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Comparative Analysis of Complete Mitochondrial DNA Control Region of Four Species of Strigiformes

XIAO Bing[1], MA Fei[1], SUN Yi[2], LI Qing-Wei[1,①]

1. *College of Life Sciences*, *Liaoning Normal University*, *Dalian* 116029, *China*;

2. *School of Life Sciences*, *Xiamen University*, *Xiamen* 361005, *China*

Abstract: The sequence of the whole mitochondrial (mt) DNA control region (CR) of four species of Strigiformes was obtained. Length of the CR was 3 290 bp, 2 848 bp, 2 444 bp, and 1 771 bp for *Asio flammeus, Asio otus, Athene noctua,* and *Strix aluco*, respectively. Interestingly, the length of the control region was maximum in *Asio flammeus* among all the avian mtDNA control regions sequenced thus far. In addition, the base composition and organization of mtDNA CR of *Asio flammeus* were identical to those reported for other birds. On the basis of the differential frequencies of base substitutions, the CR may be divided two variable domains, Ⅰ and Ⅲ, and a central conserved domain, Ⅱ. The 3′ end of the CR contained many tandem repeats of varying lengths and repeat numbers. In *Asio flammeus*, the repeated sequences consisted of a 126 bp sequence that was repeated seven times and a 78 bp sequence that was repeated 14 times. In *Asio otus*, there were also two repeated sequences, namely a 127 bp sequence that was repeated eight times and a 78 bp sequence that was repeated six times. The control region of *Athene noctua* contained three sets of repeats: a 89 bp sequence that was repeated three times, a 77 bp sequence that was repeated four times, and a 71 bp sequence that was repeated six times. *Strix aluco*, however, had only one repeated sequence, a 78 bp sequence that was repeated five times. The results of this study seem to indicate that these tandem repeats may have resulted from slipped-strand mispairing during mtDNA replication. Moreover, there are many conserved motifs within the repeated units. These sequences could form stable stem-loop secondary structures, which suggests that these repeated sequences play an important role in regulating transcription and replication of the mitochondrial genome.

Key words: Strigiformes; mitochondrial DNA; control region (CR); tandem duplication; molecular evolution

The control region (D-loop) is the only major noncoding segment in the vertebrate mitochondrial genome. It is also the most variable part of the mtDNA and evolves three to five times more rapidly compared with the rest of the mitochondrial genome[1]. In Aves, the mitochondrial control region is located between the $tRNA^{Glu}$ gene and the $tRNA^{Phe}$ gene[2], and its primary function is usually believed to be the regulation of replication and transcription of the mitochondrial genome[3]. In particular, sequence variation of the control region may result in length differences in bird mitochondrial genomes. In addition, many studies have shown that the extensive size variation in the mtDNA control region in birds is attributed to the insertion-deletion of some segments and/or the variation of the copy number-length of tandem repeat sequences within its 5′ and 3′ ends. Therefore, a better understanding of the structural property of the control region is important for studies on avian phylogeny and population.

Over the past ten years, with the sequencing of the mitochondrial control region of many Classes, such as Cyclostomata, Pisces, Amphibia, Reptile, Aves, and Mammalia, a great deal of attention has been paid to the evolutionary mechanism of the control region and its application in molecular phylogenetic analysis. Currently, the mitochondrial control region sequences of 22 avian species have been de-

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① Corresponding author. E-mail: Liqw@263.com; Tel & Fax: +86-411-8582 7799

posited in the GenBank. However, there are only five incomplete sequences from Strigiforme species in the GenBank, namely, *Strix occidentalis* (DQ087169), *Strix aluco* (DQ092611), *Ninox novaeseelandiae* (AY309457), *Strix nigrolineata* (AY830859), and *Strix uralensis* (AY836774). To further elucidate the structural character of the complete mitochondrial control region of Strigiformes, in this study, the sequence of the complete mtDNA control region of four owls is reported and the base composition and the organization of the control region is analyzed. Furthermore, the origin and the evolution of tandem repeats of the mtDNA control region as well as their function and application are discussed.

1 Materials and Methods

1. 1 Samples

Asio flammeus and *Asio otus* were collected from the National Natural Protective Zone of Lao Tie Hill, Dalian. *Strix aluco* and *Athene noctua* were collected from Kunming, Yunnan Province. Fresh liver tissue samples were extracted and frozen at −80℃.

1. 2 DNA extraction

Mitochondrial DNA of the four owls was extracted from frozen liver tissue according to the procedure described by Arnason *et al* [5].

1. 3 DNA amplification and sequencing

Primers (Table 1) were designed for the four owls. PCR products were sequenced using the methods of Long-PCR and primer walking. Complete mtDNA control region including all adjacent segments were sequenced bidirectionally until overlapping adjacent segments of approximately 80−120 bp were obtained to ensure accuracy. All sequencing reactions were carried out on an ABI 377 DNA automated sequencer (PE Applied Biosystems).

1. 4 Sequence analysis

The locations of genes were determined by comparisons with sequences of the mtDNA control region of *Gallus gallus* (X52392), *Ninox novaeseelandiae* (AY309457), and *Buteo buteo* (AF380305).

Clustal X 1.8[6] software, Sequencher software, and DNASTAR software were used to analyze nucleotide composition and sequence alignment to determine the length of the control region, as well as to search conserved sequence motifs. Potential secondary structures of the nucleotide sequence of the control region were analyzed using RNAstructure4.2 program. MEME-Motif discovery tool was used to analyze conserved motif in the repeated sequences.

2 Results

2. 1 The length of the control region

Sequencing results showed that the control region of *Asio flammeus, Asio otus, Athene noctua,* and *Strix aluco* was 3 290 bp, 2 848 bp, 2 444 bp, and 1 771 bp long, respectively. Thus, the control region of the four owls is much longer than that of other birds (about 1−1.5 kb). It is also interesting that the length of the control region is maximum in *Asio flammeus* among all the avian mtDNA control regions sequenced thus far.

2. 2 The organization of the control region

The base composition and organization of the

Table 1 Primers for amplification

	Primers	Nucleotide sequence (5′→3′)	Derived source
1	S-Cyt*b*-13354F	GCTGACTACTCCGCAACCTACACGCAAATG	This study
2	S-12S-256R	GAATGTAGCCCATCTCTTCCACCTCATAGG	This study
3	S-ND6-527F	AAGCCGCCGTTAACTCACCC	This study
4	S-Cty*b*-221R	GTGGTGGGAGCATTAGGATT	This study
5	L-Cty*b*-1592F	GGTGTAGGAGGGGGGAAA	This study
6	L-12S-211R	CCTTGGGTGTTCTGTGGTGA	This study

Note: 1 and 2 were the primers for *Strix aluco* and *Athene noctua*; 3 and 4 were the primers for *Asio flammeus*; 5 and 6 were the primers for *Asio otus.*

control region of the four owls are very similar to those reported for other birds, i.e., low G content and considerably AT-rich content. The nucleotide composition of the mtDNA control region of the four owls is listed in Table 2.

On the basis of the differential frequencies of the base substitutions, the mitochondrial control region may be divided into three domains[7–9]: two variable domains, Ⅰ and Ⅲ, and a central conserved domain, Ⅱ. The general structure of the mitochondrial DNA control region of the four owls is shown in Fig. 1. Some conserved sequence motifs are also found in all the three domains, including ETAS-1, ETAS-2, and CSB-1-like motif in domain Ⅰ; B-box, C-box, D-box, E-box, F-box, and bird similarity box in domain Ⅱ; and CSB-1 motif in domain Ⅲ[10]. However, the four owls lack heavy strand replication origin OH and the bidirectional transcription promoter LSP/HSP. Furthermore, CSB-2 and CSB-3 motifs that have been identified in some vertebrates have not been found in the four owls. A comparison of the conserved sequence block is shown in Table 3. It is very clear that the homologies of the conserved sequence blocks in domain Ⅱ are higher than those in domains Ⅰ and Ⅲ, suggesting that domain Ⅱ is more conservative compared with domains Ⅰ and Ⅲ.

2. 3 Tandem duplication sequences

The 3′ end of the control region contains many tandem duplication sequences. In *Asio flammeus*, the repeated sequences consisted of a 126 bp sequence that was repeated seven times and a 78 bp sequence that was repeated 14 times, the base composition of which were identical. Between the two repeat units, there was a 99 bp spacer, which was an incomplete copy of the two repeated units. In *Asio otus* also, there were two repeated sequence units, a 127 bp sequence that was repeated eight times and a 78 bp sequence that was repeated six times, with a 55 bp spacer. The control region of *Athene noctua* contains three sets of repeats: an 89 bp sequence that was repeated three times, a 77 bp sequence that was repeated four times, and a 71 bp sequence that was repeated six times. The 89 bp unit represented an entire 77 bp unit plus an extra 12 bp unit to the 3′ end, with only a 2 bp spacer between the two sets of repeat sequences. The 77 bp repeats and 71 bp repeats were separated by 64 bp incomplete copies. *Strix aluco*, however, had only one repeated sequence, a 78 bp sequence that was repeated five times. The sequences of the repeated units are shown in Table 4.

Low sequence homologies exist between the repeated units of the four owls and those of the other

Table 2 Nucleotide composition of mtDNA control region of the four species of Strigiformes

Species	%A	%C	%G	%T	%A+T	Repeated sequences %A+T
Asio flammeus	32.30	29.01	10.61	28.07	60.37	63.42
Asio otus	32.16	25.39	9.62	32.83	64.99	70.89
Athene noctua	32.76	25.88	11.51	29.85	62.61	67.52
Strix aluco	30.06	26.72	13.39	29.83	59.89	67.69

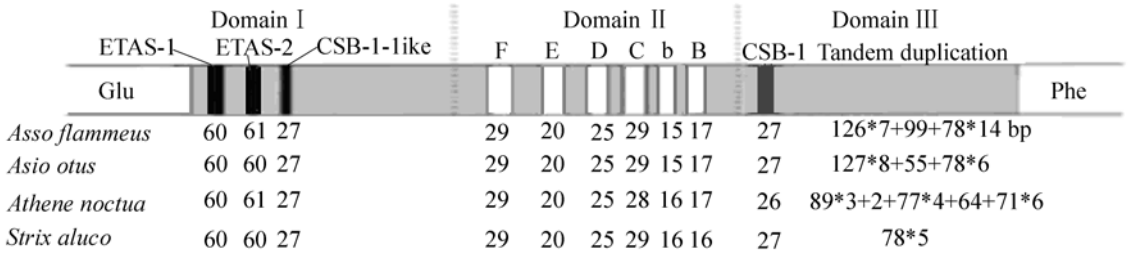

Fig. 1 General structure of mitochondrial DNA control region of the four species of Strigiformes

GLU = $tRNA^{Glu}$; PHE = $tRNA^{Phe}$; b = Bird similarity box. The number beneath the conserved sequence blocks denotes its length; 126*7 + 99 + 78*14 indicates a 99 bp interval between seven repeats of 126 bp and 14 repeats of 78 bp.

Table 3 Comparison of CR conserved sequence block of the four species of Strigiformes

Species	ETAS-1	ETAS-2	CSB-1-like
1	TCGGTATGTACTGCTGTGCATCACACTACCACCCCATAACACACTACATCCATGTCCCAC	TACCAACATACAACCCC-ATGCACCTATCACATGCACTACATGC--AC-CAAGAGTATCCCAACCC	TATAAAATCCATG-ACCGACAAACATGC
2	TCGCTATGTATTATTGTACATTACATTATTAACCCCATATACATCACATTAATGTACTGC	TACAAACATAATATCC--ATGCTCAA-GACCAGATTATGCATGC--CCTCAAATACAACCCATTC-	CATACTATTCATG-TTTGATAAACATAC
3	TCGGGATGTATAAATGTGCATTAGACTATTTTCCACATAAGCATAATATGTATGTACTAG	CAATGACTTAT-ATATCCATGTA-CCTCACCATACCCTCCATGTAATTGGATAAACCTACCATG--	CATACCATCCATG-TACAATAGACTTAC
4	TTGGGATGTATTATTGTACATTACACTATCCACCACATACACATACCATTAATGGACCAA	GACCGACATACCATCC--ATGTTCTCCAAACATATCATGTATGT--AC-CAAAAACACCCTAATTC	TATACCATCCATG-TCCGCCAAACACAC
	* * ***** *** *** * ** ** * ** ** *** * * ** * * *** ** * *** * * * *** ** **** * ** *		

Species	F-box	E-box	D-box	C-box	Bird-similarity-box	B-box
1	GAGCTTCTCACGTGAAATCAGCAACCCGG	ACGCCCAGCTTCAGGCCCAT	CCTCTGGTTCCTATTTCAGGGCCAT	TTGTTCTTCACCGAGACATCTGGTTGGCT	-CACTGATGCACTTTG	TTCCATTCAGTCTTGGT
2	GAGCTTCTCACGTGAAATCAGCAACCCCG	ACGCCCAGCTTCAGGCCCAT	CCTCTGGTTCCTATATCAGGGCCAT	TTGCTTTTCACCGAGACATCTGGTTGGCT	-CACTGATGCACTTTG	TTCCATTCAGTCTTGAT
3	GGGCTTCTCACGTGAAACCAGCAACCCCG	ACGACTAGCTTCAGGCCCAT	CCTCTGGTTCCTTTTTCAGGGCCAT	TTGCCTTTCAC-GAGGCATCTGGTTGGCT	ACACTGATGCACTTTG	TTGCATCTGGTTATGGT
4	GGACTCCTCACGAGAAATCAGCAACCCGG	GCTCCTAGCTTCAGGCCCAT	CCTCTGGTTCCTCGGTCAGGGCCAT	TTGCTCTTCACCGATACATCTGGTTGGCT	ACACTGATGCACTTTG	TTCCATTTGGC-TTGGT
	* ** ****** **** ********* * * * ********** ********** ********** *** ***** ** ********** ********** ** *** * ** *					

Species	CSB-1
1	TATTCAGTTAATGGTTGCGGGACATGC
2	TATTTACTTAATGGTCACAGGACATAA
3	TATA-GTTTAATGGTTACGGGACATGT
4	TATTCAGTTAATGATTCTGGAACATGC
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Note: 1 denotes *Asio flammeus;* 2 denotes *Asio otus;* 3 denotes *Athene noctua;* 4 denotes *Strix aluco*. * denotes identical base among the four species .

Table 4 Nucleotide sequences of repeated units in the control regions of the four species of Strigiformes

Species	Repeated units	Sequences
Asio flammeus	126 bp unit	5′-TAACATCGGCGCCCAAATTCCGCTAACAATTTTGATCCCATCTATCCATTTTTCTAT TCAACTTTCATCAAAACTTCTAGGCAAACTCTAACTGTTTGCCATTCGATCTTATCA CTCACCTTACCC-3′
	78 bp unit	5′-TAGGCAAACTATAACAATCAATTATCGTCCACCATCAAAAATTTTACCGATCAATC GAACGAACGATCAAAAACCTTC-3′
Asio otus	127 bp unit	5′-TAGGCAAACTATAACTGTTTGCCAATCAACTTTATCGTTAATTCTTATCATAATATC GGCACCCAAATCCCGCTAATAAATCTATCTTTTATCCATTTATTTTTTACCCAAATTTA CTCGACACTTT -3′
	78 bp unit	5′-CAAACGAACGATAAGAAACTTTCTAGACAAACTATAACAATCAATTATCATCCAT CATCAAAAATTTTATTGATCAAT-3′
Athene noctua	89 bp unit	5′-AAACTTATCCTCAATATAACCCTAAGTTTGCCTACAGAAATTAAACACGAATTCTA TTATCCATTCTATTTTCCATTTATTTTAATCTT - 3′
	77 bp unit	5′-AAACTTACCCTAAATACAACCCTAAGTTTGCCTGCATAGATTAGGTATCGATCCTA TCATCCATCCAAACTTTACAC-3′
	71 bp unit	5′-TAAAAGGTTGACCAAACAACCAAGCACTAATCAGAAATTCTTAACAAAAACCAA TACTCATAATCGTAACT -3′
Strix aluco	78 bp unit	5′-AACAAACCATAGGGGAATTCTAGAGGAATTGCAACGATCATTTTGTATTCATCAC CAAAAATTTTTATCGATCAATCA-3′

birds. Between *Asio flammeus* and *Asio otus*, the homology between the 126 bp (127 bp) unit and the 78 bp unit was also low. However, the corresponding repeat units exhibited very high homology: the homology of the 78 bp unit was about 88.5% and that of the 126 bp (127 bp) unit was about 75.8%. Moreover, the homology of the 78 bp unit between *Strix aluco* and *Asio flammeus* or *Asio otus* was about 70%. Because the 89 bp unit in *Athene noctua* was essentially a 77 bp unit plus a 12 bp unit, it was compared to the 78 bp unit, and the homology was approximately 74%.

Potential secondary structures of the repeated sequences in the control region were constructed using RNAstructure4.2 program (Fig. 2). Results showed that this region was able to form a stable stem-and-loop structure. MEME-Motif discovery tool was used to analyze the eight repeated units. Results seemed to indicate that there were many conserved motifs in the repeated sequences, which are identified in color in Table 5.

3 Discussion

3. 1 Basic characteristics of the control region

The size of the control region of avian mtDNA is usually 1–1.5 kb. Few vertebrates have a control

Table 5 The conserved motif of repeated units in the CR of the four owls

Name	Start	*P*-value	Sites
1	78	6.73e−12	TAGGCAAACTCTAACTGTTTGCCATTCGATCTTATCACTCA CCTTACCC
2	1	1.32e−16	TAGGCAAACTATAACAATCAATTATCGTCCACCATCAAAAA TTTTACCGAT
3	1	1.09e−11	TAGGCAAACTATAACTGTTTGCCAATCAACTTTATCGTTAA TTCTTATCAT
4	24	9.56E−17	TAGACAAACTATAACAATCAATTATCATCCATCATCAAAAA TTTTATTGAT
5	6	3.44e−12	TATCCTCAATATAACCCTAAGTTTGCCTACAGAAATTAACA CGAATTCTAT
6	6	8.57e−12	TACCCTAAATACAACCCTAAGTTTGCCTGCATAGATTAGGT ATCGATCCTA
7	10	3.00e−09	GACCAAACAACCAAGCACTAATCAGAATTCTTAACAAAAAC CAATACTCAT
8	21	4.08e−12	TAGAGGAATTGCAACGATCATTTTGTATTCATCACCAAAAA TTTTTATCGA

Note: In the column Name, 1 and 2 denote the 126 bp and 78 bp repeated units of *Asio flammeus*, respectively; 3 and 4 denote the 127 bp and 78 bp repeated units of *Asio otus*, respectively; 5, 6, and 7 denote the 89 bp, 77 bp, and 71 bp repeated units of *Athene noctua*, respectively; and 8 denotes the 78 bp repeated unit of *Strix aluco*. The number in the Start column denotes the site of the repeated unit.

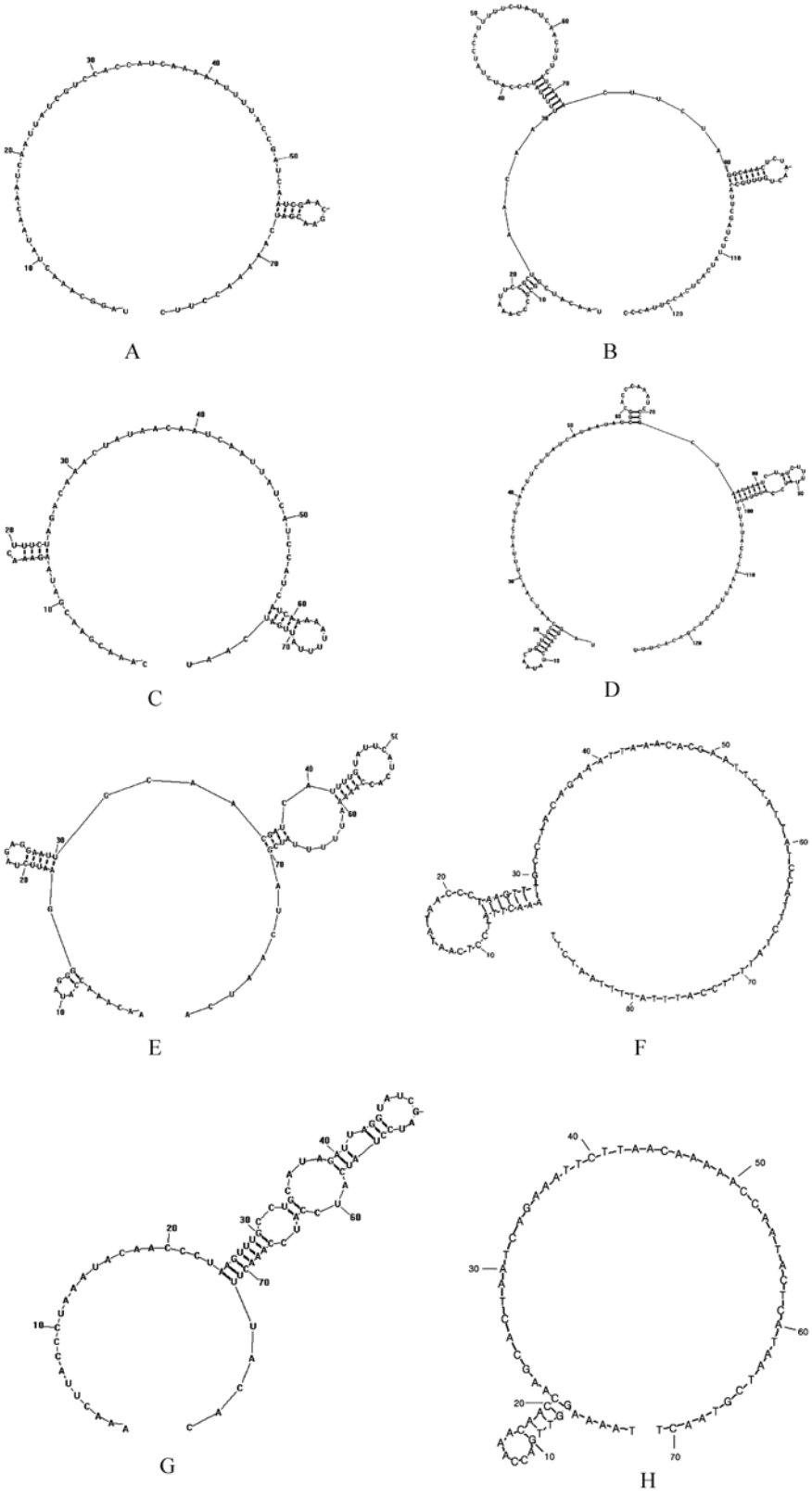

Fig. 2 Secondary structure of repeat units of the four owls

A: the 78 bp repeat unit of *Asio flammeus*; B: the 126 bp repeat unit of *Asio flammeus* ; C: the 78 bp repeat unit of *Asio otus*; D: the 127 bp repeat unit of *Asio otus* ; E: the 78 bp repeat unit of *Strix aluco* ; F: the 89 bp repeat unit of *Athene noctua* ; G: the 77 bp repeat unit of *Athene noctua*; H: the 71 bp repeat unit of *Athene noctua.*

region that is longer than 3 kb. The longest reported control region in vertebrates is that of *Myxine glutinosa*[11] (3 628 bp, AJ404477). In this study, the complete mtDNA control region of four species of Strigiformes has been determined, and it was observed that the length of the control region was unusually long, ranging from 1 771 bp to 3 290 bp. It is noteworthy that *Asio flammeus* has the longest control region (3 290 bp) among all sequenced avian mitochondrial DNA control regions, as shown in this study.

The general organization of the control region of the four species is similar to that reported for other birds. The control region may be divided into three domains, and some conserved sequence motifs could be found in all the three domains. The $tRNA^{Glu}$-adjacent domain Ⅰ contains terminal-associated sequences (TAS) and CSB-1-like conserved sequences. TAS is associated with termination of H-strand replication, whereas the CSB-1-like motif is considered to be unique to Aves [12]. B-box, C-box, D-box, E-box, and F-box, present in domain Ⅱ in most vertebrates, also exist in the four owls. They may be associated with regulating H-strand synthesis. In addition, some considered that B-box, D-box, and F-box were prevalent in Aves but that C-box and E-box existed only in some Aves[13], which implied that B-box, D-box, and F-box might be more essential in H-strand replication compared with C-box and E-box. Bird similarity box could be also identified in the four owls, and exhibited the highest similarity among homologous sequences in domain Ⅱ (Table 3). It suggests that bird similarity box may play a key role in the replication and transcription of the mitochondrial genome in Aves. Furthermore, CSB-1 motif, being located in the 5′ end of domain Ⅲ, is also conserved in the four owls. However, CSB-2 and CSB-3 motifs identified in mammals have not been found in the four species [14]. Short conserved sequence blocks (CSB-1, CSB-2, CSB-3) are usually considered to regulate the initiation of replication and transcription of the mitochondrial genome[15]. These above-mentioned results seem to indicate that CSB-1 plays a more dominant role than does CSB-2 and CSB-3. Furthermore, the four owls lacked heavy-strand replication origin OH and the bidirectional transcription promoter LSP/HSP, which were extremely important in heavy-strand replication and initiation of the bidirectional transcription of mitochondrial genome in most vertebrates. It is speculated that these four species may form special secondary structures to carry out these tasks.

3. 2 Origin and evolution of tandem duplication sequences

The results of this study suggest that the large size of the mitochondrial control region of the four owls may be attributed to numerous repeated sequences within the 3′ end of their control regions. Extensive tandem repeats in the mtDNA control region have been found in many vertebrate species (Table 6). Nevertheless, such repeated sequences herein may be a peculiar event. These repeat units in the CR were compared among the four species and with other vertebrates (Table 6). However, because of the lack of data for mtDNA control region in other Strigiformes, it is yet to be confirmed whether multiple repeated sequences are a common feature in Strigiformes.

The results of this study also showed that very high sequence homology existed in the 126 bp (127 bp) and 78 bp units in *Asio flammeus, Asio otus,* and *Strix aluco*, which suggested that these corresponding repeated units might have a common origin. They might have existed before species divergence, although independent evolution may have led to difference in these corresponding repeated units in the four owls. In addition, because the 89 bp unit is a 77 bp unit with an extra 12 bp added to the 3′ end in *Athene noctua*, the 89 bp and 77 bp units may also originate from an ancestral sequence despite some sequence differences.

Molecular mechanisms, such as recombination and transposition[17], unequal crossing-over (gene conversion)[18], and slipped-strand mispairing[19-21], have been proposed on the origin of tandem repeated sequences and subsequent generation of mtDNA

Table 6 Comparison of the repeat units in CR between the four owls and the other vertebrates

Species	Repeat unit in CR	Derived source
Asio flammeus	126 bp ×7, 78 bp ×14	This study
Asio otus	127 bp ×8, 78 bp ×7	This study
Athene noctua	89 bp ×3, 77 bp ×4, 71 bp ×6	This study
Strix aluco	78 bp ×5	This study
Buteo buteo	27 bp ×2, 97 bp ×2, 11 bp ×4, 11 bp ×13 (+8 bp) 9 bp ×3, 11 bp ×2, 23 bp ×3	AF380305
Falco peregrinus	68 bp ×3, 56 bp ×5(+11 bp)	NC000880
Ciconia ciconia	71 bp ×3(+43 bp)	AB026818
Gallus gallus	53 bp ×2(8 bp spacer), 19 bp ×2	NC001323
Rhea americana	26 bp ×2(+43 bp), 4 bp ×18	NC000846
Smithornis sharpei	56 bp ×5(+24 bp)	NC000879
Alligator mississippiensis	22 bp ×3, 21 bp ×14	Y13113
Diceros bicornis	10 bp ×4,10 bp ×11	L22010
Halichoerws grypus	6 bp ×54, 14 bp ×3	X72004
Phoca vitulina	6 bp ×40, 8 bp ×11, 8 bp ×7	X63726
Didelphis virginiana	19 bp ×3, 9 bp ×12, 19 bp ×8	Z29573
Ornithorhynchus anatinus	9 bp ×5, 11 bp ×2, 17 ×16	X83427

Note: The number in parentheses denotes one incomplete repeat.

length variants. Although recombination is usually thought of as the dominant mechanism that causes variation in nuclear genomes, direct evidence for the same is yet to be found in animal mitochondrial genomes. The results of this study seem to indicate that the origin of tandem repeats of mtDNA control region may result from slipped-strand mispairing during mtDNA replication. It is proposed that the origin of a new repeated sequence may be generated through the following three steps. First, an extraneous and mutational sequence should have been deleted from the original sequence; second, the sequence loops out because of strand contraction; and third, the sequence expands to form a repeated sequence. As a result, the extraneous sequence is not deleted but a new sequence is generated by slipped-strand mispairing. Wilkinson and Chapman argued that because of unidirectional replication of the H-strand in the con- trol region, duplication of motifs should occur only in one direction[22]. According to this hypothesis, repeats at the 5′ end can be duplicated or deleted, whereas repeats at the 3′ end cannot be duplicated and are usually fragmentary due to partial deletion. In this study, spacers of variable lengths have been found between two repeat units (99 bp in *Asio flammeus*, 55 bp in *Asio otus*, and 64 bp in *Athene noctua*). They are composed of incomplete copies of the 5′ end of the first repeat unit and the 3′ end of the second repeat unit. This could be due to the duplication of the two repeated sequences in opposite directions. Therefore, according to Wilkinson-Chapman hypothesis, the latter repeat sequences may be generated by the replication of the L-strand.

The sequence polymorphism within the repeated arrays results in an intermediate stage in the replication process, and its observation requires a balance between two opposing evolutionary forces: point mutations and insertion-deletion mutations [23]. The former causes divergence, whereas the latter causes homology. In this study, all 14 copies of the 78 bp unit are exactly the same in *Asio flammeus*, which may imply that insertion-deletion mutations play a more dominant role than point mutations.

3. 3 Application of tandem duplication sequences

Several researchers believed that tandem repeated sequences are redundant in the mitochondrial genome[24]. However, others hold opposing views [25]. In addition, palindromic and hairpin sequences located within the repeated sequences have been reported in many species and are capable of folding into complex secondary structures[24]. The repeated

sequences in the control region of the four owls are able to form a stable stem-and-loop structure, which is believed to be functionally essential in terminating mitochondrial genome replication[12]. In addition, there are many conserved motifs in the repeated sequences (Table 6), which may offer multiple copies of functionally important sequences and even confer replicative advantages to mtDNA molecules with multiple copies of replication signals. In this regard, repeated sequences may also be involved in the formation of complex secondary structures of the control region and strengthen the replication and transcription of mtDNA. The results of this study showed that the repeated sequences of the control region exhibited some differences between species, even between two individuals of the same species. It is therefore suggest that the mtDNA control region may be used as a molecular marker to study population evolution.

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鸮形目 4 种鸟类线粒体调控区全序列的测定与比较研究

肖 冰[1], 马 飞[1], 孙 毅[2], 李庆伟[1]

1. 辽宁师范大学生命科学学院，大连 116029；

2. 厦门大学生命科学学院，厦门 361005

摘 要: 利用 Long-PCR 和 Primer Walking 的方法对鸮形目的短耳鸮、长耳鸮、纵纹腹小鸮、灰林鸮 4 种鸟类的线粒体调控区进行了全序列测定。结果表明：短耳鸮的调控区长度为 3 290 bp；长耳鸮为 2 848 bp；纵纹腹小鸮为 2 444 bp；灰林鸮为 1 771 bp。短耳鸮的调控区长度是 4 种鸮中最大的，并且是目前已知最大的鸟类线粒体调控区。这 4 种鸮类调控区的基本结构和其他鸟类相似，按照碱基变化速率的不同可以分为 3 个区: 碱基变化速率较快的外围区域Ⅰ、Ⅲ和保守的中间区域Ⅱ。这 4 种鸟类调控区的 3′ 端均存在大量的串联重复序列，短耳鸮为 126 bp 单元重复 7 次和 78 bp 单元重复 14 次；长耳鸮为 127 bp 单元重复 8 次和 78 bp 单元重复 6 次；纵纹腹小鸮有 3 个重复单元，分别为 89 bp 单元重复 3 次、77 bp 单元重复 4 次和 71 bp 单元重复 6 次；灰林鸮仅有 1 个单元的串联重复为 78 bp 重复 5 次。调控区中串联重复序列可能是由链的滑动错配产生，另外这些重复序列都能形成热力学稳定的多重茎环二级结构，而且在重复序列中还发现一些保守基序，这说明重复序列可能具有一定的生理功能，影响调控区的调控功能从而影响线粒体基因组的复制和转录。

关键词: 鸮形目；线粒体基因组；调控区；重复序列

作者简介: 肖冰（1980−），女，吉林人，硕士，研究方向：细胞生物学。E-mail: xiaobing19800516@sina.com