Cytochrome Subunit b and NADH Oxido-reductase Subunit I Genes of Mitochondrial Genome from the Eri Silkworm, Samia cynthia ricini and Phylogenetic Analysis

Xing-jia Shen¹, Qiao-ling Zhao¹, Zhi-fang Zhang¹.*, Yi-ren Li¹ and Jia-lu He¹

Abstract Mitochondrial DNA of eri silkworm, Samia cynthia ricini was prepared from newly-hatched larvae with the method described previously. After mtDNA and pBluescript SK(+) (Amp') vector were doubly digested by EcoR I and Xba I respectively, a 3.3-kb restriction fragment was cloned and screened out. It contains partial 16S-rRNA gene, complete genes of tRNA^{Leu} (UUA), NADH oxido-reductase subunit I (nd-1), tRNA^{Ser} (AGU), cytochrome subunit b (cytb) and partial NADH oxido-reductase subunit VI (nd-6) gene orderly. The sequences of cytb and nd-1 of S. c. ricini were compared with those of nine published species of Insecta and a phylogenetic tree was constructed based on their sequences. The results indicated that S. c. ricini has closest evolutionary relationship with Bombyx mori and B. mandarina. Meanwhile the possible secondary structures of tRNA^{Leu} (UUA) and tRNA^{Ser} (AGU) of S. c. ricini were inferred from their sequences.

Key words: cytochrome subunit b, NADH oxido-reductase subunit I, mitochondrial DNA, eri silkworm, Samia cynthia ricini

Introduction Telephone and Company of the Company o

Mitochondrial genomes of animal contain thirteen polypeptide-encoding genes including cytochrome subunit b gene (*cytb*), three cytochrome oxidase subunit genes (*cox-1*, *cox-2* and *cox-3*), seven NADH oxido-reductase subunit genes (*nd-1*, *nd-2*, *nd-3*, *nd-4*, *nd-4L*, *nd-5* and *nd-6*) and ATP synthase subunit VI and VIII genes (*atp-6* and *atp-8*). Their expression products are components of oxidative phosphorylation

system on mitochondrial inner membranes and join in the process of energy metabolism. They are, therefore, significant to cells and organisms (Dey et al, 2000). In these thirteen genes, cytb, cox-1, cox-2 and cox-3 are the most conservative ones with about 80% of nucleotide sequence similarity among different animal species. They are useful for studying molecular evolution and classification of species (Garcia-Machodo et al., 1999; Gaunt et al., 2002). Studies of molecular evolution have been carried out on the basis of nucleotide sequences of these

¹ Key Laboratory of Silkworm Biotechnology, Ministry of Agriculture, Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212018, China

^{*}To whom correspondence shoud be addressed. Key Laboratory of Silkworm Biotechnology, Ministry of Agriculture, Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212018, China. Tel: +86-511-5616659; Fax: +86-511-5615044; E-mail: zjsbsri@public.zj.js.cn

conservative genes (Lo Galbo *et al.*, 2002; Prychitko *et al.*, 2000; Howell, 1989; Bernasconi *et al.*, 2000; Koulianos *et al.*, 2000; Miya *et al.*, 2000; Wei *et al.*, 2002).

The eri silkworm, S. c. ricini, is one of important industrial insects living mainly on leaves of castor-oil plant, Ricinus comunis and economically significant as Antheraea pernyi. Many researches have been performed on domestication and improvement of breeds. The S. c. ricini has relatively close relationship with B. mori, so molecular studies on mitochondrial genome of S. c. ricini are expected to provide us insights on evolution of both B. mori and S. c ricini. Recently, a primary restriction endonuclease map has been drawn (Ling et al., 1993). Further, the cox3, 12S-rRNA and a few other genes were published or registered (Genbank accession No.: AF288145, AY037829). In this research, as the first time we reported the sequences of cytb of S. c. ricini and from which the evolutionary relationship with other insect species was discussed.

Materials and Methods

Eri silkworm and reagents

The eri silkworms for mtDNA preparation is named as Hualan preserved in our laboratory. The cloning vector pBluescript SK(+) (Amp') and host bacterial *E. coli* JM109 are also house-keeping. Restriction endonucleases and T4 DNA ligase were purchased from Gibco-BRL Ltd. The sequences of mitochondria genes of eri silkworm *S. c. ricini* were determined from our research and registered into Genbank (Accession No.: AF527774). The other data for phylogenetic research were retrieved from the GenBank.

Cloning and screening

MtDNA was extracted from newly-hatched larvae of eri silkworm with the method described previously (Tamura *et al.*, 1988; Shen *et al.*, 2000). Before cloning, both mtDNA and cloning vector were doubly digested with *EcoR* I and *Xba* I, respectively. And the large fragment of vector was obtained by agarose gel electrophoresis segregation. Then they were ligated by T4 DNA ligase at 14~16 °C and the resultant

product was transformed into *E. coli* JM109 (Sambrook *et al.*, 1989). Bacterial colonies were cultured in LB medium with ampicillin and screened with the method as described previously (Beuken *et al.*, 1998).

Sequencing and analysis

Primer-walking method was employed in sequencing of the recombinant nucleotide. By comparison and online retrieve of Genbank, the order of genes in the fragment of *S. c. ricini* mtDNA have been determined. The gene structure and phylogenetic analyses were carried out with Dnastar software.

Results and Discussion

Identification of 3.3 kb fragment of mtDNA of eri silkworm

By analyzing plasmid DNAs with endonulease digestion and electrophoresis, finally one recombinant DNA was found. When the recombinant DNA digested by *EcoR* I or *Xba* I, respectively, only one band about 6.3 kb was observed. But, when it doubly digested by *EcoR* I and *Xba* I, there were two bands, about 3 kb of the vector DNA and about 3.3 kb of an insert DNA. Sequence analysis confirmed that the 3.3 kb insert was a fragment of eri silkworm mtDNA.

Gene structure analysis

The cloned fragment was 3296 bp long (Fig. 1). By comparison with genes of mitochondrial genome of other species of Insecta such as B. mori and Drosophila, we deduced genes and their order in the sequence as follows: ① From the 1st to 723rd nt is one-second sequence of 16S-rRNA (the 3' end part) ending by AGA: ②From the 726th to 798th nt is tRNA^{Leu} (UUA): ③From the 789th to 1718th nt is NADH oxsidoreductase subunit 1 (Scrnd-1); (4) From the 1719th to 1784th nt is the complementary sequence of tRNA^{ser} (AGU); ⑤From the 1814th to 2962nd nt is the complementary sequence of cytochrome oxidease b (Scrcytb); ⑥From the 2969th to 3296th nt is the complementary sequence of the 3' end part of nd-6 (Scrnd-6). The tRNA^{Leu} and Scrnd-1 are overlapped for 9 nts, while short intervening sequences exist

1 GAATT

181 TAATA

451 TGO

631 TITTA

721 AGAAA 811 TTATTA

901 TAATA 991 TITIGI

1081 GGAGT 1171 ATTAO

1261 TATTT 1351 ACTOC 1441 ATATTS

1531 TTTCT

1711 TAATA

1801 TATTAA 1891 AATTGT

1981 ATTAAT 2071 CTCCTA

2161 GGAATO 2251 ATATGT

2341 TTATTO 2431 CAATTA

2521 ATAACO 2611 GGCCA

2701 TAGTTA 2791 TAAAGA

2881 AGGGG

2971 ATTTAA 3061 AATTAA

3151 TATTAA 3241 TTTAAA

Fig.1. N

	16S rPNA
1	GAATTCGGCAAAAATTTATATTCACTTGTTTATCAAAAAACATGTCTTTTTTGTTAATAATTTAAAGTCTAATCTGCCCACTGATGTAATATTGAAG
91	GCCTCCACTATTTTCACCTCTACAAAAAATTTATCAACAAAAACATGTCTTTTTGTTAATAATTTAAAGTCTAATCTGCCCACTGATCTAATATTTCAAC
181	GGCTGCAGTATTTTGACTGTACAAAGGTAGCATAATCATTAGTCTCTTAATTGAAAGTCTAATTTGAAGGTCTAATCTGCCCACTGATGTAATATTTGAAG TAATATTTAATTGAATTTAATTTTTTTT
	TAATATTTAATTGAATTTAATTTTATTTAAAAAAGTTAAAATAAT
361	ATTATTTATATATATATATATATATATATATATATATA
451	ATGAATTTTTGATCCAATTTTATTGATTAAAAGAAAAATTACCTTAGGGGTGACAAAAAAAA
541	TGCGACCTCGATGTTGGATTAAGATAAAATTTAAATGCAAAAGTTTAAAATTTTTGATCTGGTCGATCATTAAAAATCTTACATGAGAAAAAAGTT AACCGGTGTAAGCCAGGTTGGTTTCTATCTTTTAAAAAATTTTAATATTTTAACTAGTCGATCATTAAAAATCTTACATGATCTGAGTTCA
	AACCGGTGTAAGCCAGGTTGGTTTCTATCTTTTAAAAATTTTAATATTTTAGTACGAAAGGATTGAATATTAAATTTAAATTTAAATTTTTAATATTTTTAATAT
On A	TTTTATTAATTTTTTTTTTATTCAAGTAAAAAATTAAAATTAAAATTTTTGGC
721	Leu-tRNA — nd-1→ AGAAAAAATGTGATGATTTTAGAAGTCATGAATGTATAATTTATTATATAGATAG
811	TTATTAATTTTAATTCTTCCCCCTAATACTACCAATTCCCCCAATTCCCCAATTCCCCCAATTCCCCCAATTCCCCCAATTCCCCCC
901	TTATTAATTTTAATTGTTGGGGTAATAGTAGGAATTGCTTTTTTGACTTTATTAGAGCGTAAAGTATTAGATTAGATTTATATTTGATTTTTATTGGA TAATAAGTTAGGTATAGTTGGGGTTATTACAACTTTTTTCTCATCCTATTAAAGTTAGTT
991	TAATAAGTTAGGTATAGTTGGGTTATTACAACCTTTTTTGATGGTATTAATTA
1081	TTTGTTATTATTTTTCTCCGGAGTTAGTTTTTTTAATATCTTTATTAATTTTGGATAGTTATTTACCAAAAGAACAACTTATCCTAATTATTCAAATTATT GGAGTTTTATTTTTTTTTT
1171	ATTACGAGCGGTAGCTCAAACAATTTCTTATCAACTAAACTAAAGTAAATTACAGTAATTATAATTGCTGGTGATCTCTAAATTCTAATTATGCTTTATTAGGGGGG
1261	TATTTTTTAAATATCAAAAACTAATTTCATTTTTTTTTT
1351	ACTCCTTTTGACTTTGCTGAAGGGGAGAGAGAGTTAGTATTTTCATATTTCATTTTGTGTTTGGGTTTCTTCAATAATGGCTGAAACTAATCGT ATATTCTAGTATTTTATTT
1441	ATATTCTAGTATTTTATTTATAACATTTTTTTTTAGCAGA
1531	ATATTCTAGTATTTTATTTATAAGATTTTTATTTGTTTTAGTTTATTTAGGGGGTTATGTTTTAAATTTAAAATTTAATATTTTTT
1621 7	TTCTTTTTTATTTATTTATTTATTTATTTGTTTTAGTTTATTTAGGGGGG
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	Ser-tRNA (complementary seq. ————————————————————————————————————
1711 T	
	Code ()
1801 T	Cytb (complementary seq. — >
1891 A	ATTAATTAAAAATTATTTATTGAATAAAAGTTTGTCTCAATAAATTCTAATTAAAGGATTTAAGATAAAATATAAAAAATATAAAAATTGTTAAT ATTGTCCTGTGATAATATAAGGAGCTCTAAGGACGACCTCAATTCAACTTAATAAAAATATAAAAATATAAAAAA
1031 A	ATTGTCCTGTGATAATATAAAGGAGCTTCTACAGGACGAGCTCCAATTCAAGTTAAAGGATTTAAGATAAAAATATAAAAAATTTGTTAAT TTAATTGGGTAAAATTGTAAACCTTGAATTTTTTTTTAAAAATCTTAAACGTAAAATAATTAAT
2071 C	TTAATTGGGTAAAATTGTAAACCTTGAATTTTTTTTAAAAATGTTAAAGGTAAAATAATTAAAATTAAAAATAAAATATAAAATTACAAAGATTCAAAAATATAAAATTG CCCTAATTTATTAGGGATTGATCGTAGAATAGCATAGGCAAAAAATGATAAAATAATAAAATTAAAAATAAAATAAAATTACAAAATAACAATTACTAAAGCAATTACTC
2161 G	CCTAATTTATTAGGGATTGATCGTAGAATAGCATAGGCAAAAAGAAAATATCATTCTGGTTGAATATGGATATTACTAAAGCAATTACTC GAATGAAATTATCAGGGATCTCCTAATAAGTAGGGGTTACATAAACTTAAAATTAAAATAGATATGGATAGGAGTAACTAATGGGTTAGCT
2251 AT	GAATGAAATTATCAGGATCTCCTAATAAGTAGGGGTTAGATAAAGTTAATATTAATAGAAGTATTAT
2341 TT	ATGTAAAATAGGGGTGAAAGGGGATTTATCTATATTACTATTAAGTCCTAAAGGGTTATTATTAGAACCTGTTTGATGTAAAATCCAATTAAAATCTTT ATTGTTAAAATTAAAATAAATAAATGGTAGAAGAAATCAAATCTATAAAATCCCCTTAAAAGGTTATTAGAACCTGTTTGATGTAAAAATAAAT
2431 CA	ATTGTTAATATTAAAATAATAAATGGTAGAAGAAAATGAAATGTATAAGTCCTAAAGGGTTATTAGAACCTGTTTGATGTAAAAATAAAT
2521 AT	ATTAACTAGTATAGTTCCTAAATAAGGGATAGCTGAAAGAAGATTAGTATAAAATCGGGTTAGGGTAGCGTTATCTACAGCAAATCCCCCTCAAATT AACCTATAAAAGCAGTTGCTATTAATATAAAATAAA
2611 GG	AACCTATAAAAGCAGTTGCTATTAATATAAAATAAATAAA
2701 TA	CCAATATGAAGATAAATACAAATAAAGAAAAAAAATAATTACTCCAATAAATCAAGTATATTTTAAGGTTAAAAAGATTCATAATAAATTCCTC GTTAACACTAAAAAAAAGCTAATTCAATATTTGCTGTATAGTATATACTTAAACAAAGAAGAGTTCAAACAATAATTTACATTTCGACAAATA
2791 TA	GTTAACACTAAAAAAAGCTAATTCAATATTTGCTGTATAGTATATAGTTAAGAAAAGACCTGTTAAAATTTGGATTATTAAACATATTTCGACAAATA AAGATCCAAAGTTTCATCATATAGAGATATTTG
A STATE OF THE STA	a respondential a
2881 AG	GGGGATGGTAAATCGATCAAAGATCCATTAATAATTTTTAAAATAGGATGAGTTTTTCGAATAGGGAGAAATTTAGTTATCATTTTTT
	THE COMMISSION OF THE COMMISSI
9071 Am	nd-6 (complementary seq. ————————————————————————————————————
2971 ATT	
PIEL TAT	TAAAAAATTTTGATTATTGTATAATTTATTTAAATTATTTTGTTTTCACTATTGCTACTAAAGTAAATAAA
2941 TM	TAATATTAAAATTTATTCAATTTAATTAATTAATTATTTATTTAAAAAA
0241 111.	AAATTAAATGAGAATAATTCATTAGAGGCAATTCTAGA

Fig.1. Nucleotide sequence of 3.3 kb fragment from EcoR I - Xba I doubly digested mtDNA of S. c. ricini

between other adjoining genes in this fragment.

Scrnd-1 is 930 nts encoding 309 amino acids, which is 15 nts shorter than that of *B. mori* (*Bmnd-1*). Its initiation and stop codon are ATA and TAA, respectively, the same as that of *B. mori*. The percentage of A+T is 76.56 similar to those of other insect species listed in Table 1.

Scrcytb is 1149 nts encoding 382 amino acids, which is 3 nts shorter than that of B. mori (Bmcytb). Both Scrcytb and Bmcytb are initiated with ATA and stopped with TAA.

There are two tRNA genes at the flanking of *nd-1* in the cloned fragment. One is tRNA^{Leu} (UUA) and the other is tRNA^{Ser} (AGU). They are 72 nts and 66 nts respectively. Their secondary structures were deduced according to their nucleotide sequences (Fig. 2a, 2b). The diagrams showed that tRNA^{Leu} (UUA) is stable. The tRNA^{Ser} (AGU) base pairs are however highly mis-paired especially in the D-loop indicated its unstable structure.

Comparison of nucleotide sequence of *nd-1* and *cytb* and phylogenetic analyses

The comparison of nucleotide sequence of nd-1 and cytb were carried out among the following 10 insects species: S. c. ricini (Scr), B. mandarina (Bma) and B. mori (Bm) belonging to Lepidoptera, Anopheles quadrimaculatus (Aq), A. gambiae (Ag), Cochilomyia hominivorax (Ch), Drosophila yakuba (Dy) and D. melenogaster (Dm) belonging to Diptera, Apis mellifera (Am) belonging to Hymenoptera and Locusta migratoria (Lm) belonging to Orthoptera, respectively. The results indicated that the similarities of nd-1 and cytb ranged from 66.9% to 97.5% except for A. mellifera, which was much lower than others. The average similarities of *nd-1* and *cytb* were 71.8% and 71.3%, respectively (Table 1). The S. c. ricini nd-1 showed the highest similarity with that of B. mori and B. mandarina (80.9% and 80.7%, respectively), and so does the S. c. ricini cytb (77.2%, both). It indicated that S. c.

Table 1. Comparison of homologies of nd-1 and cytb from mtDNAs of 10 insect species

%	TAMPAM	2	3	4	5	6	7	8	9	10
1. Scr	/	71.1	57.5	68.9	80.7	80.9	71.9	70.7	71.6	68.3
2. Ag	71.9	1	57.3	89.2	69.7	68.6	72.7	73.9	74.8	70.5
3. Am	55.1	57.4	/	57.3	60.1	59.2	54.1	57.4	57.7	56.6
4. Aq	72.8	90.4	57.1	1	71.0	69.7	75.5	75.8	77.7	71.2
5. Bma	77.2	74.2	58.0	74.9	1	95.0	70.5	72.0	73.5	72.1
6. Bm	77.2	75.0	58.2	75.1	97.5	/	70.3	71.2	72.9	71.4
7. Ch	71.7	77.1	54.8	78.7	70.8	70.7	1	81.4	82.9	74.1
8. Dm	71.9	80.2	56.6	79.3	72.9	72.9	80.0	1	91.0	73.3
9. Dy	72.8	79.8	57.2	79.7	71.9	71.7	81.5	93.2	/	74.1
10. Lm	65.7	72.1	53.5	72.7	69.0	70.0	69.0	71.2	71.0	1

Note: Above the diagonal are homologies of nd-1 genes and below are homologies of cytb genes.

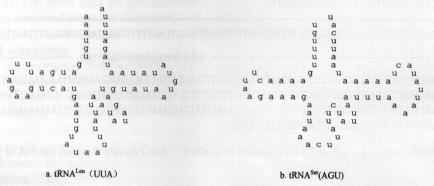


Fig. 2 The deduced secondary structures of tRNA^{Leu} and tRNA^{Ser} of S. c. ricini mtDNA

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ricini has a close evolutionary relationship with *B. mori* and *B. mandarina*. According to the results of sequences alignment by Dnastar software, we recognized that differences of cytb and nd-1 among these species increased greatly at both ends of their sequences. In contrast, in the middle parts, about 1100 nts in cytb and 880 nts in nd-1, were conservative. It suggested that the middle parts are related to the activity domain of their expression products.

The cytb, cox-1, cox-2 and cox-3 are the most conservative genes in mitochondrial genomes of animals and followed by nd-1, atp-6, atp-8, control region and so on. So cytb and cox genes are useful for studying molecular evolution among species (Howell 1989; Zardoya 1996; Lo Galbo et al, 2002; Gaunt et al., 2002). Here, a comparative phylogenetic tree is constructed from combined sequences of cytb and nd-1 in mitochondrial genomes from ten insect species (Fig. 3). When we used cytb and nd-1 sequences separately and combined for construction of phylogenetic trees, they showed the similar patterns (data not shown). In this tree, nine of the ten species belonging to Orthopetra, Diptera and Lepidoptera are distributed into one group. This group can be divided into two sub-groups further. One sub-group consists of six species of Orthopetra and Diptera. The other sub-group consists of three Lepidopteran species, i. e. S. c. ricini, B. mori and B. mandarina. These results are in accordance with that of classical morphological taxonomy. Exceptionally, A. mellifera formed an independent clade in this tree. The comparison of nucleotide sequences showed that single cytidine or adenosine was inserted at the 479th nt, 725th nt and 754th nt of A. mellifera nd-1 sequence, respectively.

Moreover, at the 104th nt, 110th nt, 114th nt, 502nd nt, 773rd nt and 848th nt sites, the absence of short sequences resulted in the movement of codon and led to lower similarities with other species. It implicated that these mutated sites are not the activity domain of nd-1. Similarly, at the 726th nt of A. mellifera cytb a single adenosine was inserted. The results indicated that the base replacements had happened much frequently on A. mellifera than those on the other species because of its specific reproductive style (David et al., 1994). A swarm of A. mellifera usually only has one queen who bears the task of producing offsprings. Once a genetic variation happens, it is easy to be fixed in the swarm and strictly follow the maternal inheritance. Their base replacement is about five to ten times as that of nuclear genome, i.e. their evolution rates are five to ten times as that of nuclear genome.

Mitochondrial genomes are an important model system for studying molecular evolution of the eukaryote (Clary et al., 1985; David et al., 1994). Up to the date, many phylogenetic trees have been constructed based on nucleotide sequences or deduced amino acid sequences of mitochondrial genes. However, the results of these molecular evolutionary analyses varied when different genes were analyzed, and often conflict to that of classical morphological taxonomy (Boore et al., 1998; Wilson et al., 2000). Thus, more comprehensive studies should be done to understand molecular evolution of the insect mtDNA.

References

Bernasconi, M. V., Valsangiacomo, C., Piffaretti,

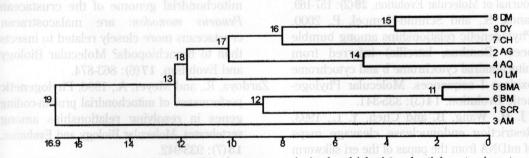


Fig. 3 Phylogenetic tree based on combined sequences of mitochondrial nd-1 and cytb from ten insect species

- J. C., and Ward, P. I., 2000. Phylogenetic relationship among Muscoidea (Diptera: Calyptratae) based on mitochondrial DNA sequences. Insect Molecular Biology, **9**(1): 67-74.
- Beuken, E., Vink, C. and Bruggeman, C. A., 1998. One-step procedure for screening recombinant plasmids by size. Biotechniques, **24**(5): 748-750.
- Boore, J. L., Lavrov, D. V. and Brown, W. M., 1998. Gene translocation links insects and crustaceans. Nature, **392**: 667-668.
- Clary, D. O., and Wolstenholme, D. R., 1985. The mitochondrial DNA molecular of *Drosophila yakuba*: nucleotide sequence, gene organization and genetic code. Journal of Molecular Evolution, **22**(3): 252-271.
- David, O. F., Shlbinsk, L., Zouros, S. E., *et al.*, 1994. Mitochondrial DNA inheritance. Nature, **368**: 817-818.
- Dey, R., Barrientos, A., Moraes, C. T., 2000. Functional constraints of nuclear-mitochondrial DNA interactions in xenomitochondrial rodent cell lines. Journal of Biological Chemistry, **275** (40): 31520-31527.
- Garcia-Machado, E., Pempera, M., Dennebouy, N., et al., 1999. Mitochondrial genes collectively suggest the paraphyly of crustacea with respect to insecta. Journal of Molecular Evolution, **49**(1): 142-149.
- Gaunt, M. W. and Miles, M. A., 2002. An insect molecular clock dates the origin of the insects and accords with palaeontological and biogeographic landmarks. Molecular Biology and Evolution, **19**(5): 748-761.
- Howell, N., 1989. Evolutionary conservation of protein regions in the protonmotive cytochrome b and their possible roles in redox catalysis. Journal of Molecular Evolution, **29**(2): 157-169.
- Koulianos, S., and Schmid-Hempel, P., 2000. Phylogenetic relationships among bumble bees (Bombus, latreille) inferred from mitochondrial cytochrome b and cytochrome oxidase I sequences. Molecular Phylogenetic Evolution, 14(3): 335-341.
- Ling, J. H., Wang, B. and Chen, Y. L., 1993.
 Restriction endonuclease cleavage maps of mtDNA from the pupas of the eri silkworm

- (J). Journal of Xiamen University (natural science), **32**(5): 641-646, (in Chinese).
- Lo Galbo, A. M., Carpenter, K. E., and Reed, D. L., 2002. Evolution of trophic types in emperor fishes (Lethrinus, Lethrinidae, Percoidei) based on cytochrome B gene sequence variation. Journal of Molecular Evolution, **54**(6): 754-762.
- Miya, M., and Nishida, M., 2000. Use of mitogenomic information in teleostean molecular phylogenetics: a tree-based exploration under the maximum-parsimony optimality criterion. Molecular Phylogenetic Evolution, 17(3): 437-455.
- Prychitko, T. M., and Moore, W. S., 2000. Comparative evolution of the mitochondrial cytochrome b gene and nuclear beta-fibrinogen intron 7 in woodpeckers. Molecular Biology and Evolution, 17(7): 1101-1111.
- Sambrook, J., Fretsch, E. F., Maniatis, T., 1989. Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press.
- Shen, X., Zhao, Q. and Zhang, Z. et al., 2000. Cloning and sequence alignment of EcoR I 1.9 kb fragment of silkworm mitochondrial DNA. Canye Kexue (Acta Sericologica Sinica), 26(2):87-90.
- Tamura, K. and Aotsuka, T., 1988. Rapid isolation of animal mitochondrial DNA by the alkaline lysis procedure. Biochemical genetics, 26 (11/12): 815-819.
- Wei, Z., Zhao, Q., Zhang, Z., et al., 2002. Characterization of the cytochrome oxidase subunit II gene of mitochondrial DNA from the eri sikworm, Simia cynthia ricini. Acta Entomologica Sinica, 45(2), 193-197.
- Wilson, K., Cabill, V., Ballment, E. and Benzie, J., 2000. The complete sequence of the mitochondrial genome of the crustacean Process more closely related to insects than to branchiopods? Molecular Biology and Evolution, 17(6): 863-874.
- Zardoya, R. and Meyer, A., 1996. Phylogenetic periodic and mischondrial protein-coding relationships among relationships among Biology and Evolution, 13 (7) 433-44.