Progress in the Researches on Insect Mitochondrial Genome and Analysis of Gene Order

Li Hu Gao Jianyu Liu Haiyu Cai Wanzhi*

(Department of Entomology, China Agricultural University, Yuanmingyuan West Road, Beijing 100193, China; *corresponding author, email: caiwz@cau.edu.cn)

Insect mitochondrial genome is a double-stranded circular genomes which range from 14, 503 bp to 19, 571 bp in size. Nearly all the sequenced insect mitochondrial genomes encode 37 genes: two for rRNAs, 13 for proteins and 22 for tRNAs. This review compares and summarizes the features of complete mitochondrial genomes from 175 sequenced species of insects in 22 orders. The genomic organization, contents, gene order, and rearrangements of gene order are analyzed.

Key words insect; mitochondrial genome; gene order; phylogeny

Mitochondrial (mt) genomes have become a major data sources for comparative genomics and play an important role in metabolism[1], apoptosis[2], disease[3] and aging[4]. In insects, mtDNA is typically a small genome, less than 16 kb, in combination with a generally conserved gene completement and rapid rate of nucleotide substitution, provide an ideal system for a wide range of comparative studies that have furthered the understanding of genome evolution.

Due to the relative ease of amplification and sequencing, in the last two decades the insect mitochondrial genome has become the most commonly used molecular marker for population genetics, phylogeography, molecular diagnostics and phylogenetic studies. In the present review, the basic features of complete mitochondrial genomes from 175 species of insects in 22 orders are surveyed and summarized with respects of their genomic organization, contents, and gene order.

1 Structure and composition of mitochondrial genomes of insects

To date the complete or near complete mitochondrial genomes have been sequenced from 175 insect species in 22 orders. Most species come from the order Diptera, Hemiptera, Hymenoptera, Lepidoptera and Orthoptera.

The mitochondria of insects contain their own double-stranded circular genomes which range from 14, 503 bp[5] to 19, 517 bp in size[6]. Despite the extraordinary range in size, the gene content of the molecule is remarkably conserved. With few exceptions it encodes 37 genes including 13 protein-coding subunits (*COI-III, CytB, ND1-6, ND4L, ATP6* and *ATP8*) from three of the oxidative phosphorylation complexes, plus the 2 ribosomal RNA (*rrnS* and *rrnL*) and 22 transfer RNA (*trnI, trnQ, trnM, trnW, trnC, trnY, trnL1, trnK, trnD, trnG, trnA, trnR, trnN, trnS1, trnE, trnF, trnH, trnT, trnP, trnS2, trnL2, trnV*) genes necessary to translate the protein-coding genes [7]. In addition, mt genomes of insects contain one large AT-rich non-coding control elements, which are involved in the initiation and regulation of mt transcription and replication, and are therefore referred to as the AT-rich region or the mitochondrial control region (CR), which is usually present between *srRNA* and *tRNA-I*. The use of the term "AT-rich region" to describe the control region should be reconsidered even though each control region has one or several more AT-rich subregions, because this region is not always the most AT-rich part of the mt genome[8].

Except for the control region, the entire genome comprises compactly arranged coding sequences with no introns and with very few intergenic nucleotides, and neighboring genes may even shortly overlap in some cases. For instance, besides the control

region there are two major non-coding regions of 232 and 273 bp in length that are located at the gene junction *tRNA Q-E* and *tRNA W-S2* in *Bothriometopus macrocnemis*[9].

Overlapping genes found among many mtDNAs leads to speculation that the "polycistron model" may not universally apply, since it would not be possible to release full-length RNAs of each overlapping message from the same transcript[10].

Both the largest and smallest mitochondrial genome comes from the order Diptera. The former is *Drosophila melanogaster*, which is 19, 517 bp in size[6, 11], and the latter is *Rhopalomyia pomum,* which is 14, 503 bp in size[5]. The reason for the difference in size of the mitochondrial genome is the different length of the control region. Generally the size of the control region of mtDNA range from 70 bp (Orthoptera: *Ruspolia dubia*)[12] to 4.6 kb (Diptera: *Drosophila melanogaster*)[6, 11]. The views that mtDNAs are selected for extreme economy of size, while apparent in some cases, is refuted by mitochondrial genomes with very large amounts of non-coding sequence, as has been found in some species.

The genome which contains the standard set of 22 tRNA genes usually presents in insects mtDNAs, but there are some exceptions (Table 1). For example, the genome of *Bothriometopus macrocnemis* encodes 25 tRNA genes, 21 of the 22 tRNA genes common to most mt genomes of insects plus three additional copies of the *tRNA-Y*, and an additional copy of the *tRNA-V*. The gene for the *tRNA-A* is lacking. In addition, remnants of two additional copies of tRNA are present. The two tRNA-like pseudogenes were found between *tRNA-N* and *tRNA-T* and between *tRNA-S2* and *COI*. In both regions a cruciform secondary structure resembling a tRNA could be found. However, neither could form a functional anticodon loop[9].

The total AT content calculated over the whole molecule ranges from 63%[13] to 88%[14]. This conforms to the well-know A+T bias of insect mitochondrial genomes. In some mtDNA all genes are transcribed from one strand such as the screamer louse *Bothriometopus macrocnemis*[9], whereas in others the genes are distributed between the two strands. In most cases, the majority of genes are located on the *plus* or J-strand, the remainder having opposite polarity and being oriented on the *minus* or N-strand. Except for different amount of genes on the two strands, there is an asymmetrical compositional bias across the two strands. In an olive fly *Bactrocera oleae*[15], protein coding genes on the J-strand show a fairly similar A% and T%, whereas those encoded on the N-strand have a much higher proportion of T than A. The presence of such a bias suggests that genes may be subject to additional substitutional pressures (directional selection) following a translocation that involves a change in the coding strand. This phenomenon may have important consequences on molecular phylogenetic studies when single mitochondrial genes are used to compare distantly related taxa, in which the same gene product may be encoded on different strands[15].

Canonical initiation codons (ATA or ATG), encoding the amino acid methionine, are usually used in most PCGs[16]. However, there are apparent exceptions to the ATN rule[15]. Some genes start with non-standard codons as it often happens in mtDNAs of insects. And the translation initiation signal for the *COI* gene is a good case in point. In *Drosophila melanogaster* and *D. yakuba*[17, 18] no ATN in-frame codon is found in the proximity of the presumed start site of the gene. Although a TCG triplet opens the *COI* gene reading frame, a tetranucleotide ATAA, positioned directly upstream was proposed as an initiator[19]. Two mechanisms that would permit translation to start at this sequence were suggested that either initiation occurs at the ATA codon following a +1 frame shift of the translation machinery to restore the reading frame and resume a correct translation, or that the anticodon of the initiation *N*-formylmethionine tRNA might permit the ATAA sequence to be recognized as a single codon.

In most cases PCGs terminate with the complete termination codon TAA or TAG, but in all other cases, stop codons are truncated (T or TA) and their functionality probably recovered after a post-transcriptional polyadenylation[20]. These abbreviated stop codons are found in PCGs that are followed by a downstream tRNA gene, suggesting that the secondary structure information of the tRNA genes could be responsible for the correct cleavage of the polycistronic transcript[21]. However, there are also direct junctions between two PCGs (ATP8- ATP6, ATP6-COIII, ND6-CytB and ND4L-ND4) where other cleavage signals, different from tRNA gene secondary structures, may be involved in the processing of the polycistronic primary transcript[22]. In this respect, it is found that possibly stem-loop structures in the 3' end of those protein-coding genes which are not flanked by tRNAs[23-25].

2 Gene order and rearrangement of mt genomes

Although animal mitochondrial sequences are known to evolve rapidly, arrangements of insect mitochondrial genes are relatively stable, with some having been conserved over long periods of evolutionary time. Because of the low rearrangement rate as well as several other characteristics, mitochondrial gene arrangements promise to be a useful data set for the study of deep divergences of insects. Indeed, several problematic relationships have already been convincingly resolved using this data set[26, 27].

Whether certain types of gene rearrangement are more common than others is a question. Genome rearrangements can be characterized in several aspects: (1) the types of genes rearranged, tRNAs only (termed minor rearrangements) or also protein-coding and rRNA genes (major rearrangements); (2) whether genes are translocated along the same strand or inverted between coding strands; and (3) the localization of the rearrangement (between local or distant gene blocks)[28]. A given gene rearrangement may exhibit one or more of these aspects. In addition, there are different mechanistic models of how genome arrangements occur.

The best supported model attributes gene rearrangements to the partial duplication of mtDNA caused by errors in replication, such as erroneous initiation or termination, or strand slippage and mispairing, followed by the loss of one copy of each duplicated gene. It is commonly assumed that the loss of one of the two copies of each duplicated gene happens at random, and the model described is referred to as the duplication-random loss model[29-31].

Another most commonly mechanism invoked is the model: mtDNA duplication and nonrandom gene loss. According to this model, the destiny of each gene copy in the duplicated region is predeterminated by its transcriptional polarity and location in the genome[32].

Because translocations could not be explained by the alternative replication-splippage-based models, the incidence of genome translocations has been used as evidence for the occurrence of intra-mitochondrial recombination[33].

As additional mt genomes have been sequenced, however, the phylogenetic utility of gene rearrangement in understanding insect evolution now appears less than previously thought because most insect mt genomes have retained the ancestral gene order and there is not enough phylogenetic signal to resolve interordinal or deeper relationships from the existing gene rearrangements.

In Insecta, mitochondrial gene rearrangements have been found in 83 species belonging to11 orders, with the others exhibiting the same gene order observed in *Drosophila yakuba*[18]. Rearrangements appear much common in tRNA genes and very rare in protein-coding genes and rRNA genes. Rearrangements of protein-coding genes were found in three orders: three species in Hemiptera[34, 37], one species in Lepidoptera [36], one species in Psocoptera[37] and one species in Coleoptera. Both rearrangements of protein-coding genes and rRNA genes were observed in the orders Thysanoptera[19] and Phthiraptera[9, 38].

Variability in gene order can be observed within groups at many taxonomic levels, and it has been found that four hemipteroid orders (Hemiptera, Thysanoptera, Psocoptera, and Phthiraptera) and Hymenoptera have increased rates of gene rearrangements in the mt genomes.

In the order Thysanoptera, rearrangements of 24 genes appear in the species *Thrips imagines*[9], and translocations are found in eight of them. This species has two very similar non-coding regions, with 18 genes rearranged between them.

Almost all 37 genes are transposed from their ancestral position in the two sequenced phthirapteran species. So lice are an ideal group in which to study rearrangements due to the heightened rearrangement rate. It is found that 11 of the 13 protein-coding genes have 3' end stem-loop structures which may allow mRNA processing without flanking tRNAs in *Bothriometopus macrocnemis* and so facilitate gene rearrangements.

Within Hemiptera, there are available a number of completely sequenced mitochondrial genomes that have variations in the gene order[8, 39]. All observed rearrangements fall into three types: A: translocations and transpositions of tRNA genes on the basis of the ancestral gene order; B: *I-Q-M-ND2-W-C-Y* block transposed to the end of the control region, with the translocation of some tRNA genes; C: transpositions of DNA fragment containing *COIII-G-ND3-A-R-N* on the basis of type B. In type C, there is variation in the mitochondrial position into which these genes are transposed. In addition, there are differences with respect to the retention of the number and the order of the excised tRNA genes at the mitochondrial location in which the genes are inserted. The maximal insertion involves all of the genes from the excised fragment in their original order *A-R-N-ND3-G-COIII*, the minimal insertion involves *ND3-G-COIII*. In all insertions, the transcription direction is altered from that in the original position [34]

Surprisingly, among ten completely sequenced mitochondrial genomes in the order Hymenoptera, most of tRNA genes are

transposed[9, 14, 40- 45]. The new orders of these genomes seem haphazard.

By contrast, rearrangements of mt genomes from many other insect orders appear stable in species and gene orders are similar to that of proposed ancestor.

Most orthopterans just contain a tRNA gene rearrangement from the insect ground plan *K-D* swapping to *D-K*[46-52], except the species *Troglophilus neglectus* with an additional *tRNA-L1*[46] and *Teleogryllus emma* with the translocation of *tRNA-N* and *tRNA-E*[53]. Three species in Neuroptera only have the translocation of *tRNA-W* and *tRNA-C*[54, 55]. In Coleoptera, the region of the mt genome consisting of genes encoding for *I-Q-M*[56, 57] and *I-Q-M-ND2-W-C-Y* can be transposed from its ancestral position to the end of the control region. The translocation of *tRNA-I-Q* and *tRNA-M* is the common rearrangements of mt genomes in Lepidoptera[36, 58-67]. Changes in the Ephemeroptera, Phasmatodea, Archaeognatha and Diptera are the gene arrangement in the location of *I-Q-M*, *W-C-Y* and *A-R-N-S1-E-F* clusters[35, 68-73].

It seems that the most common changes in mt genomes are of genes near the large non-coding region or of the tRNAs of regions: *I-Q-M*, *W-C-Y, K-D* and *A-R-N-S1-E-F*, especially the region *A-R-N-S1-E-F*. This points to a role for the origins of replication in gene order translocation and, perhaps, suggests a closer look be taken for a second-strand origin in arthropod mtDNA near the *A-R-N-S1-E-F* region[27].

Rearrangements appear much more common in insects and variability in gene order can be found within groups at many taxonomic levels. With few exceptions, gene arrangements are relatively stable within major groups, but variable between them, and comparisons of these gene arrangements have great potential for resolving some of the deepest branches of insect phylogeny.

3 Conclusions

Complete mitochondrial genome sequences are being determined at an ever-increasing rate, providing many new opportunities for comparing genome structure. MtDNA data have played a major role in studies of molecular evolution that have helped to refine models of evolution and understand how rates and patterns of substitution vary across sequences and over time. With the completion of whole genome sequencing projects accelerating, this will serve as a model for interpreting broader aspects of genome evolution and insect phylogeny. In addition to expanding this data set with broader taxonomic representation, it is important that studies address the molecular mechanism leading to rearrangement and develop better theoretical models and computer reconstructions.

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References

[1] Brand MD. Regulation analysis of energy metabolism. J Exp Biol. 1997, 200: 193-202.

[2] Kroemer G, Dallaporta B, Resche-Rigon M. The mitochondrial death/life regulator in apoptosis and necrosis. Annu Rev Physiol. 1998, 60: 619-642.

[3] Graeber MB, Muller U. Recent developments in the molecular genetics of mitochondrial disorders. J Neurol Sci. 1998, 153: 251-263.

[4] Wei YH. Oxidative stress and mitochondrial DNA mutations in human aging. P Soc Exp Biol Med. 1998, 217(1): 53-63.

[5] Beckenbach AT, Joy JB. Evolution of the mitochondrial genomes of gall midges (Diptera: Cecidomyiidae): rearrangement and severe truncation of tRNA genes. Genome Bio Evol. 2009, 1(1): 278-287.

[6] Lewis DL, Farr CL, Kaguni LS. *Drosophila melanogaster* mitochondrial DNA: completion of the nucleotide sequence and evolutionary comparisons. Insect Mol Biol. 1995, 4(4): 263-278.

[7] Boore JL. Animal mitochondrial genomes. Nucleic Acids Res. 1999, 27(8): 1767-1780.

[8] Hua JM, Li M, Dong PZ, Cui Y, Xie Q, Bu WJ. Comparative and phylogenomic studies on the mitochondrial genomes of Pentatomomorpha (Insecta: Hemiptera: Heteroptera). BMC Genomics. 2008, 9: 610. doi: 10.1186/1471-2164-9-610.

[9] Cameron SL, Johnson KP, Whiting MF. The mitochondrial genome of the screamer louse *Bothriometopus* (Phthiraptera: Ischnocera): effects of

extensive gene rearrangements on the evolution of the genome. J Mol Evol. 2007, 65(5): 589-604.

[10] Montoya J, Gaines G, Attardi G. The pattern of transcription of the human mitochondrial rRNA genes reveals two overlapping transcription units. Cell. 1983, 34(1): 151-159.

[11] Lewis, DL, Farr CL, Farquhar AL, Kaguni LS. Sequence, organization, and evolution of the A+T region of *Drosophila melanogaster* mitochondrial DNA. Mol Biol Evol. 1994, 11(3): 523-538.

[12] Zhou Z, HuangY, Shi F. The mitochondrial genome of *Ruspolia dubia* (Orthoptera: Conocephalidae) contains a short A+T-rich region of 70 bp in length. Genome. 2007, 50(9): 855-866.

[13] Comandi S, Carapelli A, Podsiadlowski L, Nardib F, Frati F. The complete mitochondrial genome of *Atelura formicaria* (Hexapoda: Zygentoma) and the phylogenetic relationships of basal insects. Gene. 2009, 439(1-2): 25-34.

[14] Wei SJ, Shi M, He JH, Sharkey M, Chen XX. The complete mitochondrial genome of *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) indicates extensive independent evolutionary events. Genome. 2009, 52(4): 308-319.

[15] Nardi F, Carapelli A, Dallai R, Frati F. The mitochondrial genome of the olive fly *Bactrocera oleae*: two haplotypes from distant geographical locations. Insect Mol Biol. 2003, 12(6): 605-611.

[16] Wolstenholme DR. Genetic novelties in mitochondrial genomes of multicellular animals. Curr Opin Genet Dev. 1992, 2(6): 918-25.

[17] Clary DO, Wolstenholme DR. The ribosomal RNA genes of *Drosophila* mitochondrial DNA. Nucleic Acids Res. 1985, 13(11): 4029-4045.

[18] Clary DO, Wolstenholme DR. The mitochondrial DNA molecular of *Drosophila yakuba*: nucleotide sequence, gene organization and genetic code. J Mol Evol. 1985, 22(3): 252-271.

[19] Shao R, Barker SC. The highly rearranged mitochondrial genome of the plague thrips, *Thrips imaginis* (Insecta: Thysanoptera): convergence of two novel gene boundaries and an extraordinary arrangement of rRNA genes. Mol Biol Evol. 2003, 20(3): 362-70.

[20] Ojala D, Merkel C, Gelfand R, Attardi G. The tRNA genes punctuate the reading of genetic information in human mitochondrial DNA. Cell. 1980, 22(2): 393-403.

[21] Ojala D, Montoyo J, Attardi G. tRNA punctuation model of RNA processing in human mitochondria. Nature. 1981, 290(5806): 470-474.

[22] Boore JL, Brown WM. Complete DNA sequence of the mitochondrial genome of the black chiton, *Katharina tunicata*. Genetics. 1994, 138(1): 423-443.

[23] Kim I, Lee EM, Seol KY, Yun EY, Lee YB, Hwang JS, Jin BR. The mitochondrial genome of the Korean hairstreak, *Coreana raphaelis* (Lepidoptera: Lycaenidae). Insect Mol Biol. 2006, 15(2): 217-225.

[24] Krzywinski J, Grushko OG, Besansky NJ. Analysis of the complete mitochondrial DNA from *Anopheles funestus*: an improved dipteran mitochondrial genome annotation and a temporal dimension of mosquito evolution. Mol Phylogenet Evol. 2006, 39: 417-423.

[25] Fenn JD, Cameron SL, Whiting MF. The complete mitochondrial genome sequence of the Mormon cricket (*Anabrus simplex*: Tettigoniidae: Orthoptera) and an analysis of the control region variability. Insect Mol Biol. 2007, 16(2): 239-252.

[26] Simon C, Buckley TR, Frati F, Stewart JB, Beckenbach AT. Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. Annu Rev Ecol Evol S. 2006, 37: 545-579.

[27] Boore JL, Brown WM. Big trees from little genomes: mitochondrial gene order as a phylogenetic tool. Curr Opin Genet Dev. 1998, 8: 668-674.

[28] Dowton M, Castro LR, Austin AD. Mitochondrial gene rearrangements as phylogenetic characters in the invertebrates: the examination of genome ‘‘morphology’’. Invertebr Syst. 2002, 16(3): 345-356.

[29] Macey JR, Lrson A, Ananjeva NB et al. Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. Mol Biol Evol. 1997, 14(1): 91-104.

[30] Madsen CS, Ghivizzavi SC, Hauswirth WW. In vivo and in vitro evidence for slipped mispairing in mammalian mitochondria. Proc Natl Acad Sci USA. 1993, 90: 7671-7675.

[31] Levinson G, George AG. Slipped-strand mispairing: A major mechanism for DNA sequence evolution. Mol Biol Evol. 1987, 4(3): 203-221.

[32] Lavrov DV, Boore JL, Brown WM. Complete mtDNA sequences of two millipedes suggest a new model for mitochondrial gene rearrangements: duplication and nonrandom loss. Mol Biol Evol. 2002, 19(2): 163-169.

[33] Dowton M, Campbell NJ. Intramitochondrial recombination is it why some mitochondrial genes sleep around? Trends Ecol Evol. 2001, 16(6): 269-271.

[34] Thao ML, Baumann L, Baumann P. Organization of the mitochondrial genomes of whiteflies, aphids, and psyllids (Hemiptera: Sternorrhyncha). BMC Evol Biol. 2004, 4: 25. doi:10.1186/1471-2148-4-25

[35] Cameron SL, Lambkin CL, Barker SC, Whiting MF. A mitochondrial genome phylogeny of Diptera: whole genome sequence data accurately resolve relationships over broad timescales with high precision. Syst Entomol. 2007, 32(1): 40-59.

[36] Yukuhiro K, Sezutsu H, Itoh M, Shimizu K, Banno Y. Significant levels of sequence divergence and gene rearrangements have occurred between the mitochondrial genomes of the wild mulberry silkmoth, *Bombyx mandarina*, and its close relative, the domesticated silkmoth, *Bombyx mori*. Mol Biol Evol. 2002, 19(8): 1385-1389.

[37] Shao R, Campbell NJ, Schmidt ER, Barker SC. Increased rate of gene rearrangement in the mitochondrial genomes of three orders of hemipteroid insects. Mol Biol Evol. 2001, 18(9): 1828-1832.

[38] Covacin C, Shao R, Cameron S, Barker SC. Extraordinary number of gene rearrangements in the mitochondrial genomes of lice (Phthiraptera: Insecta). Insect Mol Biol. 2006, 15(1): 63-68.

[39] Song N, Liang AP. Complete mitochondrial genome of the small brown planthopper, *Laodelphax striatellus* (Delphacidae: Hemiptera), with a novel gene order. Zool Sci. 2009, 26(12): 851-860.

[40] Crozier RH, Crozier YC. The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. Genetics. 1993, 133(1): 97-117.

[41] Hong MY, Cha SY, Kim DY, Yoon HJ, Kim SR, Hwang JS, Kim KG, Han YS, Kim I. Presence of several tRNA-like sequences in the mitochondrial genome of the bumblebee, *Bombus hypocrita sapporoensis* (Hymenoptera: Apidae). Genes Genom. 2008, 30(4): 307-318.

[42] Cha SY, Yoon HJ, Lee EM, Yoon MH, Hwang JS, Jin BR, Han YS, Kim I. The complete nucleotide sequence and gene organization of the mitochondrial genome of the bumblebee, *Bombus ignitus* (Hymenoptera: Apidae). Gene. 2007, 392(1-2): 206-220.

[43] Dowton M, Cameron SL, Dowavic JI, Austin AD, Whiting MF. Characterization of 67 mitochondrial tRNA gene rearrangements in the Hymenoptera suggests that mitochondrial tRNA gene position is selectively neutral. Mol Biol Evol. 2009, 26(7): 1607-1617.

[44] Wei SJ, Tang P, Zheng LH, Shi M, Chen XX. The complete mitochondrial genome of *Evania appendigaster* (Hymenoptera: Evaniidae) has low A+T content and a long intergenic spacer between atp8 and atp6. Mol Biol Rep. 2009, in press.

[45] Castro LR, Ruberu K, Dowton M. Mitochondrial genomes of *Vanhornia eucnemidarum* (Apocrita: Vanhorniidae) and *Primeuchroeus* spp. (Aculeata: Chrysididae): evidence of rearranged mitochondrial genomes within the Apocrita (Insecta: Hymenoptera). Genome. 2006, 49(7): 752-766.

[46] Fenn JD, Song H, Cameron SL, Whiting MF. A preliminary mitochondrial genome phylogeny of Orthoptera (Insecta) and approaches to maximizing phylogenetic signal found within mitochondrial genome data. Mol Phylogenet Evol. 2008, 49(1): 59-68.

[47] Ding FM, Shi HW, Huang Y. Complete mitochondrial genome and secondary structures of lrRNA and srRNA of *Atractomorpha sinensis* (Orthoptera, Pyrgomorphidae). Zool Res. 2007, 28(6): 580-588.

[48] Liu Y, Huang Y. Sequencing and analysis of complete mitochondrial genome of *Chorthippus chinensis* Tarb. Chin J Biochem Mol Bio. 2008, 24(4): 329-335.

[49] Fenn JD, Cameron SL, Whiting MF. The complete mitochondrial genome sequence of the Mormon cricket (*Anabrus simplex*: Tettigoniidae: Orthoptera) and an analysis of control region variability. Insect Mol Biol. 2007, 16(2): 239-252.

[50] Ma C, Liu C, Yang P, Kang L. The complete mitochondrial genomes of two band-winged grasshoppers, *Gastrimargus marmoratus* and *Oedaleus asiaticus*. BMC Genomics. 2009, 10: 156. doi:10.1186/1471-2164-10-156.

[51] Flook PK, Rowell CH, Gellissen G. The sequence, organization, and evolution of the *Locusta migratoria* mitochondrial genome. J Mol Evol. 1995, 41(6): 928-941.

[52] Shi HW, Ding FM, Huang Y. Complete sequencing and analysis of mtDNA in *Phlaeoba albonema* Zheng. Chin J Biochem Mol Bio. 2008, 24(7): 604-611.

[53] Ye W, Dang JP, Xie LD, Huang Y. Complete mitochondrial genome of *Teleogryllus emma* (Orthoptera: Gryllidae) with a new gene order in Orthoptera. Zool Res. 2008, 29(3): 236-244.

[54] Beckenbach AT, Stewart JB. Insect mitochondrial genomics 3: the complete mitochondrial genome sequences of representatives from two neuropteroid orders: a dobsonfly (order Megaloptera) and a giant lacewing and an owlfly (order Neuroptera). Genome. 2009, 52(1): 31-38.

[55] Cameron SL, Sullivan J, Song H, Miller KB, Whiting MF. A mitochondrial genome phylogeny of the Neuropterida (lace-wings, alderflies and

snakeflies) and their relationship to the other holometabolous insect orders. Zool Scr. 2009, 38(6): 575-590.

[56] Li X, Ogoh K, Ohba N, Liang X, Ohmiya Y. Mitochondrial genomes of two luminous beetles, *Rhagophthalmus lufengensis* and *R. ohbai* (Arthropoda, Insecta, Coleoptera). Gene. 2007, 392(1-2): 196-205.

[57] Friedrich M, Muqim N. Sequence and phylogenetic analysis of the complete mitochondrial genome of the flour beetle *Tribolium castanaeum*. Mol Phylogenet Evol. 2003, 26(3): 502-512.

[58] Hu J, Zhang DX, Hao JS, Huang DY, Cameron S, Zhu CD. The complete mitochondrial genome of the yellow coaster, *Acraea issoria* (Lepidoptera: Nymphalidae: Heliconiinae: Acraeini): sequence, gene organization and a unique tRNA translocation event. Mol Biol Rep. 2010, in press.

[59] Lee ES, Shin KS, Kim MS, Park H, Cho S, Kim CB. The mitochondrial genome of the smaller tea tortrix *Adoxophyes honmai* (Lepidoptera: Tortricidae). Gene. 2006, 373: 52-57.

[60] Liu Y, Li Y, Pan M, Dai F, Zhu X, Lu C, Xiang Z. The complete mitochondrial genome of the Chinese oak silkmoth, *Antheraea pernyi* (Lepidoptera: Saturniidae). Acta Biochim Biophys Sin. 2008, 40(8): 693-703.

[61] Kim SR, Kim MI, Hong MY, Kim KY, Kang PD, Hwang JS, Han YS, Jin BR, Kim I. The complete mitogenome sequence of the Japanese oak silkmoth, *Antheraea yamamai* (Lepidoptera: Saturniidae). Mol Biol Rep. 2009, 36(7): 1871-1880.

[62] Yang L, Wei ZJ, Hong GY, Jiang ST, Wen LP. The complete nucleotide sequence of the mitochondrial genome of *Phthonandria atrilineata* (Lepidoptera: Geometridae). Mol Biol Rep. 2009, 36(6): 1441-1449.

[63] Jiang ST, Hong GY, Yu M, Li N, Yang Y, Liu YQ, Wei ZJ. Characterization of the complete mitochondrial genome of the giant silkworm moth, *Eriogyna pyretorum* (Lepidoptera: Saturniidae). Int J Biol Sci. 2009, 5 (4): 351-365.

[64] Cameron SL, Whiting MF. The complete mitochondrial genome of the tobacco hornworm, *Manduca sexta*, (Insecta: Lepidoptera: Sphingidae), and an examination of mitochondrial gene variability within butterflies and moths. Gene. 2008, 408(1-2): 112-123.

[65] Salvato P, Simonato M, Battisti A, Negrisolo E. The complete mitochondrial genome of the bag-shelter moth *Ochrogaster lunifer* (Lepidoptera: Notodontidae). BMC Genomics. 2008, 9: 331. doi: 10.1186/1471-2164-9-331.

[66] Coates BS, Sumerford DV, Hellmich RL, Lewis LC. Partial mitochondrial genome sequences of *Ostrinia nubilalis* and *Ostrinia furnicalis*. Int J Biol Sci. 2005, 1(1): 13-18.

[67] Hong MY, Lee EM, Jo YH, Park HC, Kim SR, Hwang JS, Jin BR, Kang PD, Kim KG, Han YS, Kim I. Complete nucleotide sequence and organization of the mitogenome of the silk moth *Caligula boisduvalii* (Lepidoptera: Saturniidae) and comparison with other lepidopteran insects. Gene. 2008, 413(1-2): 49-57.

[68] Zhang J, Zhou C, Gai Y, Song D, Zhou K. The complete mitochondrial genome of *Parafronurus youi* (Insecta: Ephemeroptera) and phylogenetic position of the Ephemeroptera. Gene. 2008, 424(1-2): 18-24.

[69] Cameron SL, Miller KB, D'Haese CA, Whiting MF, Barker SC. Mitochondrial genome data alone are not enough to unambiguously resolve the relationships of Entognatha, Insecta and Crustacea sensu lato (Arthropoda). Cladistics. 2004, 20(6): 534-557.

[70] Podsiadlowski L. The mitochondrial genome of the bristletail *Petrobius brevistylis* (Archaeognatha: Machilidae). Insect Mol Biol. 2006, 15(3): 253-258.

[71] Carapelli A, Lio P, Nardi F, van der Wath E, Frati F. Phylogenetic analysis of mitochondrial protein coding genes confirms the reciprocal paraphyly of Hexapoda and Crustacea. BMC Evol Biol. 2007, 7 Suppl 2, S8. doi:10.1186/1471-2148-7-S2-S8.

[72] Beard CB, Hamm DM, Collins FH. The mitochondrial genome of the mosquito *Anopheles gambiae*: DNA sequence, genome organization, and comparisons with mitochondrial sequences of other insects. Insect Mol Biol. 1993, 2(2): 103-124.

[73] Mitchell SE, Cockburn AF, Seawright JA. The mitochondrial genome of *Anopheles quadrimaculatus* species A: complete nucleotide sequence and gene organization. Genome. 1993, 36(6): 1058-1073.