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Keywords
Ostrinia nubilalis, Ostrinia furnicalis, mitochondrial variation

Disciplines
Entomology | Plant Breeding and Genetics

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Partial mitochondrial genome sequences of *Ostrinia nubilalis* and *Ostrinia furnicalis*

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**Abstract**

Contiguous 14,535 and 14,536 nt near complete mitochondrial genome sequences respectively were obtained for *Ostrinia nubilalis* and *Ostrinia furnicalis*. Mitochondrial gene order was identical to that observed from *Bombyx*. Sequences comparatively showed 186 substitutions (1.3% sequence divergence), 170 CDS substitutions (131 at 3rd codon positions), and an excess of transition mutation likely resulting by purifying selection ($d_\text{N}/d_\text{S} \approx 0.15$). Overall substitution rates were significantly higher at 4-fold (5.2%) compared to 2-fold degenerate codons (2.6%). These are the 3rd and 4th lepidopteran mitochondrial genome reference sequences in GenBank and useful for comparative mitochondrial studies.

**Key words**

*Ostrinia nubilalis*, *Ostrinia furnicalis*, mitochondrial variation

**Author biography**

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1. Introduction

Mitochondrial genomes of 16 insect species are completely sequenced and published with a majority from the order Diptera; D. yakuba [1], Ades gambiae [2], Anopheles quadrivitatus [3], D. melanogaster [4], Ceratitis capitata [5], Coccinolita hominivorax [6], D. simulans [7], and Bactrocera oleae [8]. Complete sequences also have been published from a hymenopteran, Apis mellifera [9], an orthopteran, Locusta migratoria [10], a phthirapteran H. macropus [11], thysanuran, T. inaginis [12], a hemipteran Triatoma dimidiata [13], coleopteran Crioceris duodecimpunctata [14], and lepidopterans Bombex mori and B. mandarina [15].

Larvae from corn borer species Ostrinia nubilalis and Ostrinia furniculis (Lepidoptera: Crambidae) are pests of agricultural crop plants and cause major crop production losses [16, 17]. Ostrinia nubilalis and O. furniculis are sister species [18, 19], with differences residing in female O. nubilalis and O. furniculis emission of E- and Z-stereoisomers of Δ11- and Δ12-tetradecenyl acetates [20]. The pheromone binding protein gene sequences showed little nucleotide variance between O. nubilalis and O. furniculis [21], and the zooyyme markers indicated a high similarity between Chinese populations of O. nubilalis and O. furniculis suggesting recent speciation [18]. Similarly, mitochondrial cyochrome c oxidase subunit II (cox1) gene alignment estimated 1.63% interspecies divergence [19]. The present study compares GenBank annotated mitochondrial genomes from O. nubilalis (accession AF442957) and O. furniculis (AF467260).

2. Materials and methods

2.1 Samples and amplification

A single bivoltine female Z-pheromone race O. nubilalis adult was collected from the Iowa State University Uthe Farm, Ames, Iowa, USA. One adult multivoltine O. furniculis female of indeterminate pheromone composition collected from Hengshui, Hebei Province, China, was contributed by Dr. Wang Zhen-ying, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China. DNA extractions used Qiagen DNeasy kits (Qiagen, Valencia, CA).

Primers combinations TY-J-1460 with TK-N-3785, J-11545 with N-12854, and N1-J-12585 with SR-N-14588 [23] were used to PCR amplify fragments 2, 9, and 10 (Fig. 1). Bombyx mori (GenBank: AF149768 and AY048187) and D. yakuba (GenBank: MIDYRRN) [1] mitochondrial genomes were aligned using AlignX software (Informax, San Francisco, CA) to identify regions of sequence similarity, from which regions PCR primers were designed to amplify the mitochondrial genome sequence using Primer3 [24]. All PCR reactions were performed in a 50 µl volume with 1.7 U of Tli polymerase (Promega Corp., Madison, WI), 100 ng DNA, 5 µl 10X thermal polymerase buffer (Promega), 2.5 mM MgCl₂, 100 µM dNTPs, and 20 pmol of each primer. Fragments 1, and 3 to 8 were amplified on a PTC-100 thermocycler (MJ Research, Watertown, MA) with denaturation at 95°C for 2 m, followed by 40 cycles at 94°C for 30 s, 50 to 54°C for 40 s, a 2.5°C/s ramp for +15°C, and 70°C for 1.5 to 3 m depending on fragment length. Fragments 2, 9, and 10 were amplified by denaturing template at 95°C for 2 m, followed by 40 cycles at 94°C for 30 s, 44°C for 1 m, 2.0°C/s ramp for +23°C, and 70°C for 3 m.

2.2 DNA sequence and analysis

PCR reaction products for fragments 1 to 10 were purified using Qiagen PCR purification columns (Qiagen), and diluted to 2.5ng/µl/100 bp of product length. Sequencing was performed in duplicate at the DNA sequencing core facility at Iowa State University, Ames, IA. Overlapping fragments were assembled into a single contiguous sequence using Contig Express software (Informax). Ostrinia nubilalis and O. furniculis mitochondrial genome sequences were aligned with B. mori (GenBank: AF149768, and GenBank AY048187) using AlignX software (Informax), and gene features were annotated using Vector NTI 7.0 (Informax). Contiguous mitochondrial DNA sequence of 14535 and 14536 nt were respectively submitted to GenBank for O. nubilalis (AF442957) and O. furniculis (AF467260).

Substitution rate and transition/transversion ratio for Ostrinia mitochondrial DNA sequences were calculated with MacClade 4.03 [25]. Twenty one tRNA gene structures were predicted with M-fold 3.1 [26], and viewed using RNAviz 2.0 [27]. Codon usage was evaluated by the Countcodon program version 4 (http://www.kazusa.or.jp/codon/ countcodon.html). Average per site rates of synonymous (dS) and nonsynonymous nucleotide substitution (dN) were calculated according to [28] using MEGA [29].

3. Results and discussion

3.1 Ostrinia mitochondrial genomes

Contiguous O. nubilalis (GenBank accession: AF442957) and O. furniculis (AF467260) mitochondrial genomes were assembled from overlapping PCR product sequence (Fig. 1). Each GenBank record includes 13 open reading frames (ORFs), a large ribosomal RNA (rrnL) gene, 21 tRNAs, and part of trnM and small ribosomal RNA (rrnS) genes (Fig. 1). Gene order and orientation were identical to Drosophila [1, 4], except for translocation of trnL to a position preceding trnM as was observed in Bombyx [15]. Major strand of O. nubilalis (41.3% A, 38.8% T, 8.0% G, and 11.8% C; 80.2% AT) and O. furniculis (41.5% A, 38.9% T, 7.9% G, and 11.7% C; 80.4% AT) showed a bias toward A and T nucleotides that is typical of insect mitochondrial genomes [30].

The O. nubilalis and O. furniculis mitochondrial genomes have 3731 codons; 3718 amino acid encoding and 13 termination codons (Table 1). Codons had a prevalence of A and T in 3rd positions and bias may reflect selection for optimal tRNA use [31], speed of genome replication, genome bias, or DNA repair efficacy (Table 2).

The O. nubilalis and O. furniculis mitochondrial peptides comparatively showed 24 predicted amino acid changes (24 of 3718; 0.646%; peptide similarity \( \cong 99.22\% \), and identity \( \cong 99.78\% \)) [32]. All ORFs were initiated by ATA or ATT codons, except cox1. Initiation of cox1 translation is ambiguous, but may occur by a TATTAG sequence in O. nubilalis and O. furniculis, that is similar to TTTTAG in the B. mori. Hexanucleotides, initiation signals TATCTA from Penaeus monodon [33], or ATTTAA from A. gambiae [2], A. quadrinaculatus [3] and C. capitata [5] have been proposed. Alternatively, an ATAA tetranucleotide sequence was predicted to initiate cox1 translation in Drosophila, L. migratoriana [10], and Daphnia pulex [34]. Termination codons were either TAA or TAG in O. nubilalis and O. furniculis, except for cox2 and atp6 that have incomplete stop codons T and TA, respectively. Incomplete stop codons may become function after polycistronic transcript cleavage and polyadenylation mechanisms [35, 36].

Complete nucleotide sequence was obtained for 21 O. nubilalis and O. furniculis mitochondrial tRNAs. Seven substitutions were observed, and 0.49% sequence divergence was estimated from 1429-shared sites. Insertion-deletion (indel) mutation occurred in loop structures of trnA, trnD, trnG, and trnT, and, except for trnR, did not affect predicted two-dimensional tRNA structures (Fig. 2). Variable mitochondrial tRNA loops in Bombyx were assumed not to affect biological function [15]. The complete rnl gene sequence was 1339 nt for O. nubilalis and O. furniculis, and alignment comparatively showed a single C to T transition. A partial rns sequence was obtained from O. nubilalis (434 nt) and O. furniculis (435 nt), and comparatively showed a single nucleotide deletion.

3.2 Nucleotide substitution pattern

A 14543 nt consensus mitochondrial genome alignment identified 186 substitutions between O. nubilalis and O. furniculis: 138 transition (ts) and 48 transversion (tv) mutations (ts:tv = \( \kappa \cong 2.88 \)). This ratio deviated significantly from neutral expectation (1:2; \( \chi^2 = 141.447, d.f. = 1, P < 0.001 \)), indicating evolutionary pressures are acting upon O. nubilalis and O. furniculis mitochondrial genomes. Excess transition mutation also was reported between D. melanogaster subgroup members (\( \kappa = 761/180 \cong 4.23 \)) and attributed to non-neutral evolutionary forces or population effects [7].

Additionally, mitochondrial protein coding sequences (CDS) comparatively showed 170 substitutions between O. nubilalis and O. furniculis: 131 at 3rd codon positions. The ratio of the rate of nonsynonymous changes at nonsynonymous sites (\( d_s \)) to synonymous changes at synonymous sites (\( d_s \)) in Ostrinia ORFs indicated a 7-fold excess of silent mutation (\( d_s/d_s = \alpha \cong 0.15 \)) [28]. High peptide similarity (\( \cong 99.22\% \)) may reflect regency O. nubilalis and O. furniculis mitochondrial genomes, but effects of purifying selection can be inferred since synonymous substitutions are very prevalent. Alternatively, similar environmental selection after speciation could lead to peptide conservation co-occurring with a background of random genetic drift at neutral nucleotide positions. The observed mutation rate at Ostrinia 4-fold degenerate codons (\( \mu_{4,664} = 5.22\% \)) was significantly higher than at 2-fold degenerate codons (\( \mu_{2,664} = 2.60\% \); \( \chi^2 = 35.157, d.f. = 1, P < 0.001 \)). Results suggest a greater susceptibility of 4-fold degenerate codons to synonymous substitution.

3.3 Divergence time estimates

The divergence time between O. nubilalis and O. furniculis mitochondrial was estimated by assuming a linear rate of substitution in short-term evolution (molecular clock) [37] of 2% per million years [38]. Nucleotides in rRNA and tRNA may lack independence due to structural dependence, and purifying selection may act at 1st and 2nd codon positions. The intergenic sequence (IGS) and 3rd codon positions only sites that are nearly neutral. IGS region and 3rd codon positions showed 3.54% nucleotide difference between O. nubilalis and O. furniculis, indicating that speciation occurred 1.8 mya [38]. Alternatively, 3rd position and IGS region data give a pairwise genetic distance of 0.3284 ± 0.0348 using the Kimura-2-parameter model [39]. Estimates of 0.1 distance unit (D) per 1.0 myr [40] suggest divergence at 3.3 mya. These molecular-based divergence time estimates are supported by highly similar morphology of O. nubilalis and O. furniculis [41].

Conflict of interest

The authors have declared that no conflict of interest exists.

References


38. Powell JR. Rates of nucleotide substitution in *Drosophila* mitochondrial DNA and nuclear DNA are similar. Proc Natl Acad Sci USA 1986; 83(23):9090–9093.

### Tables and Figures

#### Table 1: Codon usage for 3718 amino acid residues and 13 nonsense codons among protein coding regions from each *O. nubilalis* (On) and *O. furnicalis* (Of) using the invertebrate mitochondrial genetic code.

<table>
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<th>Codon</th>
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<th>Codon</th>
<th>On</th>
<th>Of</th>
<th>Codon</th>
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* Includes stop codons from *cox2* (T) and *atp6* (TA), completed by adenylation.

#### Table 2: Nucleotide frequencies partitioned among *O. nubilalis* (On) and *O. furnicalis* (Of) mitochondrial genome regions. IGS = non-coding intergenic spacer regions.

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<th>2nd position</th>
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<th>rrmS</th>
<th>tRNAs</th>
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<td>Of</td>
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<td>Of</td>
<td>On</td>
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<td>17.8</td>
<td>17.9</td>
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**Figure 1.** *Ostrinia* mitochondrial genome map of sequenced regions. Protein coding genes represented by arrows indicating direction with left-facing arrows on major strand. The tRNA genes are labeled by single letter codes and * indicating coding sequence on minor strand. Underscores indicate positions of ten overlapping PCR amplified genome fragments.

**Figure 2:** Predicted secondary structures for A) *O. nubilalis* and B) *O. furnicalis trnR*. 

![mtDNA Map](image)

![Secondary Structures](image)