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Organization and Variation of the Mitochondrial Control Region in Two Vulture Species, *Gypaetus barbatus* and *Neophron percnopterus*

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We report the first entire mitochondrial DNA (mtDNA) control region sequences in two endangered vulture species, the bearded vulture (*Gypaetus barbatus*) and the Egyptian vulture (*Neophron percnopterus*). Results showed that the general organization of vulture control regions was very similar to other birds, with three distinct domains: a left variable domain (DI), a central conserved one (DII) including the F, E, D, and C boxes, and a right domain (DIII) containing the CSB1 sequence. However, due to the presence of long tandem repeats, vulture control regions differed from other avian control regions both in size and nucleotide composition. The Egyptian vulture control region was found to be the largest sequenced so far (2031 bp), due to the simultaneous presence of repeats in both DI (80 bp) and DIII (77 bp). Low variation was found in vulture control regions, particularly in *G. barbatus*, as the probable result of populations declines in the last few centuries.

The vertebrate mitochondrial genome (mtDNA) is a closed, maternally inherited circular molecule (15–20 kb) composed of about 37 genes coding for 22 transfer RNAs (tRNAs), 2 ribosomal RNAs (rRNAs), and 13 messenger RNAs (mRNAs). Although genetically conserved, gene order within the mtDNA has been shown to vary among taxa (Bensch and Harlid 2000; Desjardins and Morais 1990; Saccone et al. 2000). Within the mitochondrial genome, different regions evolve at different rates, with the control region having the highest nucleotide substitution rate, probably due to the lack of coding constraints. For this reason, this portion has been useful in a great number of studies at inter- and intraspecific levels.

The control region of birds differs from other vertebrates in that it is flanked by the genes $tRNA^{Glu}$ and $tRNA^{Phe}$ (Desjardins and Morais 1990). More recently, Mindell et al. (1998) described a new gene order in several bird groups in which the control region is flanked by $tRNA^{Thr}$ and $tARN^{Pro}$. Study of the avian control region is of growing interest, mainly to address population structure using small parts of the control region (e.g., Pitra et al. 2000; Wenneberg 2001), but also to describe the organization and variation of the entire region at several taxonomic levels (Desjardins and Morais 1990; Haring et al. 2001; Sorenson et al. 1999). Recently Ruokonen and Kvist (2002) extensively described the structural and evolutionary characteristics of 68 avian species, but information is still lacking on Falconiformes species. Lower intra- and/or interspecific variation was found in birds compared to mammals, but more bird sequences are needed to assess the level and extent of similarity at different taxonomic levels.

Large size variations have been described in some avian control regions as the result of tandemly repeated sequences located in domain I or domain III (e.g., Berg et al. 1995; Ritchie and Lambert 2000). Such tandem sequences have been described in a great variety of taxa. Slippage during DNA replication has been proposed as a likely mechanism for the generation of length polymorphism (Wenink et al. 1994), but it still remains unclear whether those repeats could potentially be informative for phylogenetic purposes.

The Egyptian vulture (*Neophron percnopterus*) and the bearded vulture (*Gypaetus barbatus*) belong to the family Accipitridae (order Falconiformes), which includes the majority of the Eurasian and African birds of prey. These two species cluster together in raptor phylogenetic reconstructions (Seibold and Helbig 1995) and are therefore considered the closest extant relatives of each other. They are two of the most endangered vulture species in Europe, where their populations have decreased drastically since the beginning of this century due to human causes. Few studies of genetic variability have been investigated in European vultures except isolated works on phylogenetic relationships between related species (Mindell et al. 1998; Wink 1995) or population structure using either nuclear loci (Kretzmann

et al. 2003; Negro and Torres 1999) or a small part of the control region (Godoy et al. 2004).

In this article we characterize the organization and variability of the entire control region sequence in *N. percnopterus* and *G. barbatus* and compare it to other avian control regions.

Materials and Methods

Tissue Samples

Insertions of mtDNA in the nuclear genome ("numts"; Lopez et al. 1994) have been observed in several birds species (Quinn 1992, 1997; Sorenson and Fleischer 1996). In order to verify that the amplifications obtained in this study were of mtDNA origin, internal fragments of the control region were amplified from either blood or tissue rich in mitochondria and compared. In addition, maternal inheritance was evaluated by sequencing mtDNA fragments in families (parents and their progeny). All results were consistent with the mtDNA origin of vulture sequences.

Sample Collection and DNA Extraction

Five bearded vultures (two from the Pyrenees, Spain; three from the former Soviet Union) and six Egyptian vultures (two from the Iberian peninsula, Navarra; two from the Canary Islands; two from the Baleares, Menorca) were sequenced for the entire control region. One additional sample comes from a separate subspecies, *Neophron percnopterus ginginianus*, from south-central India. Genomic DNA was extracted either from blood stored in lysis buffer (Seutin et al. 1991) or frozen tissues (liver) using a lithium chloride method adapted from Gemmell and Akiyama (1996).

Polymerase Chain Reaction (PCR) Primers and Amplification

In order to determine gene order within vulture species, we initially attempted the amplification of the entire control region using primers designed from the consensus sequence of several birds species in the known flanking regions, the tARN-Glu and tARN-Phe genes (Baker and Marshall 1997). No amplification products of the size expected were obtained with these primers using our vulture samples. We therefore designed new primers in the regions tThrF and tProR that flanked the new gene order recently described by Mindell et al. (1998). This time we amplified a product of the expected size in *G. barbatus* and *N. percnopterus*. The PCR was performed in 20 μl reaction volumes containing 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 μM of each primer, 1 U *Taq* polymerase, and approximately 10–50 ng of total DNA. The control region of *N. percnopterus* was amplified using *Pwo* polymerase (Roche Diagnostics) following the manufacturer's protocol. PCR conditions were similar for both species and included an initial step at 92°C for 2 min, 34 cycles at 92°C for 30 s, at 64°C for 30 s, and at 72°C for 30 s. Following the last cycle, a final extension step at 72°C for 5 min was performed.

Cloning and Sequencing

Polymerase chain reaction products of the entire control region were extracted from agarose gels using the GFX Gel Extraction Kit (Amersham Life Science). *G. barbatus* purified products were cloned into a pMOS blue vector (Cloning Kit, Amersham Life Science) following the manufacturer's instructions, while *N. percnopterus* purified products were cloned in a blunt end vector pCAPs digested with MluN1 and transformed into XL1Blue *Escherichia coli* competent cells (Roche Diagnostics). Plasmids from both species were isolated and purified using the Nucleospin plasmid extraction kit (Macherey-Nagel) and 1 μl of the final eluate was loaded onto a 1% gel to estimate DNA concentration. We found more than one length control region variant per individual (size heteroplasmy) in both vulture species, but a single clone was sequenced.

Sequencing of the clones in both directions was first carried out using flanking universal primers (T7 and U19, *G. barbatus*; S1 and S2, *N. percnopterus*) using 50–200 ng of plasmid DNA. Since regions difficult to sequence (poly-C, secondary structure) prevented the correct reading of the whole control region, we decided to amplify and sequence PCR fragments with internal primers. Amplified PCR products were purified using the GFX Gel Extraction Kit and about 50 ng of PCR product was sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem) in an ABI Prism TM 310 Genetic Analyzer. In *G. barbatus*, the major part of the mitochondrial control region (~1450 bp) was sequenced at least twice and was thus determined without ambiguities. However, due to a poly-C region, roughly the first 100 bp were correctly read for only one out of five individuals. In *N. percnopterus*, all clones were successfully sequenced in both strands.

Sequence Analysis

For each of the species, sequences were aligned using the program Sequencer 4.1.2 (Gene Codes Corp.) and corrected manually. Nucleotide compositions were computed using the program Mega 1.01 (Kumar et al. 1993). The program DNAsp (Rozas and Rozas 1999) was used to calculate values of nucleotide diversity (π). Percent similarities between both vultures were estimated using the algorithm Align in the DnaStar software package (Lasergene Inc.).

Results and Discussion

Characterization of Bearded and Egyptian Vulture Control Region

Gypaetus barbatus and *Neophron percnopterus* control regions both show the gene order recently described by Bensch and Härlid (2000) and Mindell et al. (1998). Both vulture species'

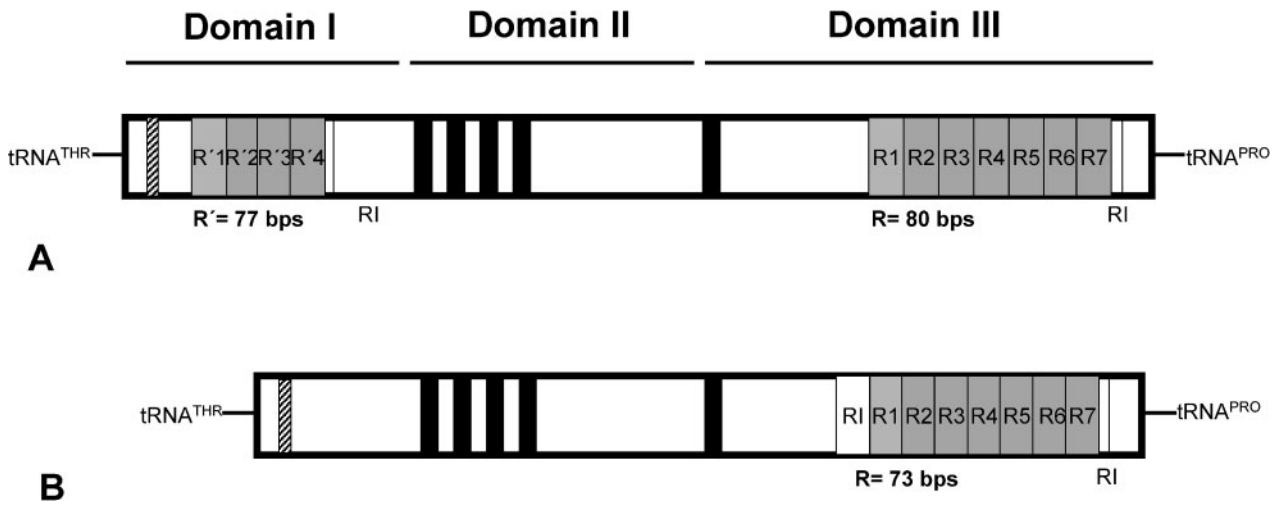

Figure 1. General scheme of the organization of the largest control region sequences in (**A**) *N. Percnopterus* and (**B**) *G. barbatus*: ETAS1 (striped box), conserved (black boxes), complete (grey boxes), and incomplete RI (white boxes) repeated sequences (R).

control regions shared many of the general characteristics that have been reported in other birds (Marshall and Baker 1997; Ruokonen and Kvist 2002). Generally three distinct domains are described in the control region, defined arbitrarily from information on genetic variability or base composition: the most variable, left domain I (DI); the conserved, central domain (DII); and the right domain III (DIII) (Baker and Marshall 1997). Similarly these three distinct domains were observed in vultures. The border between DII and DIII was placed before CSB1, following Sbisa et al. (1997) (Figure 1). The overall base compositions were similar between both species' control regions and in agreement with previous observations in avian species, with A + T > G + C, a CT-rich DII, and a DIII very poor in G and with high proportions of AT nucleotides (Baker and Marshall 1997; Ruokonen and Kvist 2002). However, the control region DI of *N. percnopterus* is not AC rich, as in most bird species, but was exceedingly rich in thymidine (33.7%) due to the presence of a tandemly repeated region.

At the 5′ end of the DI, we found an interrupted poly-C sequence (Figure 2). This structure seems to be a conserved feature across many species and was described in birds including Struthioniformes, Galliformes, Falconiformes, and Sphenisciformes (Haring et al. 2001; Ritchie and Lambert 2000). Although it could potentially form a stable hairpin structure (Quinn 1992; Quinn and Wilson 1993), its function has never been determined. In addition, conserved palindromic motifs 5′-TACAT-3′ (Saccone et al. 1991) and 5′-TATAT-3 were found at the 5′ end of both vulture species' control regions (Figure 2). The four conserved F, E, D, and C boxes located in the central domain and the CSB1 region were identified in both species (Figures 1 and 2). However, the conserved blocks CSB2 and CSB3, previously reported in other vertebrates and believed to be involved in the initiation of DNA synthesis, could not be mapped unambiguously (Sbisa et al. 1997). Similarly these boxes were absent in other Falconiformes and Ciconiiformes species such as *Falco peregrinus* (Mindell et al. 1998), *Buteo buteo* (Haring et al. 2001), and *Ciconia ciconia* (Yamamoto et al. 2000). Sbisa et al. (1997) identified two long conserved blocks in DI—ETAS1 and ETAS2—potentially involved in the control of H strand synthesis. Interestingly, in both the DI of *G. barbatus* and *N. percnopterus*, a 31 bp long sequence of high similarity (71%) with the ETAS1 sequence could be identified (Figures 1 and 2). However, the ETAS2 sequence could not be found without ambiguity. In their study, Sbisa et al. (1997) observed that the ETAS1 sequence was common in many vertebrates species, but in contrast, the ETAS2, CSB2, and CSB3 conserved regions were absent in many of them.

One of the main characteristic of the vulture control region is its large size, due to the presence of long tandem repeats. Repetitive sequences occur in DIII in both species, but the major difference between species comes from the presence of tandem repeated sequences, also in the DI of *N. percnopterus* (Figures 1 and 2). Therefore the length of the control region varies among individuals within species, with the size depending on the number of tandem repeats and also, within individuals (size heteroplasmy). According to this, the length of the control region in *G. barbatus* ranges from 1564 bp (five repeats, DIII) to 1750 bp (seven repeats, DIII) and from 1710 bp (four repeats, DI; three repeats, DIII) to 2031 bp (four repeats, DI; seven repeats, DIII) in *N.*

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Figure 2. Nucleotide sequence of the largest mitochondrial control region in (**A**) *N. percnopterus* and (**B**) *G. barbatus*. Several conserved motifs were identified along this fragment: putative ETAS1 elements (underlined), palindromic motifs (TACAT, TATAT), boxes (F, E, D, C), blocks (CSB1), and repeated sequences (*). GenBank numbers are AY542899 and AY542900.

A

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TTGGTCTTGTAAACCAAAGAATGAAGGCTCACCCCTTCTTAGAGTTTCCTCGCCTCAACGGGCTTCATCCACTTAAACTT 80
                                                                ETAS1
GACCCCCCCCCCCCCTTCCCCCCCGTACAATTATCATAACTATGTATTATTGTACATTAAACTATATGCCCCATTATAAATG 160
                                                                                  *
CAACTCTTTATGTCATACATAACAATGTATGTACTGTATCCATAACTATATAACCGGACATACTCTTATCTCTTACTACC 240
                                                                                *
TACTTTCAAGGATTTATCAGTGCTTGCTCTAAAGAATTTACTCTACTTATCGGGTCTCCTCTGCTTGAGGCCTACTCTAC 320
                                                                              *
TTTCAAGGATTTATCAGTGCTTGCTCTAAAGAATTTACTCTACTTATCGGGTCTCCTCTGCTTGAGGCCTACTCTACTTT 400
                                                                            *
CAAGGATTTATCAGTGCTTGCTCTAAAGAATTTACTCTACTTATCGGGTCTCCTCTGCTTGAGGCCTACTCTACTTTCAA 480
                                                                          *
GGATTTATCAGTGCTTGCTCTAAAGAATTTACTCTACTTATCGGGTCTCCTCTGCTTGAGGCCTACTCTACTTTCAAGGA 560
AGCTCTCTGCATGGTTCAGGACTAGAGATTCTCTGCCCTCGGTCCCAAGACGGTTACGGCTCGATCTGTACAGTACCTGC 640
TTGCCCCTACGGATATGCTTGAGGATATGCTGCCCATGGTAACTGGTCATCCCCTGAAATCCTGCTTGTAGGACCGGTAG 720
                                F BOX
CTATCGGACCAGGTTATCTCTGAGTCGGTCTTCTCACGAGAAATCAGCAACCCGCTGCCCGGAAGATCCTCTACGACTAG 800
   E BOX                                                                D BOX
CTTCAGGACCATTCTTTCCCCCTACACCCCTAGCACTCCTTGCACTTTTGCGCCTCTGGTTCCTATGTCAGGGCCACGAC 880
                              C BOX
TCGCTTAGTCCTACGAACTTGCTCTTTACGAATACATCTGGTAGGCTCTTGATCACCATGTGGCCTCTTAATCGCGGCAT 960
CCGAAAGCCTTTGCGCTGTTGGTTCCCTTTTTTTTTTGGGGCGTCTTCACAGGAGACCCTTCCAAGTGCGGCGGGTAAAT 1040
ACAATCTGTTGACGTGAGCATACAATGGTATACGGTCGGTTTCTCATCCTCAGGAATTAATTAATGAGACGGTTGGCGTA 1120
TTGGGGGAATCATTTTAACACTGATGCACTGTCAGGGCCATTGATAATGGTAGATCACGGACCTTACTTATGGTGCAATT 1200
          CSB1
TAGTGAATGCTTGTGGGACATGCTATTTCACTTTTTTTTTCCTCTTACTTTTTAACCAACACTAGTCGCTTTTCAACTAA 1280
AACGCTAGTCGTATTTTTTTACAAATTTTATCGATAATTTTTTTACACTTCTTTGTTTGTTAAATGCACTAAAATTTCAT 1360
                                *
TAATAACTCAAACTCTTTCGTTTGTTGTTTTCGTCATCGTTTACCATGCATTTTTCACAACCCTTACCTTAAATTTCCGC 1440
                                *
TAAAATTACATTAACAATCCTTTACACACTTTCTTACCGTTTACCATGCATTTTTCACAACCCTTACCTTAAATTTCCGC 1520
                                *
TAAAATTACATTAACAATCCTTTACACACTTTCTTACCGTTTACCATGCATTTTTCACAACCCTTACCTTAAATTTCCGC 1600
                                *
TAAAATTACATTAACAATCCTTTACACACTTTCTTACCGTTTACCATGCATTTTTCACAACCCTTACCTTAAATTTCCGC 1680
                                *
TAAAATTACATTAACAATCCTTTACACACTTTCTTACCGTTTACCATGCATTTTTCACAACCCTTACCTTAAATTTCCGC 1760
                                *
TAAAATTACATTAACAATCCTTTACACACTTTCTTACCGTTTACCATGCATTTTTCACAACCCTTACCTTAAATTTCCGC 1840
                                *
TAAAATTACATTAACAATCCTTTACACACTTTCTTACCGTTTACCATGCATTTTTCACAACCCTTACCTTAAATTTCCGC 1920
                                *
TAAAATTACATTAACAATCCTTTACACACTTTCTTACCGTTTACTGCCCCCCAGCAACCGCTCAGAGAAAGAGGAATCAA 2000
ACCTCTATCACCAACTCCCAAAGCTGGAATT 2031
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B

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TTGGTCTTGTAAACCAAAGATTGAAGGCTTACCCCTTCTTAGAGTTCTGGCCCACTAGGGGCCATAATTGCCAAATTTTA 80
                                                                ETAS1
ACTAGACACCCCCCTAGATAGCCCACCCCCCCCTACCCCCCCCACCCCCCAGCTATGTATTATTGTACATTAAACTATAT 160
GCCCCATTATAATGCACTATTCTATGTTATACATTAATGTATGTACTATAGTCATATATATATGTAATCGGGCATATC 240
ACTCTCTGGGCCATCTACACTTTCAAGTACAGGTCGAGACTCCATGAGGCAGGGAATTTCCTACTACCTTCCTCGGACTA 320
AACCCTAGACCAAAGCTTAATTGGATAATCCTACTCTTCTCCATATGGATATTCTTGGGGACAAGTTCTGTATGGTAGCT 400
GGACATAAATCGCCATTTCCTGTCGAACCTACCTTGCCATTTCTTATAGTACCTGTTTCTGTCGGACTGGGTTATTTATT 480
              F BOX                                   E BOX
GATCGTTCACCTCACGAGAAACCAGCAACCCGGTGTCAGGAAGATCCTCTACTACTAGCTTCAGGACCATTCTTTCCCCC 560
                                        D BOX
TACACCCCTCGCACGACTTGCACTTTTGCGCCTCTGGTTCCTATGTCAGGGTCACGACTCGGTTGATCCTACGAAATTGC 640
       C BOX
TCTTTACGAATACATCTGGTAGGCTATTGATCACCATTTGGCCTCTTAATCGCGGCATCTAGCCTCTTTTGCGCTGTTGG 720
TTCCCTTTTTTTTTCTGGGCGTCCTCACAGGAGACCCTTCCAAGTGCAACGGGTAAATACAATCTGTTGACGTGAGCATC 800
TCGATGGCATGCGGTCTGGGGCCGGCACTCAGGAGTTAATTAATGAGACGGTTGGCTTGTTAGGGGAATCATCATACACA 880
                                                                CSB1
CTGTGCACTGTCAGGGCCATTTGGCTATGGCGCGTCCACTAACCCTACCTATGGGGCTATTTGGTGAATGCTTGTTGGAC 960
ATGTTTTATTACTTTTCTTTTCCTCTTACTTTTTAAGCATCACTAGTCGCTTCCTAACTAGAACGCTAGACGTATTTTTT 1040
CACAAATTTTATCGATACTTTTTTCACATTTTTTTGTTCGTCAAATCCTCTAAAGTTTCATTAATAAATTAAACCTCTAT 1120
                *                                                               *
CATTCGTCATTTTATCGTATCGTTTGTTGTTGTTTCAACAAACACAACCTCAATAACCCCTAGAACTTTCCCACCACAAA 1200
                                                                              *
CCATCATATATCGTTTGTTGTTTGTTTCAACAAACACAACCTCAATAACCCCTAGAACTTTCCCACCACAAACCATCATA 1280
                                                                            *
GTATCGTTTGTTGTTTGTTTCAACAAATACAACCTCAATAACCCCTAGAACTTTCCCACCACAAACCATCATAGTATCGT 1360
                                                                          *
TTGTTGTTTGTTTCAACAAATACAACCTCAATAACCCCTAGAACTTTCCCACCACAAACCATCATAGTATCGTTTGTTGT 1440
                                                                        *
TTGTTTCAACAAATACAACCTCAATAACCCCTAGAACTTTCCCACCACAAACCATCATAGTGTCGTTTGTTGTTTGTTTC 1520
                                                                      *
AACAAATACAACCTCAATAACCCCTAGAACTTTCCCACCACAAACCATCATAGTATCGTTTGTTGTTTGTTTCAACAAAC 1600
                                                                    *
ACAACCTCAATAACCCCTAGAACTTTCCCACCACAAACCATCATAGTGTCGTTTGTTGTTTGTTTCAACAAATACAACCT 1680
CAATAACCCCTAGAACTTTCCCACCACAATTTCAAAGAGAGAGGGGTCAAACCTCCATCATTGGGATTAT  1750
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percnopterus (Figure 1). Compared to other avian species, the control region of *N. percnopterus* is the largest sequenced so far (Delport et al. 2002; Ruokonen and Kvist 2002).

Levels of Sequence Variation Among and Within Vulture Species

Because of the presence of high interspecific sequence variation in DI and DIII and the presence of distinct tandemly repeated sequences, the control region of the two species could not be aligned properly. We therefore calculate sequence similarity between portions of the control region. Results showed high heterogeneity of percent similarity across the control region. Within DI, high interspecific variability was found, but interestingly, the first 100 bp were well conserved (79% similarity). In agreement with previous studies, the central DII, including all the conserved boxes (F, E, D, C, and CSB) was highly conserved, with 86% similarity. In total, 980 bp out of the common 1200 bp of the control region presented 83% of the similarity between both vulture species. However, we found no sequence similarities between the repeat units in the two vultures.

Within species, control region diversity was low compared to other avian species (Delport et al. 2002; Randi and Luchini 1998; Ruokonen and Kvist 2002). Low intraspecific variability was found in *G. barbatus* with 1.8% of polymorphic sites along 1160 bp of the control region (we exclude the repeated sequences from the analysis). Roughly double this variability was found in the control region (1102 bp) of *N. percnopterus* (4%). However, DI and DIII were the most variable (3.3% and 8.8%, respectively) and DII the least variable (0.8% in both species) domains, as previously reported in most avian species (Marshall and Baker 1997; Ruokonen and Kvist 2002). A past bottleneck of vulture populations could explain this reduced variability. Alternatively, the control region in vultures may not evolve as rapidly as in other taxa (Ruokonen and Kvist 2002).

Tandem Repetitive Sequences in DI and DIII

All individuals analyzed showed long tandemly repeated sequences (Figures 1 and 2). Berg et al. (1995) reported these long tandem repeats were frequent in Ciconiiformes species, ranging in length from 50 to 200 bp and generally located 2–20 bp from the 5′ end of the $tRNA^{Phe}$. However, compared to other bird species (see Table 6 in Haring et al. 2001), the *N. percnopterus* control region contains the highest percentage (45.7%) of repeated sequences, with long repeats present both in DI (77 bp) and DIII (80 bp). The simultaneous existence of both of these repeats in DI and DIII is rare in birds, but has been previously reported in another raptor species, *Falco peregrinus* (Mindell et al. 1997, 1998).

In *G. barbatus*, repeated sequences in DIII consist of an incomplete repeat (RI) followed by five or seven copies of a 73 bp sequence repeated in tandem. Comparison of repeats within and among individuals indicated several interesting features. First, in *N. percnopterus*, repeated sequences in DI were more variable both among and within individuals than were tandem repeats located in DIII. For example, the 80 bp repeats in DIII were identical in most *N. percnopterus* individuals except the one from India, while repeats in DI were highly variable, both within and among individuals. Similarly, in shrew species, no sequence variation was found among DIII repeats from all specimens, while much more variation was found among DI repeats (Fumagalli et al. 1996; Yamagata and Namikawa 1999).

In *G. barbatus*, sequence variation in the repeated region was low, both among and within individuals. The incomplete (RI) and first complete repeat (R1) were distinct from other repeats, but were similar among almost all individuals, while adjacent remaining repeats within an individual were closely related. Buroker et al. (1990) proposed a model in which length mutations may originate through replication slippage due to a competitive displacement between the two strands (H strand and D loop) in relation to stable secondary structures formed by the repeated motifs. This model generally fits well with most of the cases described to date, but more complex mechanisms were also proposed to explain other observed patterns of variation (Brzuzan 2000; Hoelzel et al. 1993; Ritchie and Lambert 2000). In our study, we found that differences were larger among homologous repeats between individuals than among tandem repeats within an individual, suggesting a pattern of intraspecific concerted evolution (homogenization of an array through reiterated cycles of insertion and deletion of repeats) (Broughton and Dowling 1994; Rand 1994). The difference in the level of variation of repeats between and within the two vultures could be due to either a different balance between the point mutation rate (generating diversity among repeats) and the rate of insertion/deletion of repeats (implicated in the homogenization of the array) as previously proposed (Fumagalli et al. 1996; Hoelzel et al. 1993), or to the intrinsic lower genome variability of *G. barbatus*.

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