 0.7% of AsPG17297 (Table 1).

3.3. Sequence analysis of As_epo1 cDNA

The nucleotide sequence of *As_epo1* was reconfirmed using cDNA prepared from the PG of *A. selenaria* and primers (Table S2) designed based on the sequence of AsPG17297_c0_seq1. *As_epo1* contained an open reading frame of 1455 bp, which encoded a protein of 484 amino acid residues with an estimated molecular mass of 55.7 kDa (Fig. S1). An analysis of the deduced As_epo1 sequence by the SOSUI program (http://harrier.nagahama-i-bio.ac.jp/sosui/) suggested one transmembrane domain at the N terminus (amino acid positions 1–20). The As_epo1 sequence contained motifs known to be conserved in P450s, such as a heme-binding region (amino acid positions at 426–435) including the proximal cysteine residue (amino acid position at 433), a distal threonine residue (amino acid position at 300) with its upstream sequence (amino acid positions at 296–299), a K-region (amino acid positions at 354–357) located between the proximal cysteine and distal threonine residue, and an aromatic region (amino acid positions 399–411) (Fig. S1).

3.4. In vitro functional assay of As_epo1

The product of the As_epo1-His-AcNPV recombinant virus was detected 36 h post-infection, but was markedly degraded 60 or 72 h post-infection (Fig. 2A). Functional assays on As_epo1 were performed using Sf9 cells 48 h post-infection when As_epo1 was not degraded and localized in cells (Fig. 2A and B). The apparent molecular weight of His-tagged As_epo1, *ca.* 57 kDa, was close to the value estimated from its amino acid sequence (56.5 kDa), suggesting that As_epo1 was expressed without any post-translational modifications (Fig. 2A and B).

In assays using Z3,Z6,Z9-19:H as the substrate, GC–MS analyses of the hexane extracts of Sf9 cells infected with As_epo1-His-AcNPV or As_epo1-AcNPV, but not with Bac1-AcNPV, detected the presence of a chemical product, the retention time of which was close to that of authentic epo3,Z6,Z9-19:H (Fig. 3A). This product was identified as epo3,Z6,Z9-19:H by comparisons of its retention time and mass spectra with those of three epoxy-ring positional isomers (epo3,Z6,Z9-19:H, Z3,epo6,Z-19:H, and Z3,Z6,epo9-19:H), as follows (Fig. 3A, B, C). Comparisons of the retention time of this compound (12.16 min) with those of Z3,epo6,Z9-19:H (11.96 min), epo3,Z6,Z9-19:H (≈12.15 min), and Z3,Z6,epo9-19:H (≈12.15 min) indicated that this compound was epo3,Z6,Z9-19:H or Z3,Z6,epo9-19:H. Since the mass spectra of this product were identical to those of epo3,Z6,Z9-19:H (Fig. 3B and C) (Ando et al., 2004; Ando and Yamakawa, 2011), it was concluded to be epo3,Z6,Z9-19:H. Collectively, these results indicated that As_epo1,

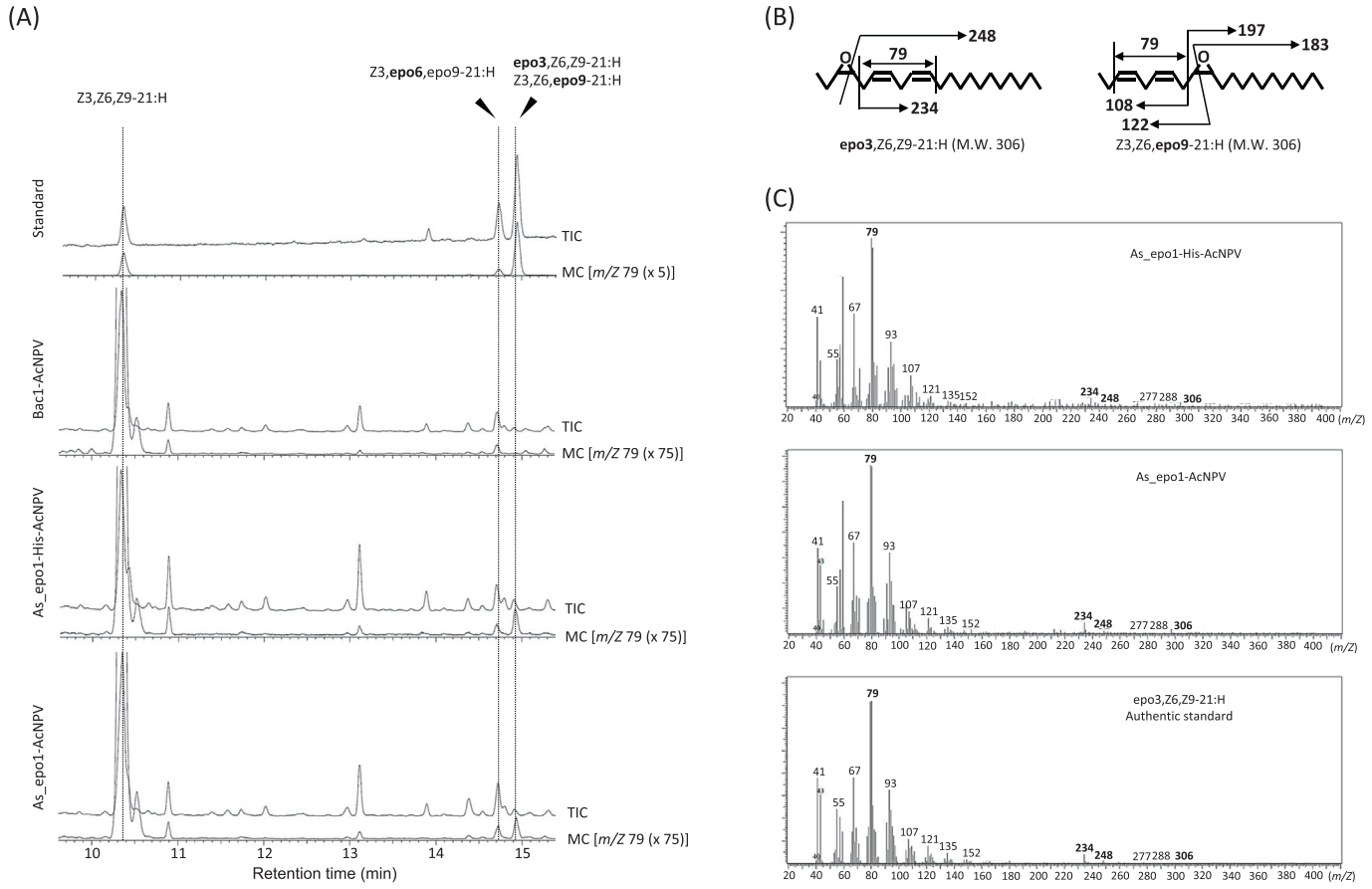

Fig. 4. Results of the functional assay of As_epo1 using Z3,Z6,Z9-21:H as the substrate. (A) Total ion chromatograms (TIC) and mass chromatograms (*m/z* 79; base ion peak of the substrate and product in this study) of a mixture of Z3,Z6,Z9-21:H and its epoxide isomers epo3,Z6,Z9-21:H, Z3,epo6,Z9-21:H, and Z3,Z6,epo9-21:H standards (upper traces), an extract from Sf9 cells infected with Bac1-AcNPV (middle traces; negative control), and an extract from Sf9 cells infected with As_epo1-His-AcNPV or As_epo1-AcNPV (lower traces). (B) Diagnostic fragment ions of two epoxide isomers of Z3,Z6,Z9-21:H (Ando and Yamakawa, 2011). These two isomers have the diagnostic ion, *m/z* 79, in common. (C) Mass spectra of the products of As_epo1-His (upper) and As_epo1 (middle), and synthetic epo3,Z6,Z9-21:H (lower). Bold figures indicate diagnostic fragment ions of epo3,Z6,Z9-21:H.

regardless of the absence/presence of the His-tag at the C terminus, catalyzed the specific epoxidation of a (*Z*)-double bond at position 3 of the triene precursor, Z3,Z6,Z9-19:H.

We next examined whether As_epo1 catalyzes the epoxidation of Z3,Z6,Z9-21:H, a non-native triene with a longer carbon chain length. GC–MS analyses of the hexane extracts of Sf9 cells infected with As_epo1-His-AcNPV or As_epo1-AcNPV, but not with Bac1-AcNPV, detected the presence of a product, the retention time of which was close to that of authentic epo3,Z6,Z9-21:H (Fig. 4A). This product was identified as epo3,Z6,Z9-21:H by comparisons of its retention time and mass spectra with those of three epoxy-ring positional isomers, epo3,Z6,Z9-21:H, Z3,epo6,Z-21:H, and Z3,Z6,epo9-21:H (Fig. 4A, B, C).

3.5. Phylogenetic analysis of As_epo1

Reconstruction of the phylogenetic tree of insect CYPs confirmed that As_epo1 belonged to CYP340, a different family from CYP341, to which Hc_epo1 and Li_epo1 belong (Fig. 5). CYP340BD2 was assigned to *As_epo1*.

4. Discussion

In the present study, we identified the Z3 double bond-specific epoxidase, *As_epo1*, from *A. selenaria*. As_epo1 also epoxidized the Z3 double bond of a non-native substrate, Z3,Z6,Z9-21:H, suggesting that the substrate specificity of As_epo1 in terms of aliphatic carbon chain length is not high. This is consistent with the findings of a previous study (Miyamoto et al., 1999), in which the specificity of epoxidase in *A. selenaria* PG was investigated using deuterium-labeled precursors. In the present study, contig AsPG17691 was disregarded because of its low expression level in the PG (Table 1); however, its enzymatic activity is of interest because it also belongs to the same CYP340 family as As_epo1 (Fig. 5). We consider the contribution of AsPG17691 to pheromone biosynthesis to be very low even if it exhibits Z3-specific epoxidase activity.

To date, more than 140 moth species have been reported/suggested to utilize epoxyalkene pheromones (Table S1; Ando et al., 2008). Most of these species belong to two superfamilies, Geometroidea and Noctuoidea. It is interesting to note the presence of phylogenetic bias in the utilization of epoxy positional isomers (Table S1). For example, 3-epoxyalkenes are almost exclusively utilized by moths belonging to the superfamily Geometroidea. In contrast, moths in the subfamily Arctiinae almost exclusively utilize 9-epoxyalkenes.

The sequences and functions of CYPs in insects have become extremely diversified by repeated duplication events and the subsequent acquirement of new functions by these duplicated genes (Calla et al., 2017; Feyereisen, 2012). The Z3 double bond-specific epoxidase, As_epo1 (CYP340BD2), belonged to the CYP340 family, whereas the Z9 double bond-specific epoxidase, Hc_epo1 (CYP341B14) and Li_epo1 (CYP341B14), belonged to the CYP341 family (Fig. 5) (Rong et al., 2014, 2019). Although CYP340 and CYP341 families belong to the same Clan 4, the deduced amino acid sequence of As_epo1 shared only 9.5% identity with that of Hc_epo1 or Li_epo1. The two CYP families are considered to have diverged long before the divergence of moth

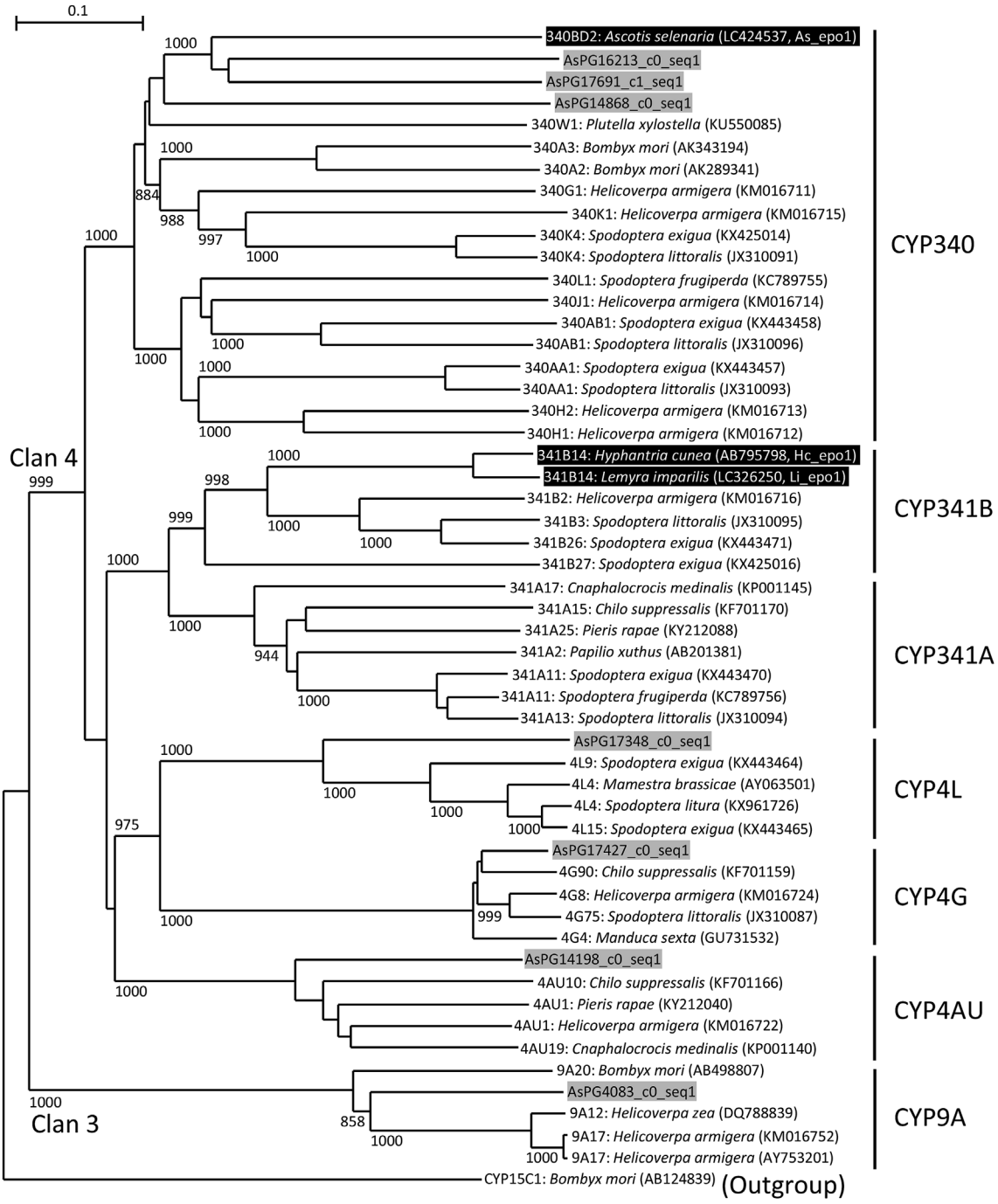

Fig. 5. Phylogenetic relationships between As_epo1, Hc_epo1, Li_epo1 and related insect CYPs. The phylogenetic tree was reconstructed using the neighbor-joining method. CYP15C1 of *Bombyx mori* (AB124839) was used as an outgroup. GenBank accession numbers and species names are shown next to the names of CYP genes. Bootstrap values after 1000 replications are shown near the branches. The branch length is drawn to the genetic distance.

families. To elucidate the evolution of the utilization of CYPs in the biosynthesis of moth sex pheromones, the identification of many more epoxidases in type-II moths, those with specificity for the Z6 double bond in particular, is needed. An analysis of CYPs expressed in the PG of moths utilizing 6-epoxyalkene pheromones is under way.

Many protein sequences belonging to CYP341 and CYP340 are deposited in the NCBI non-redundant protein sequence database (Fig. 5). It is remarkable that CYP341 and CYP340 sequences are also reported from type-I moths, such as *Helicoverpa armigera* and *Spodoptera littoralis*, which do not utilize epoxyalkene sex pheromones, and, thus, epoxidation is not included in their pheromone biosynthetic pathways. Most of these CYP sequences are deduced from genome sequences. Therefore, neither the functions nor tissue expression patterns of these genes are known. Further studies are warranted to clarify whether the proteins encoded by these genes exhibit epoxidase activity. Since moth species belonging to relatively recently diverged groups (Geometridae and Erebidae) utilize type-II pheromones, the production of type-II pheromones and, thus, the utilization of epoxidase in pheromone production, are considered to be derived traits. Information on the roles of CYP genes in type-I pheromone producers may provide an insight into how CYP genes became involved in the biosynthesis of type-II pheromones.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibmb.2019.03.002.

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