The Complete Mitochondrial Genome of the Wild Type of Antheraea pernyi (Lepidoptera: Saturniidae)

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ABSTRACT Antheraea pernyi (Guérin-Ménéville) (Lepidoptera: Saturniidae), a well-known economically important insect, was domesticated from its wild type. In this study, the complete mitochondrial genome (mitogenome) of the wild type of A. pernyi was determined and compared for nucleotide variation with its domesticated type. The mitogenome of the wild type of A. pernyi is 15,537 bp in size, thus 29 bp smaller than that of the domesticated type. The gene content, order, and orientation of the complete mitogenome of the wild type are identical to those of the domesticated type, as are those of the other completely sequenced lepidopteran mitogenomes. A striking difference between the two mitogenomes was found in the A+T-rich region because of the numbers of tandem repeat units. The wild type has five tandem repeat units, whereas the domesticated type has six. Comparative analysis of the two mitogenomes revealed a relatively lower level of sequence divergence (1.70%). Within the two mitogenomes, there are no significant differences in nucleotide substitution rate for the 13 protein-coding genes except for the nad4L gene, which is different from those differences observed between the domesticated silkmoth Bombyx mori (L.) (Lepidoptera: Bombycidae) and its wild-type ancestor Chinese B. mandarina Moore. The divergence time between the two Antheraea mitochondrias was estimated to be between 0.74 ± 0.13 and 0.97 ± 0.17 million years ago, based on the genes cox1 + cox2 sequences. To our knowledge, this is the first report on sequence variation of the complete mitogenomes between the domesticated insect and its wild-type ancestor, within a single species.

KEY WORDS Antheraea pernyi, mitochondrial genome, nucleotide substitution pattern, divergence time

The mitochondrial deoxyribonucleic acid (mtDNA) of most animals is a self-replicating, circular deoxyribonucleic acid (DNA) molecule ≈14–20 kb in size, which encodes a conserved set of 37 mitochondrial genes (13 protein-coding genes, 22 transfer ribonucleic [tRNA] genes, and 2 ribosomal ribonucleic acid [rRNA] genes) (Wolstenholme 1992). It also contains a control region, known as the A+T-rich region in insects. Because of its maternal inheritance, small size, lack of recombination, and presence of polymorphisms, the genome data have been used widely as an informative marker for insect phylogenetic and evolutionary studies (Nardi et al. 2003, Arunkumar et al. 2006). The large amount of insect mitochondrial genome (mitogenome) data has been used for deep-level phylogenetic studies (Jiang et al. 2009, Kim et al. 2009, Zhou et al. 2009, Hu et al. 2010, Yin et al. 2010, Son and Kim 2011). And, some mitogenome sequences have been used for comparative genomics studies to investigate the nucleotide variation and evolutionary patterns between species, such as two Ostrinia species (Coates et al. 2005), three Nasonia species (Oliveira et al. 2008), 15 species of beetles (Pons et al. 2010), and members of the Drosophila melanogaster (Meigen) subgroup (Ballard 2000a). The sequence variation of the complete mitogenomes within distinct D. simulans Sturtevant haplotypes of a single species also has been investigated (Ballard 2000b). The complete mitogenome of the mulberry silkworm Bombyx has been used to look for the selection evidence on mtDNA (Li et al. 2010a). However, little attention has been paid to the nucleotide variation pattern at the complete mitogenome level between the domesticated insect and its ancestor relatives.

The purpose of this study is to examine nucleotide variation in the complete mitogenome for the wild and domesticated type of Antheraea pernyi (Guérin-Ménéville) (Lepidoptera: Saturniidae). This insect is one of the most well-known wild silkmoths used for silk production. Silk production based on this species is an economically significant industry in rural economies of many populous nations including China, India, Korea, and Indonesia. The silkworm, including larva, pupa, and adult, also is used for a high quality insect-
protein food (Zhou and Han 2006). According to historic records, the domesticated type of *A. pernyi* is believed to have been domesticated successfully from the wild type in the 16th century, although the commercial use of its cocoon started from the Han Dynasty (40 B.C.) in China (Liu et al. 2010). This insect is still thought to be semidomesticated. This is the first study to investigate the sequence variation of complete mitogenomes within a single species between the domesticated insect and its wild-type ancestor.

The wild type of *A. pernyi* used in this study was collected from Yunnan Province, China. It has been identified as the wild type of *A. pernyi*, according to its high similarity with the domesticated populations by morphology and DNA barcoding (Zhu et al. 2009), although a few differences are observed between them: 1) the wild population has only a yellow–cyan cuticle color in larvae, whereas the domesticated counterparts have four kinds of cuticle color including yellow–cyan, yellow, white, and blue. Presently, the varieties of yellow–cyan cuticle color are distributed in north China, whereas the majority of yellow cuticle color cultivars are in south China; 2) the wild population behaves as both univoltine and bivoltine in nature, whereas the domesticated counterparts are exclusively either univoltine or bivoltine as a result of breeding and selection; and 3) some individuals of the wild population can remain in pupal diapause >2 yr before the silk moth emerges and starts the life cycle, whereas no individuals from the domesticated populations can delay the diapause break. Usually, the univoltines, which are distributed in south China, complete only one life cycle in a calendar year, and enter into pupal diapause around the second week of July. Thus, the moth emergence starts around the first week of April of the second calendar year. Similarly, the bivoltines complete two life cycles in a calendar year and stay in diapause for a much shorter time than the univoltines. Because the domesticated and wild populations are believed to have originated from a common ancestor (ancient *Antheraea perynii*) and to have undergone different selection histories, it is of interest to ascertain the nucleotide variation and evolutionary pattern of the complete mitogenomes between the domesticated and wild type.

The domesticated silkworm, *Bombyx mori* (Lepidoptera: Bombycidae), is the only truly domesticated lepidopteran insect. *Bombyx mori* is thought to have been domesticated from its ancestor relative, Chinese *B. mandarina*, ≈5,000–10,000 yr ago (Goldsmith et al. 2005). The nucleotide variation and evolutionary patterns at the complete mitogenome level already have been comparatively investigated between *B. mori* and its ancestor relatives, Japanese *B. mandarina* Moore and Chinese *B. mandarina* (Yukuhiro et al. 2002, Li et al. 2010b). The two samples are considered to be from different species after long-term divergence from a common ancestor, although these two species can crossbreed and yield fertile hybrid offspring.

In this study, the complete mitogenome of the wild type of *A. pernyi* was determined. This mitogenome was compared with that derived from the domesticated type to examine nucleotide variation including sequence divergence, substitution pattern and nonsynonymous (*Ka*)/synonymous (*Ks*) value for selection effect. Finally, the divergence time between the wild and domesticated type of *A. pernyi* mitochondria was assessed, based on the molecular clock of the swallowtail butterflies *Papilio* (Lepidoptera: Papilionidae) (Zakharov et al. 2004).

**Materials and Methods**

**Sample and DNA Extraction.** The cocoons of the wild type of *A. pernyi* were collected from Yunnan Province, China in 2001, which has been identified as the wild type of *A. pernyi* according to its high similarity with the domesticated populations by morphology and DNA barcoding (Zhu et al. 2009). The mtDNA was extracted from a single pupa by the alkaline lysis procedure (Koichiro and Tadashi 1988).

**Polymerase Chain Reaction (PCR) Amplification and Sequencing.** The complete mitogenome of the domesticated type of *A. pernyi* has been determined in our laboratory (Liu et al. 2008). The full mitogenome of the wild type was amplified in eight overlapping fragments by using the same strategy (Liu et al. 2008). The PCR products were purified and directly sequenced. Sequencing was performed with an automatic DNA sequencer ABI 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA).

**Sequence Analysis.** The nucleotide sequence of protein and ribosomal RNA coding genes in the wild type of *A. pernyi* mitogenome were identified through BLAST search in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/), and confirmed by homology comparison to the sequence of the domesticated type. The tRNA genes and their secondary structures were predicted using the tRNAscan-SE Search Server (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe and Eddy 1997). The GenBank accession number of the wild type of *A. pernyi* mitogenome is HQ264055.

**Phylogenetic Relationships.** To reconstruct the phylogenetic relationship of Saturniidae species including *A. pernyi*, the other complete mitogenomes from Bombycoidea species including *Antheraea yamamai* (Guérin-Ménville) (EU726630), *Saturnia boisduvalii* Eversmann (EF622227), *Eriogyna pyretrorum* Westwood (FJ685653), and *Manucca sexta* L. (EU286785) were obtained from the GenBank database. The complete mitogenomes of *B. mori* (AB070264) and Chinese *B. mandarina* (AY301620) were used as outgroups. The complete nucleotide sequences were aligned with Chustal X (Thompson et al. 1997) using default settings, and Neighbor-Joining (NJ) trees were constructed with MEGA 4.0 (Tamura et al. 2007). Maximum Parsimony method also was used for phylogenetic reconstruction.

**Nucleotide Substitution.** The alignment of the wild and domesticated type of *A. pernyi* was analyzed by
MEGA software version 4.0 (Tamura et al. 2007). The frequency of nonsynonymous (\(K_a\)) and synonymous (\(K_s\)) alignment was calculated using \(p\)-distance algorithm and Nei-Gojobori model (Nei and Gojobori 1986). Taking the gap sites into consideration, the pairwise-deletion option was selected.

### Molecular Dating

The \(\text{cox}1\) and \(\text{cox}2\) genes were aligned, respectively, and subsequently combined into a concatenated data set. Pairwise genetic distance was calculated using the Kimura-two-parameters model (Kimura 1980) for the nucleotide substitution including transition and transversion. Substitution rates for the mitochondrial genes \(\text{cox}1\)/\(\text{H}11001\) \(\text{cox}2\) of the genus \(\text{Papilio}\) with a rate of \(7.8 \times 10^{-9}\) per site per year (Zakharov et al. 2004), was used as a molecular clock. 

\(\text{Antheraea pernyi}\) (Lepidoptera: Saturniidae) is closely related to \(\text{Papilio}\) (Lepidoptera: Papilionidae), the later is, to date, the lepidopteran insect with the most thoroughly studied molecular phylogeny and evolution, including fossil records (Zakharov et al. 2004). Divergence time between the wild and domesticated type of \(\text{A. pernyi}\) mitochondrias was estimated. Time since divergence (\(T\)) estimates were derived from the molecular clock \((r)\) and genetic distance \((K)\) by using the simple equation \(T = K/2r\) (Fu and Li 1997).

### Results

Characterization of the Mitochondrial Genome of the Wild Type of \(\text{A. pernyi}\). The complete mitogenome of the wild type of \(\text{A. pernyi}\) is 15,537 bp in length, thus 29 bp smaller than that of the domesticated type (Liu et al. 2008). The gene content, order and orientation in this mitogenome are identical to that of the domesticated type (Table 1), both of which agree with those properties of the other completely sequenced lepidopteran mitogenomes.

### Table 1. Organization of the wild type of \(\text{A. pernyi}\) mitogenome

| Gene     | Direction | Location | Length | Anticodon | Non | OL | Start codon | Stop codon |
|----------|-----------|----------|--------|-----------|-----|----|-------------|------------|
| \(\text{tRNA}^{\text{Met}}\) | F         | 1-67     | 67     | CAT       | 9   |    |             |            |
| \(\text{tRNA}^{\text{Ile}}\) | F         | 77-140   | 64     | CAT       | 3   |    |             |            |
| \(\text{tRNA}^{\text{Gln}}\) | R         | 138-206  | 69     | TTG       | 57  |    |             |            |
| \(\text{nad}2\) | F         | 264-1277 | 1014   | G        | 8   |    | ATT         | TAA        |
| \(\text{tRNA}^{\text{Trp}}\) | F         | 1286-1353| 68     | TCA       | 8   |    |             |            |
| \(\text{tRNA}^{\text{Cys}}\) | R         | 1346-1406| 61     | GCA       | 19  |    |             |            |
| \(\text{tRNA}^{\text{Tyr}}\) | R         | 1426-1491| 66     | GTA       | 2   |    |             |            |
| \(\text{cox}1\) | F         | 1494-3028| 1531   |          |     |    | TTAG        | T-\(\text{tRNA}\) |
| \(\text{tRNA}^{\text{Met}}(\text{\(\text{UUR}\)}\)) | F         | 3029-3094| 66     | TAA       |     |    |             |            |
| \(\text{cox}2\) | F         | 3095-3776| 682    | ATG       |     |    |             |            |
| \(\text{tRNA}^{\text{Asp}}\) | F         | 3777-3856| 71     | GCT       | 23  |    |             |            |
| \(\text{tRNA}^{\text{Ser}}\) | F         | 3851-3943| 73     | TGC       |     |    |             |            |
| \(\text{atp}5\) | F         | 3944-4111| 183    |          | 7   |    | ATT         | TAA        |
| \(\text{atp}6\) | F         | 4105-4781| 677    | ATG       |     |    |             |            |
| \(\text{cox}3\) | F         | 4782-5570| 789    | TAA       | 2   |    |             |            |
| \(\text{tRNA}^{\text{A}10007}\) | F         | 5573-5638| 66     | TCC       |     |    |             |            |
| \(\text{nad}3\) | F         | 5639-5990| 354    |          |     |    | ATT         | T-\(\text{tRNA}\) |
| \(\text{tRNA}^{\text{A}10009}\) | R         | 5951-6075| 66     | TGC       | 1   |    |             |            |
| \(\text{tRNA}^{\text{Trp}}\) | F         | 6056-6121| 66     | TCG       |     |    |             |            |
| \(\text{tRNA}^{\text{Cys}}\) | R         | 6122-6186| 65     | GCT       | 1   |    |             |            |
| \(\text{tRNA}^{\text{Asp}}\) | F         | 6255-6319| 65     | TTC       | 10  |    |             |            |
| \(\text{tRNA}^{\text{Tyr}}\) | R         | 6329-6399| 70     | GAA       |     |    |             |            |
| \(\text{nad}5\) | R         | 6400-8140| 1741   |          |     |    | ATT         | T-\(\text{tRNA}\) |
| \(\text{tRNA}^{\text{His}}\) | R         | 8141-8207| 67     | GTG       | 13  |    |             |            |
| \(\text{nad}4\) | R         | 8221-9561| 1341   |          | 5   |    | ATG         | TAA        |
| \(\text{nad}4L\) | R         | 9567-9857| 291    |          | 7   |    | ATG         | TAA        |
| \(\text{tRNA}^{\text{Thr}}\) | F         | 9685-9929| 65     | TGT       |     |    |             |            |
| \(\text{tRNA}^{\text{Pro}}\) | R         | 9930-9994| 65     | TGC       | 2   |    |             |            |
| \(\text{nad}6\) | R         | 9997-10533| 537   |          | 2   |    | ATG         | TAA        |
| \(\text{cyt}b\) | F         | 10536-11684| 1149  |          | 29  |    | ATG         | TAA        |
| \(\text{tRNA}^{\text{Leu}}(\text{\(\text{UCN}\)}\)) | F         | 11714-11781| 68   | TAA       | 19  |    |             |            |
| \(\text{nad}1\) | R         | 11801-12739| 939   |          | 2   |    | ATG         | TAA        |
| \(\text{tRNA}^{\text{Leu}}(\text{\(\text{CUN}\)}\)) | R         | 12742-12809| 68   | TAG       |     |    |             |            |
| \(\text{lrRNA}\) | R         | 12810-14179| 1370  |          |     |    |             |            |
| \(\text{srRNA}\) | R         | 14180-14246| 67   | TAC       |     |    |             |            |
| \(\text{A}+\text{T-rich}\) | R         | 15247-15021| 775  |          |     |    |             |            |

F, forward; R, reverse; Non, noncoding region; OL, overlapping region.
The 13 protein-coding genes (PCGs) in the mitogenome of the wild type of *A. pernyi* were found to share common initiation and termination codons with those of the domesticated type. The mitogenome of the wild type encodes 3,727 amino acid residues, identical to the domesticated type. The codon usage of the PCGs and the relative synonymous codon usage values in the mitogenome of the wild type are almost the same as those of the domesticated type. Only codon CTG is not represented in the mitochondrial PCGs sequence of the wild type, whereas two codons, CTG and CCC, are not represented in the domesticated type.

The structures of tRNA$^{\text{Ala}}$, tRNA$^{\text{Arg}}$, tRNA$^{\text{Glu}}$, tRNA$^{\text{Pro}}$, tRNA$^{\text{His}}$, tRNA$^{\text{Leu}}$, and tRNA$^{\text{Val}}$ are different between the mitogenome of the wild and domesticated type of *A. pernyi* (Supp. Fig. 1 [online only]). Statistical analysis revealed that changes of the secondary structure are because of a total of six transition and seven indels (insertions and deletions) of nucleotide in sequence. The tRNA$^{\text{His}}$ is the most variable with five nucleotide positions, including four indels and one transition.

The srRNA and lrRNA genes in the wild type of *A. pernyi* were identified to be 775 and 1,370 bp long, respectively, and showed 99% homology with those of the domesticated type. The alignment of the lrRNA gene showed only a single nucleotide deletion.

The A+T-rich region in the wild type of *A. pernyi* was determined to be 516 bp in length, thus being 36 bp shorter than that of the domesticated type. Sequence alignment of the A+T-rich region revealed a 97% nucleotide sequence similarity between the wild and domesticated type. The most striking difference between the two mitogenomes is in the A+T-rich region. The wild type has five tandem repeat units in a 221-bp fragment (Fig. 1). The repeat unit is ∼38 bp in length, which contains a 20-bp core motif flanked by 9-bp perfect inverted repeats (Arunkumar et al. 2006). We also amplified and sequenced the A+T-rich region from other 11 varieties of the domesticated type, confirming the presence of six repeat elements, rather than five repeat elements (unpublished data). The A+T-rich region length differences between the two mitochondrias are essentially because of variation in a highly repetitive section of several imperfect copies of a 38-bp-long repeat unit.

The presence of one or several such repetitive segments has already been reported for many animal species for which the sequence variation of the control region has been investigated. For example, a tandem triplication of a 126-bp fragment was detected in this region for Japanese *B. mandarina*, whereas *B. mori* and Chinese *B. mandarina* have only one, a 126-bp fragment (Yukui et al. 2002, Pan et al. 2008).

**Phylogenetic Inference.** To place the mtDNA of the wild type of *A. pernyi* in perspective relative to the other Saturniidae insect mitogenomes, a data set containing eight complete nucleotide sequences from Bombycoidea species was generated. The final alignment resulted in 11,266 conserved sites, 4,684 variable sites, and 2,928 informative sites for parsimony. The phylogenetic analysis, based on the complete mitogenome in this study, strongly supports the placement within the superfamily Bombycoidea, which is consistent with the previous findings by morphological analysis (Kristensen and Skalski 1999) and by molecular analysis based on some nuclear genes (Kawahara et al. 2009). The topology of tree reconstructed by the NJ method is shown in Fig. 2. The topology based on the Maximum Parsimony method is identical to that based on NJ method (data not shown). The phylogenetic analysis of the two mulberry-consuming species, *B. mori* and *B. mandarina*, belonging to the Bombycidae, and the other five nonmulberry-consuming wild silkworms, *A. pernyi* domesticated, *A. pernyi* wild, *A. yamamai*, *S. boisduvalii*, and *E. pyretorum*, belonging to the Saturniidae, independently formed distinct groups, with the highest level of node support (Fig. 2). In the phylogenetic tree, the wild type of *A. pernyi* is closely related to the domesticated type of *A. pernyi*, followed by *A. yamamai*, further confirming the identification of the wild type of *A. pernyi* based on morphology and DNA Barcoding (Zhu et al. 2009).

**Nucleotide Substitution Pattern.** The full mitogenome sequences between the wild and domesticated type of *A. pernyi* were aligned to produce a 15,581 nt
consensus mitogenome alignment, of which 264 (1.70%) nucleotide sites, including the indels, were variable. Two hundreds and five substitutions were detected between the two genome sequences (Table 2): 165 transition (ts) and 40 transversion (tv) mutations (ts:tv = 165/40 = 4.13). This value deviates significantly from neutral expectation (1:2, $\chi^2 = 203.20$, d.f. = 1, $P < 0.001$), indicating that evolutionary pressures are acting upon the two mitogenomes. Relatively excess transition mutations was also reported between the D. melanogaster subgroup members ($\kappa = 761/180 = 4.23$) (Ballard 2000a), O. nubilalis (Hubner) and O. furnicalis (Guenee) ($\kappa = 138/48 = 2.88$) (Coates et al. 2005), B. mori and Japanese B. mandarina ($\kappa = 414/100 = 4.14$) (Li et al. 2010b), and this has been thought to be attributed to non-neutral evolutionary forces or population effects.

Historical records and phylogenetic analysis make it clear that B. mori was directly domesticated from Chinese B. mandarina, rather than from Japanese B. mandarina (Goldsmith et al. 2005, Arunkumar et al. 2006, Pan et al. 2008). Between B. mori C108 (AB070264) and Chinese B. mandarina (AY301620), a 15,722 nt consensus alignment identified 484 variable nucleotide sites (3.08% sequence divergence), and 381 substitutions ($\kappa = 230/151 = 1.52$) that also deviated significantly from neutral expectation (1:2, $\chi^2 = 125.30$, d.f. = 1, $P < 0.001$). Compared with the data observed between B. mori and Chinese B. mandarina (Li et al. 2010b), the sequence divergence between the wild and domesticated type of A. pernyi is significantly smaller (1.70% versus 3.08%; $\chi^2 = 64.28$, d.f. = 1, $P < 0.001$), but the transition mutation ratio is nearly identical (4.13 versus 4.14). These findings indicate that the evolutionary pattern between the wild and domesticated type of A. pernyi is distinct from that between B. mori and B. mandarina.

Comparative analysis of the mitochondrial PCGs showed 171 substitutions between the wild and domesticated type of A. pernyi, including 131 at third, 32 at first and eight at second codon positions. Mitochondrial peptides comparatively showed 34 predicted amino acid changes between them indicating a fivefold excess of silence mutations. The 34 predicted amino acid changes resulted in nonsynonymous substitutions that are related to 11 PCGs exceptions for the cox2 and nad4L genes.

The pattern of nucleotide substitutions between the major and minor strands of the two mitogenomes was investigated. There are no significant differences in transition rates for the PCGs encoded by the major strand (25 A-G and 66 C-T transitions in 6,901 bp) when compared with that encoded by minor strand (31 A-G and 19 C-T transitions in 4,312 bp) ($\chi^2 = 0.54$, d.f. = 1, 0.25 < $P < 0.25$). However, on the major strand there are relatively more changes for T-C transitions in PCGs than observed on the minor strand ($\chi^2 = 9.38$, d.f. = 1, $P < 0.01$), whereas on the minor strand there are relatively more changes for A-G transitions than observed on the major strand ($\chi^2 = 6.79$, d.f. = 1, $P < 0.01$). These data are consistent with those seen in Drosophila and Bombyx mitogenomes (Ballard 2000a, Yukihiro et al. 2002, Li et al. 2010b).

Regional variation in nucleotide substitution rate between the two mitogenomes was also investigated. The rRNA genes are the most conserved among genes or regions (Table 2). Nucleotide sequence diver-
In the A+T-rich region is the third highest compared with those of PCGs. Two genes, atp6 and atp8, show higher nucleotide sequence divergence than the A+T-rich region. According to Zhang and Hewitt (1997), in terms of nucleotide substitution, the A+T-rich region might not be the fastest evolving region of the mtDNA of insects. These observations of nucleotide sequence divergence showed that the A+T-rich region is not the fastest evolving gene or region in Antheraea mitogenomes, providing direct evidence for the above mentioned assumption of Zhang and Hewitt (1997). Moreover, in the Antheraea mitogenome, there are no significant differences in nucleotide substitution rate for the 13 PCGs, except for the nad4L gene ($\chi^2 = 10.13, \text{d.f.} = 11, P > 0.5$), which is different from those observed between B. mori and B. mandarina (Li et al. 2010b). In Bombyx mitogenomes, the average substitution rates of the five genes surrounding the A+T-rich region and two rRNA genes (nad1, cox1, cox2, atp6, and nad2) are significantly smaller than those of the four genes (cox3, nad4, nad5, and cytB) (Yukihiro et al. 2002), whereas in Drosophila mitogenomes four disjunct regions have a significantly different nucleotide substitution process (Ballard 2000a). These results suggest that the Antheraea mitogenome is not under the common evolution-pro cess of Bombyx and Drosophila.

The frequency of nonsynonymous substitution (Ka) and synonymous substitution (Ks) (Nei and Gojobori 1986) was calculated to investigate the substitution rates in each protein-coding locus (Table 3). The atp6 gene shows the highest Ks value followed by the atp8 gene, whereas the nad6 gene shows the highest Ka value followed by the atp8 gene. The Ka or Ks values of both cox1 and nad5 genes are intermediate among these PCGs, although the two show the most substitution mutations (Table 2). According to the most common mode of selection (Hughes 1999), a $Ka/Ks$ ratio of one is assumed to indicate neutrality, $Ka/Ks > 1$ is a signature of positive selection at the amino acid level, and $Ka/Ks < 1$ is indicative of purifying selection. The highest $Ka/Ks$ value (0.2328) was found in the nad2 gene, followed by the gene nad6 (0.1599). The overall $Ka/Ks$ ratio of 13 PCGs between the wild and domesticated type of A. pernyi mitog- enome is very low ($Ka/Ks = 0.0640$). These results indicate that the PCGs within the A. pernyi mitogenome are under purifying selection, and these PCGs may reflect different purifying selection pressure. However, in B. mori, a strong signal of positive selection was discovered in the gene cytB (Li et al. 2010a).

**Divergence Time Estimation.** In Lepidoptera, the most detailed studies of molecular phylogeny and divergence time of the swallowtail butterflies concerns mitochondrial genes coxl+cox2 and the nuclear protein coding gene elongation factor one alpha (EF-1α). On the basis of fossil records of Papilionidae, the substitution rate for the genus Papilio mitochondrial genes coxl+cox2 was suggested to be $7.8 \times 10^{-9}$ per site per year (Zakharov et al. 2004). To estimate the divergence time between the wild and domesticated type of A. pernyi, we used the substitution rate of 7.5–10.2 $\times 10^{-9}$ per site per year from the mitochondrial genes coxl+cox2 of the swallowtail butterflies, closely related to A. pernyi, as a molecular clock. The genes for cox1 and cox2 were aligned, respectively, and subsequently combined into a concatenated data set. The pairwise genetic distance of 0.0151 ± 0.0027 was obtained using the kumartwo-parameter model for nucleotide substitution. The divergence time between the wild and domesticated type of A. pernyi then was estimated to be between 0.74 ± 0.13 and 0.97 ± 0.17 million years ago.

**Discussion**

The complete mitogenome sequence from the wild type of A. pernyi determined in this study is, to our knowledge, the first mitogenome sequence of an economically important insect to be compared with that of its domesticated type. The comparative genomics of mtDNA between the wild and domesticated type of A. pernyi is important because it can improve our understanding of the microevolution in the current stage of domestication of an economically important insect. The mitogenome sequence of the domesticated silkworm, B. mori has already been compared with its most probable ancestor, Chinese B. mandarina (Li et al. 2010b). However, they have been considered as different species after long-time divergence. It is clear that the present A. pernyi was domesticated from the wild type of A. pernyi $\approx 400$ yr ago (Liu et al. 2010). The current study shows that the organization and characterization of the mitogenome of the wild type of A. pernyi is almost identical to that of the domesticated type. Comparison of the mitochondrial nucleotides and peptides showed 98% and 99% identity, respectively, indicating that the time divergence after domestication is relatively short and that they are closely related. The largest difference between them is in the A+T-rich region where the wild type of A. pernyi includes five tandem repeat units while the domesticated type contains six. Analysis of the A+T-rich region from other 11 varieties of domesticated A. pernyi showed that none of them contain five tandem repeat units, indicating all of them are derived from a common domesticated ancestor after divergence from

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**Table 3. The Ka and Ks values of the PCGs between the wild and domesticated type of A. pernyi**

| Gene | Synonymous substitution | Ks | Nonsynonymous substitution | Ka | Ka/Ks |
|------|--------------------------|----|-----------------------------|----|-------|
| nad2 | 5                        | 0.0262 | 5 | 0.0061 | 0.2328 |
| cox1 | 23                       | 0.0685 | 3 | 0.0025 | 0.0365 |
| cox2 | 7                        | 0.0521 | 0 | 0.0 |
| atp6 | 4                        | 0.1747 | 1 | 0.0072 | 0.0412 |
| nad3 | 5                        | 0.0803 | 1 | 0.0035 | 0.0401 |
| nad4 | 19                       | 0.0554 | 7 | 0.0051 | 0.0921 |
| nad4L| 0                        | 0.0709 | 3 | 0.0041 | 0.0578 |
| nad5 | 15                       | 0.0644 | 4 | 0.0044 | 0.0653 |
| nad6 | 15                       | 0.0588 | 4 | 0.0094 | 0.1599 |
| nad5L| 19                       | 0.0554 | 7 | 0.0051 | 0.0921 |
| nad6 | 19                       | 0.0712 | 3 | 0.0028 | 0.0393 |
| nad4L| 0                        | 0      | 0 | 0.0001 | 0.0 |
| nad1 | 14                       | 0.0644 | 4 | 0.0044 | 0.0653 |

Ka and Ks values for the PCGs between the wild and domesticated type of A. pernyi.
wild type A. pernyi. Lower level of nucleotide sequence divergence in the mitogenome between them are consistent with the short-term domestication.

In this study, we also showed that the evolutionary pattern between the wild and domesticated type of A. pernyi is distinct from that between B. m. and B. m. mandarina. Comparative genomics of mtDNA reveals a lower level of sequence divergence (1.70%) and an excess transition mutation (tstv = 4.13) between the wild and domesticated type of A. pernyi, whereas its wild type of A. pernyi was domesticated from Chinese A. pernyi. The distinct nucleotide content between the domesticated and wild ancestor mutations indicate that evolutionary forces are non-neutral between the domesticated and wild ancestor populations (Coates et al. 2005). The sequence divergence reflects the split time after domestication. These data of sequence divergence are consistent with their historic records and archaeological evidence. Bombyx m. was domesticated from Chinese B. m. mandarina ~5,000–10,000 yr ago (Goldsmith et al. 2005), whereas A. pernyi was domesticated from the wild type of A. pernyi only ~400 yr ago (Liu et al. 2010). The excess transition mutations indicate that evolutionary forces are non-neutral between the domesticated and wild ancestor populations (Coates et al. 2005). The distinct nucleotide substitution patterns between the domesticated type versus its wild type of A. pernyi and B. m. versus its wild type B. m. mandarina show that they are attributed to different evolutionary forces. From evolutionary biology it is commonly held that excess amounts of transitions are found between mitochondrial-nucleotide sequences of closely related species caused by biased mutation pressure. It has been suggested that the A+T richness in the mitogenome will cause an apparent lower transition bias ratio in closely related species (Tamura 1992, Yu et al. 1999, Liao and Lu 2000, Arunkumar et al. 2006). However, investigation of the A+T content of the mitogenome from the four samples showed that they were almost identical to each other (from 80.11% in wild A. pernyi to 81.68% in Chinese B. m. mandarina), indicating that the heavy bias toward A+T is not suitable to explain this case. A possible explanation is a difference in selection effect resulting from natural environmental selection as opposed to artificial selection (Li et al. 2010). Both the wild and domesticated type of A. pernyi are nearly naturally grown. In contrast, B. m. mandarina is naturally grown, whereas B. m. is grown under artificial conditions. Growing in the natural environment provides much more opportunity for adaptability to occur. The different environmental selection after domestication could lead to a different adaptive mechanism. After domestication, B. m. underwent a very much stronger artificial selection than did domesticated A. pernyi (Liu et al. 2002). In a previous study, we have suggested that the effect of environmental selection for A. pernyi may exceed that of artificial selection (Liu et al. 2006). It is well known that artificial selection has been responsible for the great variety of breeds of domesticated animals. Therefore, an excess transition mutation in the mitogenome of Antheraea may be mainly be subjected to environmental selection pressure, whereas a relatively excess transversion mutation in mitogenomes from Bombyx may mainly be subjected to artificial selection pressure. In the future, the reasons for driving this divergence are noteworthy to be investigated, particularly the action of artificial selection.

We estimated the divergence time between the wild and domesticated type of A. pernyi to be from 0.74 ± 0.13 to 0.97 ± 0.17 million years ago. This divergence time is slightly smaller than that (1.08 ± 0.15–1.41 ± 0.24) estimated between the domesticated B. m. and its probable ancestor Chinese B. m. mandarina (Pan et al. 2008). According to the historic records and archaeological evidence, the use and domestication of A. pernyi was much later than that of B. m. (Goldsmith et al. 2005, Liu et al. 2010). Therefore, this divergence time estimate is acceptable. However, according to the historic records, A. pernyi was successfully domesticated in China only 400 yr ago. If the earlier use of the cocoons of A. pernyi is taken into account, the time can only be traced to ~3,000 yr ago. This time course is also much shorter than even 0.74 million years ago. The molecular-based excess divergence time also was reported between B. m. and Chinese B. m. mandarina (1.08 ± 0.18–1.41 ± 0.24 million years ago versus 5,000 yr), and is attributed to the artificial selection that might act on the mitogenome during the domestication (Pan et al. 2008, Hu et al. 2010).

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