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The complete mitochondrial genome of the wild silkworm moth, *Actias selene*

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ABSTRACT

The complete mitochondrial genome (mitogenome) of *Actias selene* (Lepidoptera: Saturniidae) was determined to be 15,236 bp, including 13 protein-coding genes (PCGs), two rRNA genes, 22 tRNA genes and a control region. The arrangement of 13 PCGs was similar to that of other sequenced lepidopterans. The AT skew of the mitogenome of *A. selene* was slightly negative, indicating a higher number of T compared to A nucleotides. The nucleotide composition of the mitogenome of *A. selene* was also biased toward A + T nucleotides (78.91%). All PCGs were initiated by ATN codons, except for the gene encoding *cytochrome c oxidase subunit 1* (*cox1*), which may be initiated by the TTAG, as observed in other lepidopterans. Three genes, including *cox1*, *cox2*, and *nad5*, had incomplete stop codons consisting of just a T. With an exception for *trnS1*(*AGN*), all the other tRNA genes displayed a typical clover-leaf structure of mitochondrial tRNA. The A + T-rich region of the mitogenome of *A. selene* was 339 bp in length, and contains several features common to the Lepidopteras, including non-repetitive sequences, a conserved structure combining the motif ATAGA and an 18-bp poly-T stretch and a poly-A element upstream of *trnM* gene. Phylogenetic analysis showed that *A. selene* was close to Saturniidae.

1. Introduction

Mitochondrial DNA (mtDNA) is maternally inherited DNA, characterized by a rapid evolutionary rate and lack of genetic recombination. It has been widely used as an informative molecular marker for diverse evolutionary studies among species, including molecular evolution, phylogenetics, population genetics, and comparative and evolutionary genomics (Boore, 1999). In the majority of insects, the mitogenome is a double-stranded, circular molecule, varying in size between 14 and 19 kilobases (kb). It contains 37 genes, including 13 protein coding genes (PCGs) (subunits 6 and 8 of the ATPase [*atp6* and *atp8*], cytochrome c oxidase subunits 1–3 [*cox1–cox3*], *cytochrome B* [*cob*], NADH dehydrogenase subunits 1–6 and 4 L [*nad1–6* and *nad4L*]), two ribosomal RNA genes encoding the small and large subunit rRNAs [*rrnL* and *rrnS*], 22 transfer RNA (tRNA) genes and a control region of variable length, known as the A + T-rich region (Boore, 1999; Moritz et al., 1987; Wolstenholme, 1992). The mitochondrial genome encodes the origin of replication and promotes the translation of both the heavy (H) and the light (L) strands, except the A + T-rich region (Kasamatsu et al., 1971).

To date, more than 200 species of the complete or near-complete mitogenomes have been sequenced from insects (http://www.ncbi.nlm.nih.gov). Lepidoptera is the 2nd most numerous order of insects, accounting for more than 160,000 insect species, but only about 50 complete or near-complete mitogenomes are currently available in GenBank (Table 1). The genomic knowledge of new lepidopteran mitogenomes will provide further insight into our understanding of diversity of this order and their evolutionary history. The silk-producing insects with economic value in Lepidoptera belong to two families of moth, Bombycidae and Saturniidae. The complete mitogenomes of three species of Bombycidae and six species of Saturniidae were sequenced: *Bombyx mori* (Yukuhiro et al., 2002), Japanese *Bombyx mandarina* (Yukuhiro et al., 2002) and Chinese *B. mandarina* (Pan et al., 2008) belonging to family Bombycidae; *Antheraea pernyi* (Liu et al., 2008), *Antheraea yamamai* (Kim et al., 2008), *Eriogyna pyretorum* (Jiang et al., 2009), *Manduca sexta* (Cameron and Whiting, 2008) and *Samia cynthia ricini* (Kim et al., 2011) belonging to the family Saturniidae. Within the Saturniidae family, *Actias selene* is an important wild, silk-spinning insect, mainly located in China, Japan, India and Southeast Asian countries. Recently, partial regions of the mitochondrial DNA of *A. selene*, the large and small ribosomal RNA and *cox 1*, was sequenced and used for phylogenetic analysis (Pu et al., 2009; Zhu et al., 2010). However, the complete mitochondrial genome (mitogenome) sequence of *A. selene* was not reported until now.

In the present paper, the complete nucleotide sequence of the mitogenome of *A. selene* was sequenced. The phylogenetic analyses to the selected species from Lepidoptera and Diptera based on the mitogenome sequences were performed using neighbor-joining (NJ) method.

Abbreviations: mitogenome, mitochondrial genome; PCGs, protein-coding genes; cox, cytochrome c oxidase; cob, cytochrome B; nad, NADH dehydrogenase; rrnS, small subunit ribosomal RNA; rrnL, large subunit ribosomal RNA.

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Table 1
The complete mitochondrial genome of Lepidoptera.

Species	Length (bp)	Accession number	References
Actias selene	15,236		This study
Caligula boisduvalii	15,360	NC_010613	Hong et al. (2008)
Eriogyna pyretorum	15,327	FJ685653	Jiang et al. (2009)
Antheraea pernyi	15,575	AY242996	Liu et al. (2008)
Antheraea yamamai	15,338	EU726630	Kim et al. (2008)
Samia cynthia ricini	15,366	JN215366	Kim et al. (2012)
Manduca sexta	15,516	EU286785	Cameron and Whiting (2008)
Adoxophyes honmai	15,680	DQ073916	Lee et al. (2006)
Coreana raphaelis	15,314	NC_007976	Kim et al. (2006)
Ostrinia nubilalis	14,535	NC_003367	Coates et al. (2005)
Artogeia melete	15,140	EU597124	Hong et al. (2009)
Bombyx mori	15,656	AB070264	Yukuhiro et al. (2002)
Chinese *Bombyx mandarina*	15,682	AY301620	Pan et al. (2008)
Japanese *Bombyx mandarina*	15,928	NC_003395	Yukuhiro et al. (2002)
Eumenis autonoe	15,489	GQ868707	Kim and Kim (2010)
Acraea issoria	15,245	GQ376195	Hu et al. (2010b)
Hyphantria cunea	15,481	GU592049	Liao et al. (2010)
Diatraea saccharalis	15,490	FJ240227	Li et al. (2011)
Spilonota lechriaspis	15,368	HM204705	Zhao et al. (2011)
Pieris rapae	15,157	HM156697	Mao et al. (2010)
Fabriciana nerippe	15,140	JF504707	Kim et al. (2011)
Grapholita molesta	15,717	HQ392511	Son and Kim (2011)
Chilo suppressalis	15,456	HQ860290	Yin et al. (2011)
Grapholita molesta	15,776	HQ116416	Gong et al. (2011)
Helicoverpa armigera	15,347	GU188273	Yin et al. (2010)
Ochrogaster lunifer	15,593	AM946601	Salvato et al. (2008)
Phthonandria atrilineata	15,499	EU569764	Yang et al. (2009)
Ctenoptilum vasava	15,468	JF713818	Unpublished
Argynnis hyperbius	15,156	JF439070	Unpublished
Libythea celtis	15,164	HQ378508	Unpublished
Sesamia inferens	15,413	JN039362	Unpublished
Cnaphalocrocis medinalis	15,377	JQ305693	Unpublished
Kallima inachus	15,183	JN857943	Unpublished
Apatura ilia	15,242	JF437925	Unpublished
Fabriciana nerippe	15,140	JF504707	Unpublished
Parnassius bremeri	15,389	FJ871125	Unpublished
Sasakia charonda	15,244	AP011824	Unpublished
Corcyra cephalonica	15,273	HQ897685	Unpublished
Ctenoptilum vasava	15,468	JF713818	Unpublished
Phalera flavescens	15,659	JF440342	Unpublished
Argynnis hyperbius	15,156	JF439070	Unpublished
Apatura metis	15,236	JF801742	Unpublished
Libythea celtis	15,164	HQ378508	Unpublished
Spindasis takanonis	15,349	HQ184266	Unpublished
Calinaga davidis	15,267	HQ658143	Unpublished
Lymantria dispar	15,569	FJ617240	Unpublished
Protantigius superans	15,248	HQ184265	Unpublished
Euploea mulciber	15,166	HQ378507	Unpublished
Calinaga davidis	15,267	HQ658143	Unpublished
Troides aeacus	15,263	EU625344	Unpublished
Teinopalpus aureus	15,242	HM563681	Unpublished
Papilio maraho	16,094	FJ810212	Unpublished
Luehdorfia chinensis	13,860	EU622524	Unpublished

2. Materials and methods

2.1. DNA extraction

The larvae of *A. selene* were collected from willow trees in Dangtu, Anhui Province, and reared in an enclosed environment with willow tree leaves until pupation. Total DNA was isolated from single specimens using the Aidlab Genomic DNA Extraction Kit (Aidlab Co., Beijing, China) according to the manufacturer's instructions. DNA from individual larvae was used for amplification of the fragments of the complete mitogenome.

2.2. PCR amplification and sequencing

For amplification of the entire mitogenome of *A. selene*, nine primer sets were synthesized (Beijing Sunbiotech Co., Ltd., Beijing, China)

Table 2
Primers used for PCR.

Primer pair	Primer sequence (5′→3′)	Size (kb)
F1	GCTTTTGGGCTCATACCTCA	1.9 k
R1	GATGAAATACCTGCAAGATGAAG	(21–1938)
F2	TGGAGCAGGAACAGGATGAAC	2.0 k
R2	GAGACCADTACTTGCTTTCAG	(1828–3774)
F3	ATTTGTGGAGCTAATCATAG	1.1 k
R3	GGTCAGGGACTATAATCTAC	(3670–4790)
F4	TCGACCTGGAACTTTAGC	2.9 k
R4	GCAGCTATAGCCGCTCCTACT	(4529–7466)
F5	TAAAGCAGAAACAGGAGTAG	3.0 k
R5	ATTGCGATATTATTTCTTTTG	(7431–10,411)
F6	GGAGCTTCTACATGAGCTTTTGG	2.2 k
R6	GTTTGCGACCTCGATGTTG	(10,733–12,939)
F7	CGGTTTGAACTCAGATCATGTAAG	1.1 k
R7	TATTGTATCTTGTGTATCAGAGTTTA	(12,838–13,896)
F8	GGTCCCTTACGAATTTGAATATATCCT	2.2 k
R8	AAACTAGGATTAGATACCCTATTAT	(12,507–14,583)
F9	CTCTACTTTGTTACGACTTATT	1.5 k
R9	TCTAGGCCAATTCAACAACC	(14,162–361)

(Table 2). Primers were designed based on the conserved nucleotide sequences of known mitochondrial sequences in Lepidoptera (Cameron and Whiting, 2008; Hong et al., 2008; Jiang et al., 2009; Kim et al., 2008; Kim et al., 2011; Liu et al., 2008; Pan et al., 2008; Yukuhiro et al., 2002) or the known sequences of fragments of the mitogenome of *A. selene* that were previously sequenced in our laboratory (GenBank accession nos. FJ358505 and FJ905474). The fragments ranging from 1.1 to 3.0 kb were amplified using Aidlab Long Taq (Aidlab Co., Beijing, China) according to the manufacturer's instructions. The PCR was performed under the following conditions: 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C and 1–3 min at 50–60 °C, and 10 min at 68 °C. The PCR products were separated by agarose gel electrophoresis (1% w/v) and purified using a DNA gel extraction kit (Aidlab Co., Beijing, China). The purified PCR products were ligated into the T-vector (TaKaRa Co., Dalian, China) and sequenced at least three times (Sunbiotech, Beijing Sunbiotech Co., Ltd., Beijing, China).

2.3. Sequence assembly and gene annotation

Sequence annotation was performed using the DNAStar package (DNAStar Inc. Madison, USA) and online blast tools available through the NCBI web site. The PCGs were identified by sequence similarity with *A. pernyi* (Liu et al., 2008). The nucleotide sequences of PCGs were translated with the invertebrate mitogenome genetic code. Alignments of PCGs for each of the available lepidopteran mitogenomes were performed using Clustal X (Thompson et al., 1997). Composition skewness was calculated according to the formulas: AT skew = [A − T]/[A + T]; GC skew = [G − C]/[G + C] (Junqueira et al., 2004). Tandem repeats in the control region were predicted using the Tandem Repeats Finder program (http://tandem.bu.edu/trf/trf.html) (Benson, 1999). Identification of tRNA genes was verified using the tRNAscan-SE program. The potential stem-loop secondary structures within these tRNA gene sequences were calculated using the tRNAscan-SE Search Server (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe and Eddy, 1997). The secondary structures of tRNA genes that could not be predicted using the tRNAscan-SE were analyzed by comparison with the nucleotide sequences of other insect tRNA sequences (Hong et al., 2008; Jiang et al., 2009; Liu et al., 2008; Salvato et al., 2008).

2.4. Phylogenetic analysis

To clarify the phylogenetic relationship among Lepidopteras, the complete mitogenomes of 15 lepidopteran species were obtained from the GenBank database. These sequences of mitogenomes were from five lepidopteran superfamilies within the lepidopteran suborder, including Bombycoidea: *A. selene*, *B. mori* (Yukuhiro et al., 2002),