

cDNA and Deduced Amino Acid Sequences of Apolipophorin-IIIs From *Bombyx mori* and *Bombyx mandarina*

Yoshio Yamauchi,¹ Charles Hoeffler,² Alvin Yamamoto,² Hajime Takeda,³ Ren Ishihara,⁴ Hideaki Maekawa,⁵ Ryouichi Sato,¹ Seong Su-II,⁶ Motoyuki Sumida,⁷ Michael A. Wells,^{2*} and Kozo Tsuchida⁵

¹Graduate School of Bio-Application and Systems Engineering, Tokyo University of Agriculture and Technology, Japan

²Department of Biochemistry and Center for Insect Science, University of Arizona, Tucson

³Department of Biological Production, Tokyo University of Agriculture and Technology, Japan

⁴Department of Agriculture, Nihon University, Japan

⁵Division of Radiological Protection, National Institute of Infectious Diseases, Japan

⁶Department of Biology, University of Suwon, Korea

⁷Kyoto Institute of Technology, Kyoto, Japan

The cDNA sequence for apolipophorin-III from two strains of *Bombyx mori* (N4 and P50) and the Japanese and Chinese strains of *Bombyx mandarina* were determined. Both the cDNA and deduced amino acid sequences of the four apolipophorin-IIIs were highly similar (95–98%). The four *Bombyx* sequences also showed significant similarity to the sequence of apolipophorin-III from another lepidopteran, *Manduca sexta* (83–84%), particularly in the five amphipathic α -helices that are proposed to play a critical role in the binding of apolipophorin-III to lipophorin. In the coding region, the nucleotide sequences for the Chinese strain of *B. mandarina* and the P50 strain of *B. mori* were identical, supporting the suggestion that P50 is the current strain most closely related to the original domesticated strain. The N4 strain of *B. mori* is more closely related to these two strains than is the Japanese strain of *B. mandarina*, suggesting that Japanese strain of *B. mandarina* separated from the Chinese strain of *B. mandarina* before domestication of *B. mori*. Arch. Insect Biochem. Physiol. 43:16–21, 2000. © 2000 Wiley-Liss, Inc.

Key words: *Bombyx mori*; *Bombyx mandarina*; apolipophorin-III

Abbreviations used: apoLp-III = apolipophorin-III.

These sequences have been deposited in GenBank under the following accession numbers: *Bombyx mori* N₄ = U59243; *Bombyx mori* p50 = U59244; *Bombyx mandarina* (Japanese strain) = U59242; *Bombyx mandarina* (Chinese strain) = AF001289.

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*Correspondence to: Dr. Michael A. Wells, Department of Biochemistry, Biological Sciences West PO Box 210088, The University of Arizona, Tucson AZ 85721-0088. E-mail: mawells@u.arizona.edu

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INTRODUCTION

Apolipoprotein-III (ApoLp-III*) plays a critical role in the transport of lipids during flight in several species of insects (reviewed by Soulages and Wells, 1994). ApoLp-III assists in the loading of diacylglycerol, generated from triacylglycerol stores in the fat body through the action of adipokinetic hormone, into the hemolymph lipoprotein lipoprotein. Such loading is an obligatory part of the pathway that transfers lipid from the fat body to the flight muscle, where the lipid is used to fuel flight. Previously, we compared the amino acid sequence of apoLp-III from distantly related insects and concluded that, although these exchangeable apolipoproteins share a common function, precise amino acid sequence identity was not as important as the conservation of a common structural feature, namely the presence of five amphipathic helices (Smith et al., 1994).

In the current study, we were interested in determining the similarity between the sequences of apoLp-III from two closely related species, *Bombyx mori* and *Bombyx mandarina*. It is generally agreed that the domestic silkworm, *B. mori*, was derived from the wild silkworm, *B. mandarina*, about 5,000 years ago in China. Thus, we have determined the cDNA and deduced amino acid sequences of apoLp-III from *B. mori* strain N4, which is a Southeast Asian strain of *B. mori*; *B. mori* P50 (Daizo), which is a Chinese strain of *B. mori*; and the Chinese and Japanese strains of *B. mandarina*. We further compared the sequence of the Bombyx sequences to another Lepidopteran, *Manduca sexta*.

MATERIALS AND METHODS

Insects

B. mandarina were reared on fresh mulberry leaves at 25°C under a 12-h light:12-h dark cycle. *B. mori* were reared on an artificial diet under the same conditions. The Chinese strain of *B. mandarina* was collected from mulberry trees in Tongxiang City, Hangzhou, People's Republic of China.

Purification of ApoLp-III and Antibody Production

ApoLp-III was purified from adult N4 *B. mori* following the procedure of Wells et al. (1985). Antibodies were prepared in rabbits.

cDNA Cloning and Sequencing

Japanese strain of *B. mandarina* ApoLp-III. Fat body from last day pupae was removed, rinsed with ice-cold PBS, quick frozen in liquid N₂, and stored at -80°C until used. mRNA was prepared from 0.1 g of pupal fat body using a QuickPrep Micro mRNA Purification Kit (Pharmacia Biotech, Piscataway, NJ). cDNA was synthesized using an oligo(dT)₁₂₋₁₈ primer and a TimeSaver cDNA Synthesis Kit (Pharmacia Biotech). The cDNA was ligated to λExCell EcoR1/CIP (Pharmacia Biotech, Piscataway, NJ) using a DNA Ligation Kit (TaKaRa, Tokyo, Japan). The DNA was then packaged in vitro using the Gigapack II Gold Packaging Kit (Stratagene, La Jolla, CA). The phage were plated onto NZYM plates with *Escherichia coli* strain NM522 (Pharmacia Biotech, Piscataway, NJ) as the host, and then transferred to nitrocellulose filters. The filters were probed with rabbit anti-*B. mori* N4 ApoLp-III antibodies and Peroxidase Conjugated Anti-Rabbit IgG Goat Serum (Jucson Lab, Tokyo, Japan). Bacteriophage containing ApoLp-III clones were used to infect *E. coli* strain NP66 (Pharmacia Biotech), which enables in vivo release of pExCell, a circular, autonomously replicating phagemid. The pExCell DNA was purified using a Plasmid Maxi Kit (Qiagen, Valencia, CA) and digested with EcoR1. The clones with the longest insert were sequenced using the BcaBest Dideoxy Sequencing Kit (TaKaRa), [α -³²P]-dCTP, and T7 and SP6 primers.

***B. mori* N4 ApoLp-III.** The procedure for mRNA extraction and cDNA synthesis was as described above. The cDNA was ligated into λ gT11, then packaged used the same kit described above. The phage were plated onto NZYM plates with *E. coli* strain Y-1090 as host and transferred to nitrocellulose and screened with the antibody described above. The ApoLp-III cDNA was subcloned into pUC 18 with a commercial kit (Pharmacia SureClone Ligation Kit) and transformed into *E. coli* JM 109 (TaKaRa). The bacteria were plated onto LB plates containing ampicillin, IPTG, and X-gal. White colonies were selected and screened for insert by restriction analysis. The clones were sequenced using the BcaBest Dideoxy Sequencing Kit (TaKaRa), [γ -³²P]-dCTP, and M13 forward and reverse primers.

	1					↓		70
BMANC	...TTCGCA	TCTCCTCCGC	ACTCAGTCTC	TCGACAGTAC	ATCATGGCCG	CCAAGTTCGT	AGTTCTCTTC	
BMORP	...TTCGCA	TCTCCTCCGC	ACTCAGTCTC	TCGACAGCAC	ATCATGGCCG	CCAAGTTCGT	AGTTCTCTTC	
BMOR4	...TTCGCA	TCTCCTCCGC	ACTCAGTCTC	TCGACAGCAC	ATCATGGCCG	GCAAGTTCGT	AGTTCTCTTC	
BMANJ	CCACTTCGCA	TCTCCTCCGC	ACTCAGTCTC	TCGACAGCAC	ATCATGGCCG	CCAAGTTCAT	AGTTCTCTTC	
	71							140
BMANC	GCCTGCATCG	CTCTGGCCCA	AGGAGCGATG	GTGCGACGCG	ACGCTCCC GA	CTTCTTCAAG	GACATCGAAC	
BMORP	GCCTGCATCG	CTCTGGCCCA	AGGAGCGATG	GTGCGACGCG	ACGCTCCC GA	CTTCTTCAAG	GACATCGAAC	
BMOR4	GCCTGCATCT	CTCTGGCCCA	AGGAGCGATG	GTACGACGCG	ACGCTCCC GA	CTTCTTCAAG	GACATCGAAC	
BMANJ	GCCTGCATCG	CTCTGGCCCA	AGGAGCGATG	GTGCGACGCG	ACGCTCCC GA	CGTCTTCAAG	GACATCGAAC	
	141							210
BMANC	ACCACACCAA	GGAGTTCCAT	AAGACTTTAG	AACAACAGTT	TAAGTTCGCTC	ACCAAGTCAA	AGGACGCACA	
BMORP	ACCACACCAA	GGAGTTCCAT	AAGACTTTAG	AACAACAGTT	TAAGTTCGCTC	ACCAAGTCAA	AGGACGCACA	
BMOR4	ACCACACCAA	GGAGTTCCAT	AAGACTTTAG	AACAACAGTT	TAAGTTCGCTC	ACCAAGTCAA	AGGACGCACA	
BMANJ	ACCACGCCAA	GGAGTTCCAG	AAGACTTTAG	AACAACAGTT	TAAGTTCGCTC	ACCAAGTCAA	AGGACGCACA	
	211							280
BMANC	GGACTTCAGC	AAGGCTTGGG	AGGACGGCTC	CGAGTCCGTG	CTGCAACAGC	TCAACGCCTT	CGCCAAGAGT	
BMORP	GGACTTCAGC	AAGGCTTGGG	AGGACGGCTC	CGAGTCCGTG	CTGCAACAGC	TCAACGCCTT	CGCCAAGAGT	
BMOR4	GGACTTCAGC	AAGGCTTGGG	AGGACGGCTC	CGAGTCCGTG	CTGCAACAGC	TCAACGCCTT	CGCCAAGAGT	
BMANJ	GGACTTCAGC	AAGGCTTGGG	AGGACGGCTC	CGACTCCGTG	CTGCAACAGC	TCAACGCCTT	CGCCAAGAGT	
	281							350
BMANC	CTCCAGGGAG	CGCTCGGAGA	CGCGAACGGC	AAGGCCAAGG	AGGCTTTGGA	ACAGTCGAGG	CAGAACATCG	
BMORP	CTCCAGGGAG	CGCTCGGAGA	CGCGAACGGC	AAGGCCAAGG	AGGCTTTGGA	ACAGTCGAGG	CAGAACATCG	
BMOR4	CTCCAGGGAG	CGCTCGGAGA	CGCGAACGCA	AAGGCCAAGG	AGGCTTTGGA	ACAGTCGAGG	CAGAACATCG	
BMANJ	CTCCAGGGAG	CGCTCGGAGA	CGCGAATGGC	AAGGCCAAGG	AGGCTTTGGA	ACAGTCGAGG	CAGAACATCG	
	351							420
BMANC	AGCGCACGGC	CGAGGAGCTC	CGCAAGGCC	ACCCTGACGT	CGAGAAGAAC	GCCACCGCCC	TCCGCGAGAA	
BMORP	AGCGCACGGC	CGAGGAGCTC	CGCAAGGCC	ACCCTGACGT	CGAGAAGAAC	GCCACCGCCC	TCCGCGAGAA	
BMOR4	AGCGCACGGC	CGAGGAGCTC	CGCAAGGCC	ACCCTGACGT	CGAGAAGAAC	GCCACCGCCC	TCCGCGAGAA	
BMANJ	AGCGCACGGC	CGAGGAGCTC	CGCAAGGCC	ACCCTGACGT	CGAGAAGAAC	GCCACCGCCC	TCCGCGAGAA	
	421							490
BMANC	GCTGCAGGCC	GCCGTGCAGA	ACACCGTGCA	GGAATCCCAG	AAGTTAGCGA	AGAAGGTGTC	CTCGAACGTG	
BMORP	GCTGCAGGCC	GCCGTGCAGA	ACACCGTGCA	GGAATCCCAG	AAGTTAGCGA	AGAAGGTGTC	CTCGAACGTG	
BMOR4	GCTGCAGGCC	GCCGTGCAGA	ACACCGTGCA	GGAATCCCAG	AAGTTAGCGA	AGAAGGTGTC	CTCGAACGTG	
BMANJ	GCTGCAGGCC	GCCGTGCAGA	ACACCGTGCA	GGAATCCCAG	AAGTTAGCGA	AGAAGGTGTC	CTCGAACGTG	
	491							560
BMANC	CAGGAGACTA	ATGAGAACT	GGCGCCCAAG	ATCAAGGCCG	CCTACGACGA	CTTCGCGAAG	AACACCCAGG	
BMORP	CAGGAGACTA	ATGAGAACT	GGCGCCCAAG	ATCAAGGCCG	CCTACGACGA	CTTCGCGAAG	AACACCCAGG	
BMOR4	CAGGAGACTA	ATGAGAACT	GGCGCCCAAG	ATCAAGGCCG	CGTACGACGA	CTTCGCGAGG	AACACCCAGG	
BMANJ	CAGGAGACTA	ATGAGAACT	GGCGCCCAAG	ATCAAGGCCG	CCTACGACGA	CTTCGCGAAG	AACACCCAGG	
	551							630
BMANC	AGGTGATCAA	GAAGATCCAG	GAGGCCGCCA	ACGCCAAGCA	GTGAGCGTCG	ATATTGAACT	CTCACAC...	
BMORP	AGGTGATCAA	GAAGATCCAG	GAGGCCGCCA	ACGCCAAGCA	GTGAGCGTCG	ATATTGAACT	CTCACAC...	
BMOR4	AGGTGATCAA	GAAGATCCAG	GAGGCCGCCA	ACGCCAAGCA	GTGAGCGTCG	ATATTGAACT	CTCACACTTA	
BMANJ	AGGTGATCAA	GAAGATCCAG	GAGGCCGCCA	ACGCCAAGCA	GTGAGCGTCG	ATATTGAACT	CTCACACTTA	
	631		657					
BMANC					
BMORP					
BMOR4	ATAAATATTT	C.....					
BMANJ	ATAAATATTT	CTTTCATATA	AAAAAAA					

Figure 1.

	1		↓		50
BMANC	<i>MAAKFVVLFA</i>	<i>CIALAQGAMV</i>	<i>RRDAPDFFKD</i>	<i>IEHHTKEFHK</i>	<i>TLEQQFNLSLT</i>
BMORP	<i>MAAKFVVLFA</i>	<i>CIALAQGAMV</i>	<i>RRDAPDFFKD</i>	<i>IEHHTKEFHK</i>	<i>TLEQQFNLSLT</i>
BMORN	<i>MAGKFVVLFA</i>	<i>CISLAQGAMV</i>	<i>RRDAPDFFKD</i>	<i>IEHHTKEFHK</i>	<i>TLEQQFNLSLT</i>
BMANJ	<i>MAAKFIVLFA</i>	<i>CIALAQGAMV</i>	<i>RRDAPDVFKD</i>	<i>IEHHAKEFQK</i>	<i>TFEQQFNLSLT</i>
	51				100
BMANC	<i>KSKDAQDFSK</i>	<i>AWKDGSESVL</i>	<i>QQLNAFAKSL</i>	<i>QGALGDANGK</i>	<i>AKEALEQSRQ</i>
BMORP	<i>KSKDAQDFSK</i>	<i>AWKDGSESVL</i>	<i>QQLNAFAKSL</i>	<i>QGALGDANGK</i>	<i>AKEALEQSRQ</i>
BMORN	<i>KSKDAQDFSK</i>	<i>AWKDGSESVL</i>	<i>QQLNAFAKSL</i>	<i>QGALGDANAK</i>	<i>AKEALEQSRQ</i>
BMANJ	<i>KSKDAQDFSK</i>	<i>AWKDGSDSVL</i>	<i>QQLNAFAKSL</i>	<i>QGALGDANGK</i>	<i>AKEALEQSRQ</i>
	101				150
BMANC	<i>NIERTAEELR</i>	<i>KAHPDVEKNA</i>	<i>TALREKLQAA</i>	<i>VQNTVQESQK</i>	<i>LAKKVSSNVQ</i>
BMORP	<i>NIERTAEELR</i>	<i>KAHPDVEKNA</i>	<i>TALREKLQAA</i>	<i>VQNTVQESQK</i>	<i>LAKKVSSNVQ</i>
BMORN	<i>NIERTAEELR</i>	<i>KAHPDVEKNA</i>	<i>TALREKLQAA</i>	<i>VQNTVQESQK</i>	<i>LAKKVSSNVQ</i>
BMANJ	<i>NIERTAEELR</i>	<i>KAHPDVEKNA</i>	<i>TALREKLQAA</i>	<i>VQNTVQESQK</i>	<i>LAKKVSSNVQ</i>
	151			186	
BMANC	<i>ETNEKLAPKI</i>	<i>KAAYDDFAKN</i>	<i>TQEVIKKIQE</i>	<i>AANAKQ</i>	
BMORP	<i>ETNEKLAPKI</i>	<i>KAAYDDFAKN</i>	<i>TQEVIKKIQE</i>	<i>AANAKQ</i>	
BMORN	<i>ETNEKLAPKI</i>	<i>KAAYDDFARN</i>	<i>TQEVIKKIQE</i>	<i>AANAKQ</i>	
BMANJ	<i>ETNEKLAPKI</i>	<i>KAAYDDFAKN</i>	<i>TQEVIKKIQE</i>	<i>AANAKQ</i>	

Fig. 2. Deduced amino acid sequences for Apolipoprotein-III from *Bombyx mori*, Strain N4 (BMOR4), *Bombyx mori*, Strain P50 (BMORP), *Bombyx mandarina*, Japanese Strain (BMANJ),

and *Bombyx mandarina*, Chinese Strain (BMANC). The signal sequence is shown in italics and the arrow shows the cleavage site, between residues 22 and 23.

B. mori p50 and the Chinese strain of B. mandarina ApoLp-III. The cDNA was produced by PCR from mRNA. Two primers, corresponding to the conserved 5' and 3' ends of the *B. mori* N4 and *B. mandarina* cDNAs, were prepared: the 5' sense primer had the sequence 5'-TTCGCATCTCCTCCGCACTCAGTCT-3' and the 3' antisense primer had the sequence 5'GTGTGAGAGTTCAATATCGACGCTC-3'. The initial cDNA was prepared as described above and PCR was performed using a commercial kit (GeneAmp RNA PCR Kit, Perkin Elmer, Foster City, CA). The PCR products from *B. mori* p50 were ligated in pUC18 using a ligation kit (Pharmacia), and XL-1-Blue *E. coli* were transformed, grown on LB plates and induced with IPTG in the presence of X-gal. White colonies were selected and screened for insert by restriction analysis. The PCR products of the Chi-

nese strain of *B. mandarina* were ligated into the pGEN-T Vector Systems (Promega, Madison, WI) and DH5 *E. coli* (TOYOBO) were transformed, grown on LB plates containing Ampicillin. Colonies were selected and checked for inserts. Five colonies containing inserts were sequenced. The clones were sequenced using the *BcaBest* Dideoxy Sequencing Kit (TaKaRa), [α - 32 P]-dCTP, and M13 forward and reverse primers.

Sequence Analysis

Sequence analysis was carried out using the Wisconsin Sequence Analysis Package (Genetics Computer Group, Inc., Madison, WI). In particular, we used the program "Pileup" to generate multiple sequence alignments. The program "Distances" was used to create a pair-wise distance matrix and the program "Growtree" used this matrix to generate a phylogenetic tree using a neighbor-joining method.

RESULTS AND DISCUSSION

Figure 1 shows the cDNA sequences for apoLp-III from *B. mori* strains N4 and P50, and from Chinese and Japanese strains of *B. manda-*

Fig. 1. Nucleotide sequences for Apolipoprotein-III cDNAs from *Bombyx mori*, Strain N4 (BMOR4), *Bombyx mori*, Strain P50 (BMORP), *Bombyx mandarina*, Japanese Strain (BMANJ), *Bombyx mandarina*, and Chinese Strain (BMANC). The initiating Met codon (ATG) and the terminating codon (TGA) are underlined, and initiation A is indicated by ↓.

	1		↓		50
BMANC	<i>MAAKF.VVLF</i>	<i>ACIALAQGAM</i>	<i>VRRDAP...D</i>	<i>FFKDIEHHTK</i>	<i>EFHKTLEQQF</i>
BMORP	<i>MAAKF.VVLF</i>	<i>ACIALAQGAM</i>	<i>VRRDAP...D</i>	<i>FFKDIEHHTK</i>	<i>EFHKTLEQQF</i>
BMOR4	<i>MAGKF.VVLF</i>	<i>ACISLAQGAM</i>	<i>VRRDAP...D</i>	<i>FFKDIEHHTK</i>	<i>EFHKTLEQQF</i>
BMANJ	<i>MAAKF.IVLF</i>	<i>ACIALAQGAM</i>	<i>VRRDAP...D</i>	<i>VFKDIEHHAK</i>	<i>EFQKTFEQQF</i>
MANS	<i>MAAKFVVVLA</i>	<i>ACVALSHSAM</i>	<i>VRRDAPAGGN</i>	<u><i>AFEEMEKHAK</i></u>	<u><i>EFQKTFSQF</i></u>
	51				100
BMANC	<i>NSLTKSKDAQ</i>	<i>DFSKAWKDGS</i>	<i>ESVLQQLNAF</i>	<i>AKSLQGALGD</i>	<i>ANGKAKEALE</i>
BMORP	<i>NSLTKSKDAQ</i>	<i>DFSKAWKDGS</i>	<i>ESVLQQLNAF</i>	<i>AKSLQGALGD</i>	<i>ANGKAKEALE</i>
BMOR4	<i>NSLTKSKDAQ</i>	<i>DFSKAWKDGS</i>	<i>ESVLQQLNAF</i>	<i>AKSLQGALGD</i>	<i>ANAKAKEALE</i>
BMANJ	<i>NSLTKSKDAQ</i>	<i>DFSKAWKDGS</i>	<i>DSVLQQLNAF</i>	<i>AKSLQGALGD</i>	<i>ANGKAKEALE</i>
MANS	<u><i>NSLVNSKNTQ</i></u>	<i>DFNKALKDGS</i>	<u><i>DSVLQQLSAF</i></u>	<u><i>SSSLQGAISD</i></u>	<u><i>ANGKAKEALE</i></u>
	101				150
BMANC	<i>QSRQNIERTA</i>	<i>EELRKAHPDV</i>	<i>EKNATALREK</i>	<i>LQAAVQNTVQ</i>	<i>ESQKLAKKVS</i>
BMORP	<i>QSRQNIERTA</i>	<i>EELRKAHPDV</i>	<i>EKNATALREK</i>	<i>LQAAVQNTVQ</i>	<i>ESQKLAKKVS</i>
BMOR4	<i>QSRQNIERTA</i>	<i>EELRKAHPDV</i>	<i>EKNATALREK</i>	<i>LQAAVQNTVQ</i>	<i>ESQKLAKKVS</i>
BMANJ	<i>QSRQNIERTA</i>	<i>EELRKAHPDV</i>	<i>EKNATALREK</i>	<i>LQAAVQNTVQ</i>	<i>ESQKLAKKVS</i>
MANS	<u><i>QARONVEKTA</i></u>	<u><i>EELRKAHPDV</i></u>	<u><i>EKEANAFKDK</i></u>	<u><i>LQAAVQTTVQ</i></u>	<u><i>ESQKLAKKVA</i></u>
	151				190
BMANC	<i>SNVQETNEKL</i>	<i>APKIKAAAYDD</i>	<i>FAKNTQEVIK</i>	<i>KIQEAANAKQ</i>	
BMORP	<i>SNVQETNEKL</i>	<i>APKIKAAAYDD</i>	<i>FAKNTQEVIK</i>	<i>KIQEAANAKQ</i>	
BMOR4	<i>SNVQETNEKL</i>	<i>APKIKAAAYDD</i>	<i>FARNTQEVIK</i>	<i>KIQEAANAKQ</i>	
BMANJ	<i>SNVQETNEKL</i>	<i>APKIKAAAYDD</i>	<i>FAKNTQEVIK</i>	<i>KIQEAANAKQ</i>	
MANS	<u><i>SNMEETNKKL</i></u>	<u><i>APKIKOAYDD</i></u>	<u><i>FVKHAEVQK</i></u>	<u><i>KLHEAATKQ</i></u>	

Fig. 3. Alignment of Lepidopteran Apolipoprotein-III. Aligned amino acid sequences for apolipoprotein-III from *Bombyx mori*, Strain N4 (BMOR4), *Bombyx mori*, Strain P50 (BMORP), *Bombyx mandarina*, Japanese Strain (BMANJ),

Bombyx mandarina, Chinese Strain (BMANC) and *M. sexta* (MANS) (Cole et al., 1987). The signal sequence is shown in italics and the arrow shows the cleavage site. The five putative amphipathic α -helices (Smith et al., 1994) are underlined.

rina. Each cDNA encodes a 186 amino acid protein and the deduced amino acid sequences are shown in Figure 2. Each protein contains a 22 amino acid signal sequence (Fig. 2). In this regard, these apoLp-IIIs are quite similar to other apoLp-IIIs which have been sequenced (Smith et al., 1994). The four cDNA sequences show only a few differences in the 558-bp coding regions and the amino acid sequences are also quite similar.

One interesting feature of the previously known apoLp-III sequences from distantly related insects, was the very low degree of sequence similarity (Smith et al., 1994). However, the four Bombycidae sequences show a very high degree of similarity to each other and a high degree of similarity to the apoLp-III from a Sphingidae, *M. sexta* (Fig. 3). As would be expected on the basis of their similarity to *M. sexta* sequence, the Bombycidae sequences show very low similarity to the other apoLp-III sequences discussed by Smith et al. (1994) (data not shown). The degree of similarity between these five mature Lepidopteran apoLp-III amino acid se-

quences is particularly high (77.8–95.2%) in the five putative amphipathic α -helices identified by Smith et al. (1994), as shown in Figure 3. The very high degree of similarity between these five putative amphipathic α -helices lends further support to their importance in the function of apoLp-III, as proposed by Breiter et al. (1991), based on the X-ray structure of ApoLp-III from *L. migratoria*.

Table 1 presents distances between the five

TABLE 1. Distances Between cDNA Sequences for Lepidopteran apoLp-IIIs (Synonymous base substitutions per 100 synonymous sites)*

	BMANC	BMORP	BMOR4	BMANJ	MANS
BMANC		0.00	0.54	3.14	73.62
BMORP			0.54	3.14	73.62
BMOR4				3.67	74.87
BMANJ					81.79
MANS					

*BMANC = *Bombyx mandarina* (Chinese strain); BMANJ = *Bombyx mandarina* (Japanese strain); BMORP = *Bombyx mori* (P50 strain); BMOR4 = *Bombyx mori* (N4 strain); MANS = *Manduca sexta*.

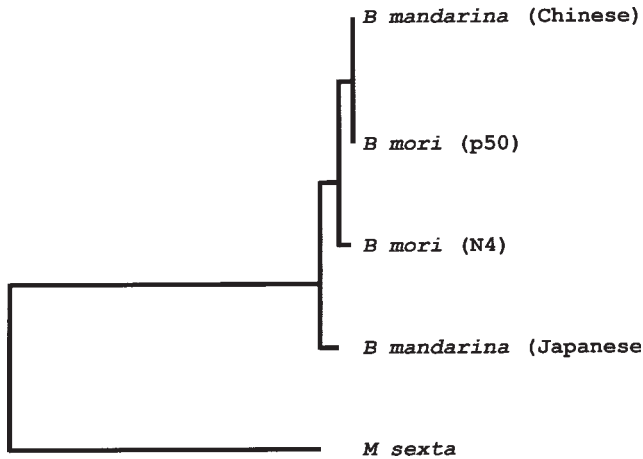


Fig. 4. Phylogenetic tree for Lepidopteran Apolipoprotein-III. This tree was constructed using the pair wise distance matrix presented in Table 1 and a neighbor-joining method.

proteins based on an analysis of amino acid substitutions, and Figure 4 shows a tree based on the amino acid-derived distances. This tree shows that *B. mori* P50 and the Chinese strain of *B. mandarina* are the most related, which is consistent with the assumption that P50 is closely related to the original *B. mori* strain derived from *B. mandarina*. The N4 strain of *B. mori* is more

closely related to these two strains than is the Japanese strain of *B. mandarina*.

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