Int. J. Indust. Entomol. Vol. 4, No. 2, 2002, pp. 155~162

International Journal of
Industrial Entomology

Molecular Cloning of Cytochrome P450 4 Family Gene Fragments from Midgut of the Beet Armyworm, *Spodoptera exigua*

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(Received 15 April 2002; Accepted 10 May 2002)

Cytochrome P450 (CYP) gene has been known to play one of the most important roles in metabolizing the exogenous materials. In insect, CYP is particularly known to detoxify toxic materials by adding oxygen molecule to the hydrophobic region of the materials. Thus, CYP-dependent metabolism is associated with the adaptation of insect to host plant chemicals. This in turn is known to be one of the driving forces for CYP diversification. In the present study, we cloned seven gene fragments of CYP 4 (*CYP4*) family from the midgut of the beet armyworm, *Spodoptera exigua*, through RT-PCT. Sequence analysis of the product showed the gene fragment to contain an open reading frame of ~ 150 amino acids, consisted of ~ 450 bp. The cloned gene fragments contained typical, conserved regions found in *CYP4* family. Pairwise comparison of the deduced amino acid sequences among seven clones ranged in divergence from 0% to 52.86% and resulted in five distinct clones. The other two clones were identical or differ by one amino acid respectively to the corresponding clone, although each differed by ten nucleotides. Analysis of correlation between GenBank-registered, full length *CYP4* and the cloned fragments resulted in statistically significant relationship ($r^2 = 0.96085$; $p < 0.001$), suggesting utility of the partial sequences as such full-length sequences. Phylogenetic analysis of the clones with GenBank-registered insect and mammal *CYP4* family sequences by parsimony and several distance methods subdivided the clones into two groups: ones belonging to *CYP4S* and the others to *CYP4M* families.

Key words : *Spodoptera exigua*, Armyworm, Cytochrome P450, *CYP4* family, RT-PCR, Phylogeny

Introduction

Living organisms are always exposed to external materials that are toxic to the host as well as essential for energy production. Living organisms, thus, have evolved to develop a system of detoxifying toxic materials. Insect is one good example that had successfully adjusted to the environmental change by developing detoxifying system against host plant (Pichersky and Gang, 2000). Once plants invent a second metabolite, which kills insects as a defense system, insects again find a key system against this metabolite. This is the mechanism by which insects and plants have co-evolved. One of such key found by insects is a system, which transforms hydrophobic second metabolite of plant into hydrophilic compound (Schuler, 1996; Scott *et al.*, 1998).

In general, hydrophobic material is absorbed into the tissue faster and remains longer in the tissue than hydrophilic material. Thus, detoxifying procedure means a process that changes the hydrophobic material into the hydrophilic material. Detoxifying procedure is divided into Phase I and Phase II. One of the enzymes representing Phase I is Mixed-Functional Oxidase (MFO). MFO includes all the enzymes that make the substrate hydrophilic by adding one molecule of oxygen to substrate (*e.g.*, cytochrome P450 and cytochrome P450

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dependent reductase). Cytochrome P450 (*CYP*) exits as families and insect has families *CYP4*, *CYP* 6, *CYP* 9, *CYP12*, and *CYP28* (Scott *et al.*, 1998). Although families 6, 9, 12, and 28 are specific to insect, family 4 is found commonly in vertebrates, plants and microorganisms. This wide distribution of *CYP4* family allowed ones to believe that all the *CYP* families were evolved from common origin. At present, many of *CYP* genes have been identified in a diverse insect species, but their detailed function is still in question. *CYP6D1* in housefly (Lee and Scott, 1992; Liu and Scott, 1996; Korytko and Scott, 1998) and *CYP6B* family in the *Papilio* species are known to have a function related to resistance to pyrethroid insecticide and to metabolism of furanocoumarin in host plant, respectively (Cohen *et al.*, 1992; Hung *et al.*, 1995). In case of *CYP4* family, it has been known that gene expression in *Drosophila* is enhanced by treatment of alkaloid, suggesting that *CYP4* family is also related to metabolism of exogenous material (Danielson *et al.*, 1998).

The beet armyworm, *Spodoptera exigua*, is a pest found in a welsh onion and has more than one hundred host plants. Thus, we think that *CYP4* family may be important genes in adaptation of *S. exigua* to various host plants. In this study, *CYP4* family gene fragments were cloned from the midgut of the species and relations of the genes to other *CYP4* subfamilies were discussed.

Materials and Methods

The larvae of the beet armyworm, *Spodoptera exigua*, were obtained from the Department of Crop Protection, The National Institute of Agricultural Science & Technology, Rural Development Administration, Korea.

RT-PCR, cloning and sequencing

Midgut of *S. exigua* was extracted and washed in 1 × SSC. It was frozen in the liquid nitrogen and ground into fine powder. Total RNA was isolated from 30 mg of midgut power by using Total RNA Extraction Kit (Promega). Total RNA was identified by electrophoresis and quantified by measuring UV absorption at 260 nm. Degenerative primer was designed on the basis of the conserved region of CYP4 family protein (Table 1). The primer sequences are as follows: *CYP4* up-primer, 5-GA(C/T) ACITT(C/T)ATGTT(C/T)GA(A/G)GGIGA(C/T)AC-3 and *CYP4* down-primer, 5-GC(A/G)AA(T/C)TGICC(A/G/T) TTIC(T/G)IGGICC-3, where I indicates inosine.

The first strand of cDNA was synthesized by PCR at the condition of 45°C for 45 min and 94°C for 2 min, followed by 40 cycles of 94°C for 30 sec, 45°C for 1 min, and 68°C for 2 min. The final extension was performed at 68°C for 9 min. PCR products were identified on 0.7% agarose gel. The expected product bands were excised from the gel and dialyzed for elusion. The PCR products for sequencing were cloned into pGEM-T vector (Promega). Plasmids were extracted by using Wizard plus SV Miniprep Kit (Promega) and successful cloning was detected both by 0.7% agarose gel and enzyme digestion (EcoR I). The plasmids were sequenced using an automatic sequencer (model 377, PE Applied Biosystems).

Data analysis

Nucleotide sequences of *S. exigua* clones were compared to protein sequences incorporated into GenBank by using BLASTX program on the NCBI Blast Server. Sequence identity among full-length *CYP4* and among partial *CYP4* were performed in the Clustal W (ver. 1.8; Jeanmougin *et al.*, 1998). PAUP (Phylogenetic Analysis using Parsimony) ver. 3.1 (Swofford, 1993) was used to infer possible phy-

Table 1. Conserved regions of *CYP4* gene of insect species utilized for primer design

Species	Upstream	Family type	GenBank Accession number	References
Tropical cockroach (*Blaberus discoidals*)	N-**DTFMFEGHDT**-C C-**GPRNCIGQKFA**-N	CYP4C1	P29981	Bradfield *et al.* (1991)
Pacific beetle cockroach (*Diploptera punctata*)	N-**DTFMFAGHDT**-C C-**GPRNCIGQKFA**-N	CYP4C7	AAC69184	Sutherland *et al.* (1998)
Fruit fly (*D. melanogaster*)	N-**DTFMFKGHDT**-C C-**GPRNCIGQKFA**-N	CYP4D2	AAB71182	Unpublished
Fruit fly (*D. melanogaster*)	N-**DTFMFEGHDT**-C C-**GPRNCIGQKFA**-N	CYP4D2	Q27589	Frolov and Alatortsev (1994)
Tabacco hornworm (*Manduca sexta*)	N-**DTFMFEGHDT**-C C-**GPRNCIGQKFA**-N	CYP4M2	S66374	Snyder *et al.* (1995)

Bold-faced letters indicate identical amino acid residues among insect species compared.
Sequences at the upper line at each column represent conserved sequences in the upstream region and those at lower line represent conserved sequences in the down stream region.

logenetic relationships among *CYP4* family sequences cloned in this study and the GeneBank-registered *CYP4* family sequences of some insects and mammals. The homologous *CYP4* family gene of house mouse, *Mus musculus* (GenBank accession number Q64462; Imaoka *et al.*, 1995), was used as an outgroup. The reliability of

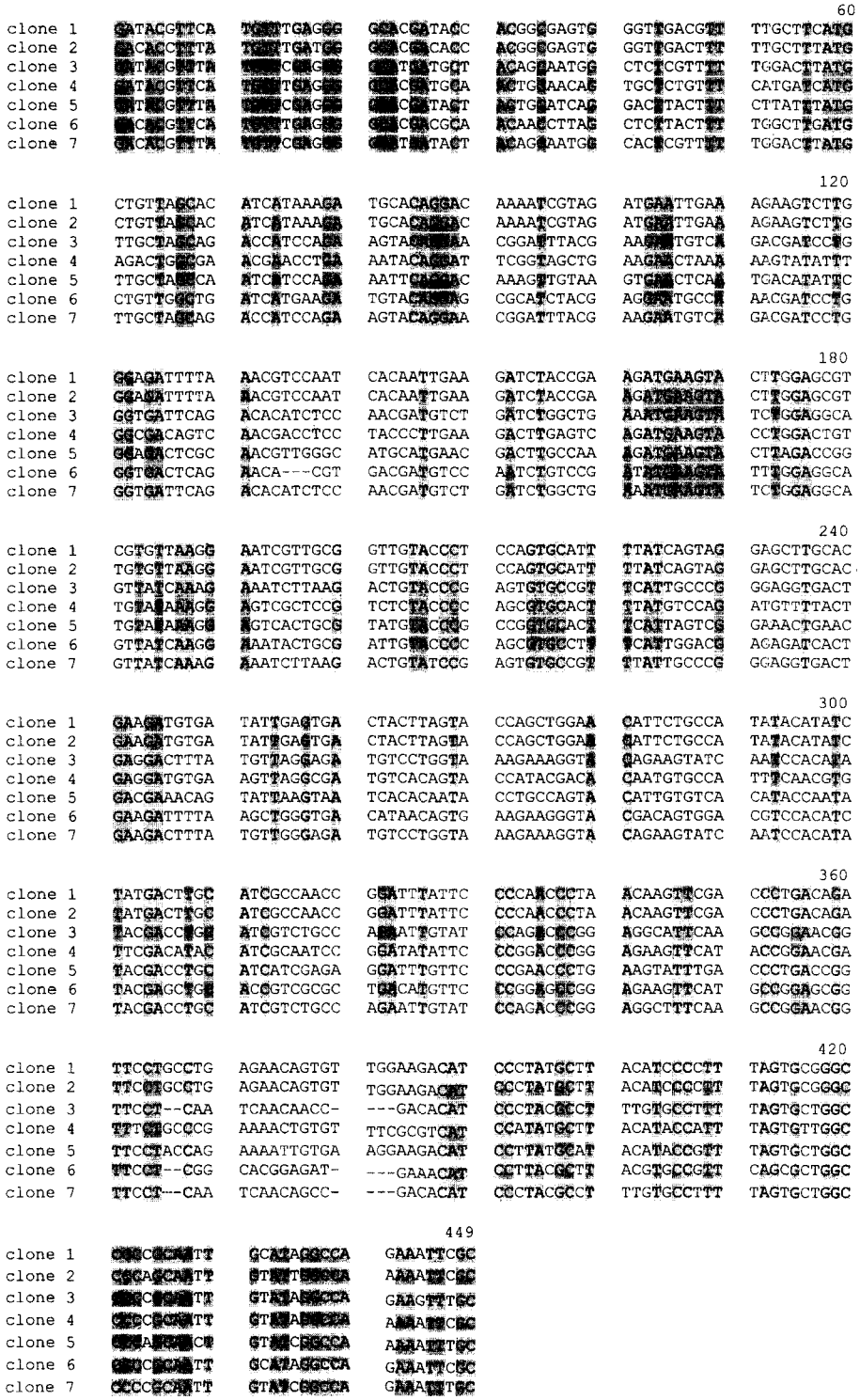

Fig. 1. Alignment of cDNA segment of seven *CYP4* gene fragments from *Spodoptera exigua*. Identical sequences are boxed with gray color.

the trees was tested by 100 iterations of bootstrapping (Felsenstein, 1985). As an alternative to the parsimony analysis, we used subprograms such as NEIGHBOR, KITSCH, and FITCH incorporated in PHYLIP (Phylogeny Inference Package) ver. 3.5c (Felsenstein, 1993). To obtain phylogenetic tree, the data set was first iterated 100 times using the subprogram SEQBOOT. Next, the iterated data set was run using the subprogram PRODIST to obtain distance matrix between pairs of amino acid sequences. Individual trees from each distance matrix were obtained using the subprograms and a consensus tree representing reliability at each branch was obtained using the subprograms CONSENSE, respectively. Input method was chosen for random input in the NEIGHBOR and global rearrangement for FITCH, but jumbling option was omitted to save computer hour in all cases.

Results and Discussion

Cloning, sequencing and characterization of the *CYP4* gene fragments

Purified CYP protein from a diverse organism showed the presence of a conserved region in the protein, called heme site (Scott *et al.*, 1994; Snyder *et al.*, 1996; Feyereisen, 1999). The site is consisted of four of its residues (F--G--C-G) and recognized as a signature motif of cytochrome P450. Along with this site, *CYP4* family has one more conserved region. This region corresponds to a portion of helix I in the tertiary structure of cytochrome P450cam, which is distal to the heme and is thought to be important in the activation of molecular oxygen and catalysis (Scott *et al.*, 1994; Snyder *et al.*, 1996; Feyereisen, 1999). Sequence alignment of a few cytochrome P450 *CYP4* subfamilies from the cockroach (*Blaberus discoidals*), the pacific cockroach (*Diploptera punctata*), the fruit fly (*Drosophila melanogaster*), and tabacco hormworm (*Manduca sexta*), in fact, revealed these two highly conserved stretches of amino acid (Table 1). In order to clone the genes of cytochrome P450 *CYP4* family from the midgut of *S. exigua*, degenerative primer was designed on the basis of these conserved sequences (Table 1).

From the midgut of *S. exigua*, we obtained a PCR product with ~ 450 bp (base pair) by RT-PCR (data not shown) and this fragment was cloned into pGEM-T vector (Promega). Twenty colonies were selected, and sequenced, and eventually provided us seven gene fragments with the size of 440 bp, 443 bp, and 449 bp (Fig. 1). Among the seven clones, clones 1 and 2 were variable in ten nucleotide sites (data not shown), but differ by only one residue in terms of amino acid (Table 2). Clones 6 and 7 also showed ten nucleotide difference (data not shown),

Table 2. Pairwise comparison among partial amino acid sequences of the seven clones of *CYP4* family gene from *Spodoptera exigua* midgut

	1	2	3	4	5	6	7
Clone 1	-	0.67	43.24	36.00	28.67	47.14	44.90
Clone 2	1	-	43.24	35.33	29.33	47.14	47.14
Clone 3	65	65	-	44.59	47.97	28.57	28.57
Clone 4	54	53	67	-	36.67	47.86	47.86
Clone 5	43	44	72	55	-	52.86	52.86
Clone 6	71	71	43	72	79	-	0
Clone 7	71	71	43	72	79	0	-

Numbers above the diagonal are percent divergence; numbers below the diagonal are actual numbers of amino acid difference.

but identical in their deduced amino acid sequences (Table 2). Excluding these similar clones (clones 2 and 7), amino acid divergence among them ranged from 28.57% (43 amino acid residues) to 52.86% (79 amino acid residues). Considering substantially large amino acid difference among the five clones, the very similar clones, 2 and 7, appear to be allelic variants to clones 1 and 6, respectively. The length of the protein ranged from 147 to 150 amino acid residues including primer sequences (Fig. 2). All of the deduced amino acid sequences contained the regions found typically in all *CYP4* families: ΦΦKE-LRΦ-P, where Φ is hydrophobic amino acid and a PERF motif (P-RF) (Fig. 2).

Correlation between partial and total fragments of *CYP4*

From the beginning, because we aimed to clone only the fragments of *CYP4* family using conserved sequences in the *CYP4* family, utility of such PCR products may not necessary reveal accurate levels of sequence homology among them and among organisms as the full-length genes do. More fundamentally, designation of the partially coned sequences may not confidentially provide the basis for the assignment of the given sequences to the existing type of the gene. This may be especially true when the partial data are utilized for the inference of evolution and multigene family diversity. Thus, we obtained ~ 40 GenBank-registered full-length amino acid sequences of *CYP4* family and investigated correlation of the levels of partial amino acid sequence homology to the full-length sequence by statistical analysis (Fig. 3). The result showed a high correlation between total and fragment amino acid sequences of *CYP4* family ($r^2 = 0.96085$) and the estimate was statistically significant at the level of $P < 0.001$. Thus, this partial sequence of *CYP4* family can be confidentially used to predict full-length sequence identity and this

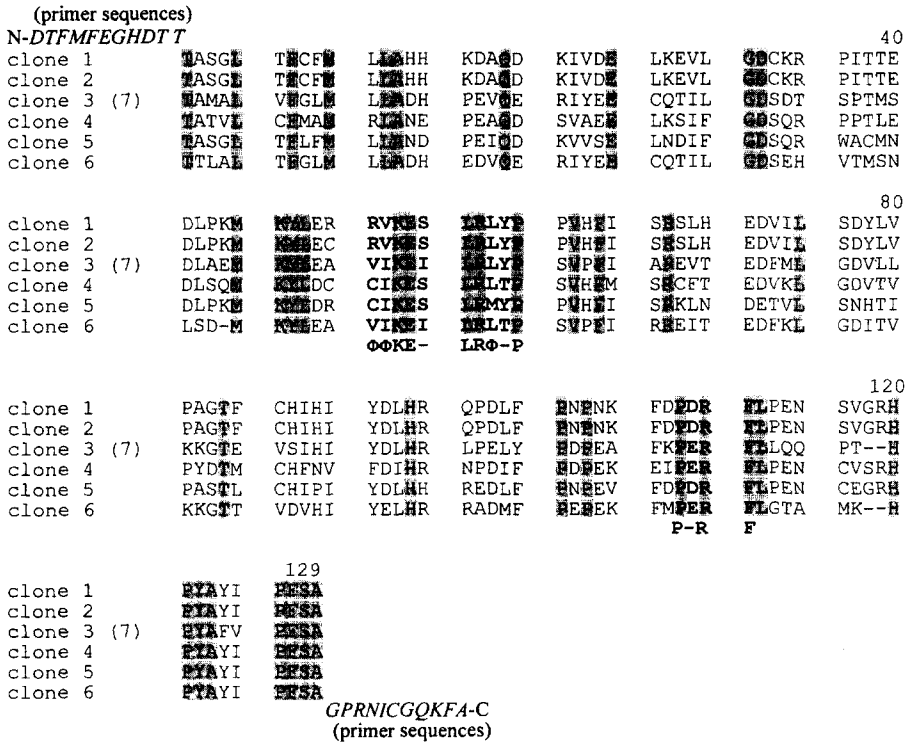

Fig. 2. Alignment of deduced amino acid sequences for the *CYP4* gene fragments from *Spodoptera exigua* isolated by RT-PCR. Identical sequences are boxed with gray color. The common "ΦΦKE-LRΦ-P" and "P-RF" amino acids in the *CYP4* gene are bold-faced, where Φ is hydrophobic amino acid.

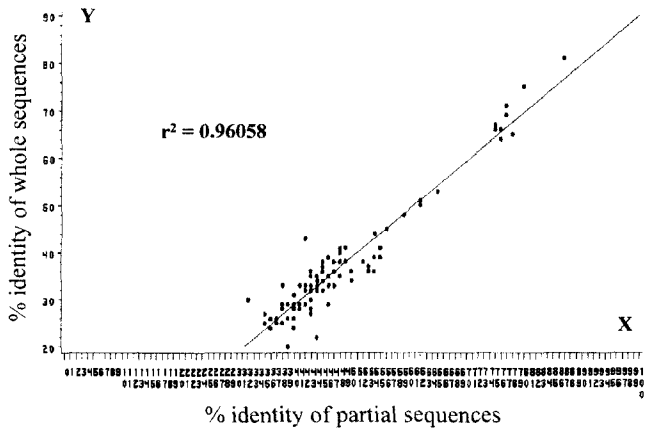

Fig. 3. Interrelationship between the percent identities of whole CYP4 proteins and the percent identity of the fragment of the proteins.

resultantly can be further utilized for the inference of the phylogenetic relationships of the cloned *CYP4* family sequences.

Relationships among clones and other insect *CYP4* family

For the understanding of the phylogenetic relationships among cytochrome P450 *CYP4* family from the diverse organisms, GenBank-registered data were extensively searched, and those with enough length were selected and trimmed for phylogenetic analysis. This effort resulted in a total of 33 amino acid sequences. These were 26 *CYP4* family gene sequences from insects, one from crayfish, and six from mammal species. Their GenBank accession number, scientific name, type of *CYP4* family, and their references are listed in Table 3. Pairwise comparison among these *CYP4* family genes including six clones from this study resulted in amino acid sequence divergence ranging from 0.33% (5 amino acid residues) to 62.9% (96 amino acid residues) (data no shown). The least divergence was observed in a comparison between two sequences of *Drosophila melanogaster* (GenBank number AAF47649 and AAB71182), belonging to the same family, *CYP4D*. The highest divergence was observed in a comparison between one of the *D. melanogaster* (GenBank number AAF48426; *CYP4S*) and *Mus musculus* (GenBank number NP_31848; *CYP4A*).

To infer phylogenetic relationships of the cloned *CYP4* family sequences to the previously reported gene family, 33-GenBank-registered *CYP4* family genes and five cloned *CYP4* family sequences (clones 1 and 2 and clones 6 and 7, respectively, were treated as each one clone because of

Table 3. List of *CYP4* family gene sequences obtained from GenBank

	GenBank Accession Number	Common name	Scientific name	Family type	Reference
1.	AAD33076	Cotton bollworm	*Helicoverpa armigera*	CYP4S1	Unpublished
2.	AAD33075	Cotton bollworm	*H. armigera*	CYP4S2	Unpublished
3.	AAD33080	Cotton bollworm	*H. armigera*	CYP4M4	Unpublished
4.	AAD33077	Cotton bollworm	*H. armigera*	CYP4G8	Unpublished
5.	AAD33078	Cotton bollworm	*H. armigera*	CYP4G9	Unpublished
6.	S66374	Tabacco hornworm	*Manduca sexta*	CYP4M2	Snyder *et al.* (1995)
7.	AAC03111	Australian termite	*Coptotermes acinaciformis*	CYP4U1	Unpublished
8.	P29981	Tropical cockroach	*Blaberus discoidalis*	CYP4C1	Bradfield *et al.* (1991)
9.	AAF70178	Red flour beetle	*Tribolium castaneum*	CYP4Q4	Unpublished
10.	AAC69184	Pacific beetle cockroach	*Diploptera punctata*	CYP4C7	Sutherland *et al.* (1998)
11.	AAF70496	Red flour beetle	*Tribolium castaneum*	CYP4Q7	Unpublished
12.	AAD41103	Red house mosquito	*Culex pipiens pallens*	CYP4	Unpublished
13.	AAD41104	Red house mosquito	*C. p. pallens*	CYP4	Unpublished
14.	AAF48049	Fruit fly	*Drosophila melanogaster*	CYP4G15	Adams *et al.* (2000)
15.	AAF58091	Fruit fly	*D. melanogaster*	CYP4AA1	Adams *et al.* (2000)
16.	AAF57098	Fruit fly	*D. melanogaster*	CYP4C3	Adams *et al.* (2000)
17.	AAF48426	Fruit fly	*D. melanogaster*	CYP4S3	Adams *et al.* (2000)
18.	AAF52233	Fruit fly	*D. melanogaster*	CYP4AC2	Adams *et al.* (2000)
19.	AAF52232	Fruit fly	*D. melanogaster*	CYP4AC1	Adams *et al.* (2000)
20.	AF52234	Fruit fly	*D. melanogaster*	CYP4AC3	Adams *et al.* (2000)
21.	Q9V559	Fruit fly	*D. melanogaster*	CYP4P3	Adams *et al.* (2000)
22.	Q27589	Fruit fly	*D. melanogaster*	CYP4D2	Frolov and Alatortsev (1994)
23.	AAF50549	Fruit fly	*D. melanogaster*	CYP4D8	Adams *et al.* (2000)
24.	O46051	Fruit fly	*D. melanogaster*	CYP4D14	Adams *et al.* (2000)
25.	AAF47649	Fruit fly	*D. melanogaster*	CYP4D20	Adams *et al.* (2000)
26.	AAB71182	Fruit fly	*D. simulans*	CYP4D2	Unpublished
27.	AF091117	Spinycheek crayfish	*Orconectes limosus*	CYP4C15	Dauphin-Villemant *et al.* (1999)
28.	U39206	Norway rat	*Rattus norvegicus*	CYP4F4	Kawashima and Strobel (1995)
29.	U39208	Norway rat	*R. norvegicus*	CYP4F6	Kawashima and Strobel (1995)
30.	AAC08589	Human	*Homo sapiens*	CYP4	Unpublished
31.	NP_001073	Human	*H. sapiens*	CYP4B4	Simpson (1997)
32.	NP_031848	House mouse	*Mus musculus*	CYP4A14	Heng *et al.* (1997)
33.	Q64462	House mouse	*M. musculus*	CYP4B1	Imaoka *et al.* (1995)

their sequence similarity or identity) were subjected to PAUP and PHYLIP analyses. Parsimony-based PAUP analysis (Fig. 4) showed that the five clones obtained in this study were subdivided into two groups: one including clones 1 (2), 4, 5 and the other including 3, 6 (7). The subdivision and clustering were somewhat robust in that each group was supported by relatively high bootstrap values (63 and 98, respectively). The first group included two types of *CYP4S* from cotton bollworm (GenBank numbers AAD33076 and AAD33075) and one type of fruit fly (AAF48426), and the second group included each one type of *CYP4M* from cotton bollworm (AAD33080) and tabacco hornworm (S66374) (Fig. 4). This result suggested us that the midgut of *S. exigua* clearly possesses at least two subfamilies of *S. exigua CYP4* sequences by this analysis. PHYLIP analysis by neighbor-joining method also supported presence of two subfamilies among the clones obtained in this study (Fig. 5). However, *CYP4S* from fruit fly (AAF48426) was placed at a different group containing several other *CYP4* subfamilies (Fig. 5). This sepa-

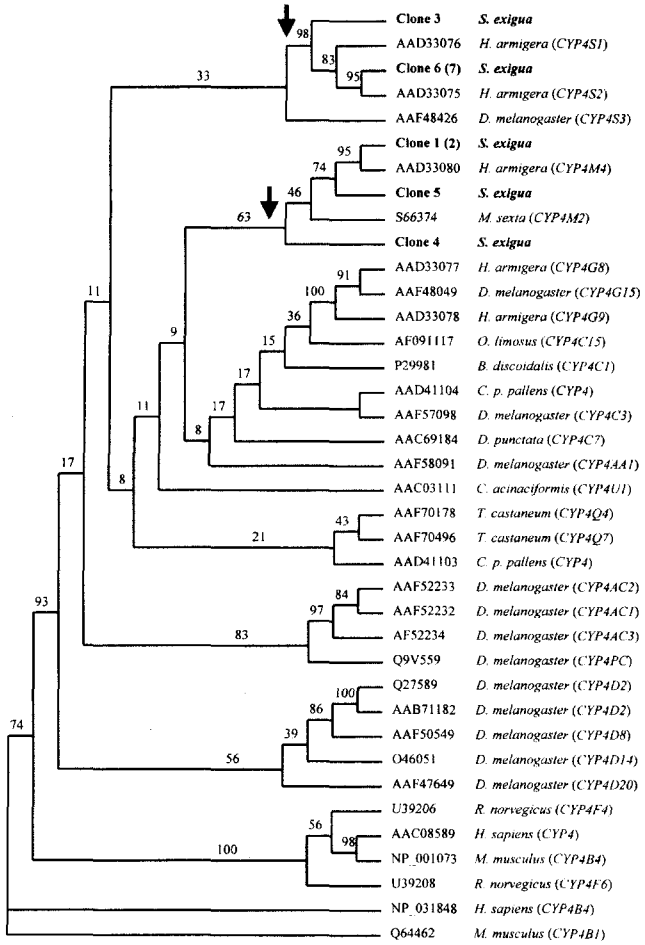

Fig. 4. PAUP analysis of 38 *CYP4* family genes. The tree shown is an unordered tree obtained with the option of "retain groups with frequency <50%" by majority-rule consensus of one most parsimonious tree from the heuristic search. The numbers shown on the branches represent bootstrap values for 100 replicates. Tree length is 1,424 steps, Consistency Index is 0.515, and Retention Index is 0.535. The arrow indicates the place of clustering of the cloned *CYP4* gene with the gene from other species.

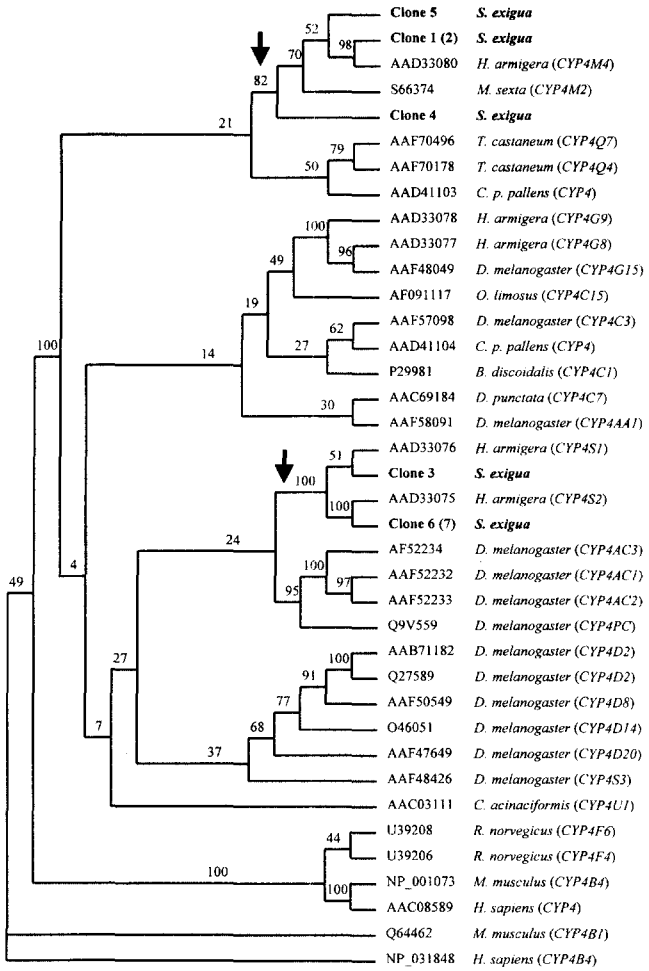

Fig. 5. PHYLIP analysis of 38 *CYP4* family genes. The tree was obtained using the subprogram NEIGHBOR incorporated in PHYLIP. The tree was rooted using Q64462 (*CYP4B1*; *Mus musculus*). The number shown on branches, which represent bootstrap values for 100 replications, were obtained using the subprogram CONSENSUS. The arrow indicates the place of clustering of the cloned *CYP4* gene with the gene from other species.

ration instead clustered the remaining members more robustly (100% of bootstrapping value). These phylogenetic analyses consistently suggest that the clones 1 (2), 4, 5 are belonged to *CYP4M* family and clones 3 and 6 (7) are belonged to *CYP4S* family. Although we also performed other distance-based analyses (*e.g.*, FITCH and KITCH), we did not present here because the topology obtained from these analyses were almost identical to the analyses provided here.

In conclusion, we report here molecular cloning of two subfamilies of *CYP4* from the midgut of the beet armyworm, *Spodoptera exigua* by RT-PCR. Seven fragments were successfully amplified using the degenerative primers designed from conserved regions of other homologous, insect gene sequences, and the presence of typical conserved region was also found. Phylogenetic analysis confirmed that clones 3 and 6 (7) are belonged to *CYP4S* family, whereas clones 1 (2), 4, and 5 are belonged to *CYP4M* family.

References

Adams, M. D. *et al.* (2000) The genome sequence of *Drosophila melanogaster*. *Science* **287**, 2185-2195.

Bradfield, J. Y., Y. H. Lee and L. L. Keeley (1991) Cytochrome

P450 family 4 in a cockroach: molecular cloning and regulation by regulation by hypertrehalosemic hormone. *Proc. Natl. Acad. Sci. USA* **88**, 4558-4562.

Cohen, M. B., M. A. Schuler and M. R. Berenbaum (1992) A host-inducible cytochrome P450 from a host-specific caterpillar: molecular cloning and evolution. *Proc. Natl. Acad. Sci. USA* **89**, 10920-10924.

Danielson, P. B., J. L. M. Foster, M. M. Mamahill and M. K. Smith (1998) Induction by alkaloids and Phenobarbital of family 4 cytochrome P450s in *Drosophilla*: evidence for involvement in host plant utilization. *Mol. Gen. Genet.* **259**, 54-59.

Dauphin-Villemant, C., D. Bocking, M. Tom, M. Maibeche and R. Lafont (1999)

Cloning of a novel cytochrome P450 (CYP4C15) differentially expressed in the steroidogenic glands of an arthropod. *Biochem. Biophys. Res. Commun.* **264**, 413-418.

Felsenstein, J. (1993) PHYLIP (Phylogenetic Inference Package). Department of Genetics, Univ. of Washington, Seattle.

Feyereisen, R. (1999) Insect P450 enzymes. *Ann. Rev. Entmol.* **44**, 507-533.

Frolov, M. V. and V. E. Alatortsev (1994) Cluster of cytochrome P450 genes on the X chromosome of *Drosophila melanogaster. DNA Cell Biol.* **13**, 663-668.

Heng, Y. M., C. S. Kuo, P. S. Jones, R. Savory, R. M. Schulz, S. R. Tomlinson, T. J. Gray and D. R. Bell (1997) A novel murine P-450 gene, Cyp4a14, is part of a cluster of Cyp4a and Cyp4b, but not of CYP4F, genes in mouse and humans. *Biochem. J.* **325**, 741-749.

Hung, C. F., M. R. Berenbaum and M. A. Schuler (1995) CYP6B3: a second furanocoumarin-inducible cytochrome P450 expressed in *Papilio polyxenes. Insect Mol. Biol.* **4**, 149-160.

Imaoka, S., T. Hiroi, Y. Tamura, H. Yamazaki, T. Shimada, M. Komori, M. Degawa and Y. Funae (1995) Mutagenic activation of 3-methoxy-4-aminoazobenzene by mouse renal cytochrome P450 CYP4B1: cloning and characterization of mous CYP4B1. *Arch. Biochem. Biophys.* **321**, 255-262.

Jeanmougin, F., J. D.Thompson, M. Gouy, D. G. Higgins and T. J. Gibson (1998) Multiple sequence alignment with Clustal X. *Trends Biochem. Sci.* **23**, 403-405.

Kawashima, H. and H. W. Strobel (1995) cDNA cloning of three new forms of rat brain cytochrome P450 belonging to the CYP4F subfamily. *Biochem. Biophys. Res. Commun.* **217**, 1137-1144.

Korytko, P. J. and J. G. Scott (1998) CYP6D1 protects thoracic ganglia of house flies from the neurotoxic insecticide cypermethrin. *Arch. Insect Biochem. Physiol.* **37**, 53-57.

Lee, S. S. T. and J. G. Scott (1992) Tissue distribution of microsomal cytochrome P-450 monooxygenases and their inducibility by phenobarbital in house fly *Musca domestica* L. *Insect Biochem. Mol. Biol.* **22**, 699-711.

Liu, N. and J. G. Scott (1996) Genetic analysis of factors controlling elevated cytochrome P450, CYP6D1, cytochrome b, P450 reductase and monooxygenase activities in LPR house flies, *Musca domestica. Biochem. Genet.* **34**, 133-148.

Pichersky, E. and R. Gang (2000) Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends Plants Sci.* **5**, 439-445.

Schuler, M. A. (1996) The role of cytochrome P450 monooxygenases in plant-insect interactions. *Plant Physiol.* **112**, 1411-1419.

Scott, J. A., F. H. Collins and R. Feyereisen (1994) Diversity of cytochrome P450 genes in the mosquito, *Anopheles albimanus. Biochem. Biophys. Res. Commun.* **205**, 1452-1459.

Scott, J. G., N. Liu and Z. Wen (1998) Insect cytochromes P450: diversity, insecticide resistance and tolerance to plant toxins. *Comp. Biochem. Physiol.* **121**, 147-155.

Simpson, A. E. (1997) The cytochrome P450 4 (CYP4) family. *Gen. Pharmacol.* **28**, 351-359.

Snyder, M. J., J. L. Stevens, J. F. Andersen, R. Feyereisen (1995) Expression of cytochrome P450 genes of the CYP4 family in midgut and fat body of the tobacco hornworm, *Manduca sexta. Arch. Biochem. Biophys.* **321**, 13-20.

Snyder, M. J., J. A. Scott, J. F. Anderson and R. Feyereisen (1996) Sampling of P450 diversity by cloning polymerase chain reaction products obtained with degenerate primers. *Method Enzymol.* **272**, 304-312.

Sutherland, T. D., G. C. Unnithan, J. F. Andersen, P. H. Evans, M. B. Murataliev, L. Z., E. A. Mash, W. S. Bowers and R. Feyereisen (1998) A cytochrome P450 terpenoid hydroxylase linked to the suppression of insect juvenile hormone synthesis. *Proc. Natl. Acad. Sci. USA* **95**, 12884-12889.

Swofford, D. L. (1993) PAUP (phylogenetic analysis using parsimony), ver 3.1. Illinois Natural History Survey, Champaign, IL.