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Preliminary molecular variability of some dove species inhabiting Saudi Arabia

Sayed Amin Mohamed Amer[1, 2]

[1]Biology Department, Faculty of Science, Taif University, Taif, Saudi Arabia
[2]Zoology Department, Faculty of Science, Cairo University, Egypt

Email address:
yasser92us@yahoo.com

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Abstract: Approximately 800 bp of the mitochondrial 12S rRNA gene were sequenced for the Eurasian collared-dove *Streptopelia decaocto* and the African collared-dove *Streptopelia roseogrisea*. The first species was collected from Taif at the eastern boarder of Sarawat mountains and from Jazan at the southwest, while the second was collected from Taif only. These obtained sequences were used to study the genetic variability between the two species. The base differences between the two species were 19 sites along the sequenced fragment, while there was no intra-specific difference. It is therefore noteworthy to conclude that the Eurasian dove is homogenous along the range of distribution throughout Sarawat mountains and the small variability between the two dove species is indicative to their morphological and genetic similarity.

Keywords: Eurasian Collared-Dove, African Collared-Dove, 12S rRNA, Mitochondrial DNA

1. Introduction

The Eurasian collared dove *Streptopelia decaocto* has an extremely large range, and the population trend appears to be increasing. The population size is extremely large and therefore it is evaluated as Least Concern [1, 2]. Eurasian Collared-Doves have plump bodies, small heads, and long tails. The wings are broad and slightly rounded. The broad tail is squared off at the tip, rather than pointed. They are chalky light brown to gray-buff birds with broad white patches in the tail. The bird's collar is a narrow black crescent around the nape of the neck. In flight and when perched, the wingtips are darker than the rest of the wing [3].

The African collared dove is also called ringed turtle dove consists of two subspecies of which *Streptopelia roseogrisea arabica* is subspecies mainly found in Saudi Arabia. It is characterized by pale creamy buff color, white chin, belly and undertail coverts, black half-collar around back of the neck, white undertail coverts, red eye, black bill and crimson feet [4].

Genetic characterization can be done by different classes of molecular markers, such as Restriction Fragment Length Polymorphisms (RFLP) [5], Single Stranded Conformation Polymorphisms (SSCP) [6], Random Amplified Polymorphic DNA (RAPD) markers [7-9], Amplified Fragment Length Polymorphisms (AFLP) [10], Single Nucleotide Polymorphisms (SNP) [11] and mitochondrial DNA. Mitochondrial DNA (mtDNA) is a circular DNA, found in the mitochondrion of the cell. It is an important molecular marker, widely used to trace domestication history [12]. Since it does not go under recombination, differences between mitochondrial sequences occur due to mutations only. It reflects only the history of the maternal lineage [13].

Recent molecular phylogenetic study conducted by Awan et al. [14] revealed that Eurasian collared dove (*Streptopelia decaocto*) and African collared dove (*Streptopelia roseogrisea*) shared a common clade with Pakistani collared dove which indicated a super-species group in *Streptopelia* genus. To the best of our knowledge, there is no molecular study on the genetic relationship within the genus *Streptopelia*. However, Fulton et al. [15] studied the basal relationships within the typical pigeons and doves by using data from nuclear and mitochondrial genomes and from intron 7 of the nuclear encoded fibrinogen beta chain (FGB). The authors found a contrast in the relationships between the data of both genomes. FGB data suggest that the cuckoodoves are more closely related to the Old World pigeons and turtle doves (*Columba* and *Streptopelia*), although the addition of mtDNA data reduces the support for

this until the relationship is no longer recovered. However, FGB-only analyses support the cuckoo-doves + *Columba* + *Streptopelia* clade strongly, yielding a hypothesis for this subfamily that, to our knowledge, has not yet been explored.

The present study aims to sequence 12S rRNA mitochondrial gene for both species Eurasian collared-dove *Streptopelia decaocto* and the African collared-dove *Streptopelia roseogrisea* to investigate their genetic variability.

2. Materials and Methods

2.1. Samples

Six individuals of the Eurasian collared-dove *Streptopelia decaocto* and the African collared-dove *Streptopelia roseogrisea* from local market of Taif and Jazan provinces, Kingdom of Saudi Arabia were used in this study. Blood samples were withdrawn from the jugular vein into heparinized tubes. 300 µL were frozen for the molecular study.

2.2. DNA Extraction and PCR Experiments

Different blood samples were numbered and labeled with full information. Mitochondrial DNA was extracted from 0.5 mL blood samples with QIAGEN spin-column kits according to the manufactur's instruction (QlAamp ® DNA Mini and Blood Mini extraction kit).

PCR was conducted in a final volume of 25 µL containing 1 µL DNA template, 0.1 µL of 10 Pmolar forward primer (5`-AGACTYAGTCCTAACCTT-3`), 0.1 µL of 10 Pmolar reverse primer (5`-CTTACCTTGTTACGACTT-3`) [16], 12.5 µL PCR master mix (Promega Corporation, Madison, WI) and 11.3 µL autoclaved deionized distilled water. PCR was carried out using a PeX 0.5 thermal Cycler with the cycle sequence at 94°C for 4 min one cycle, followed by 35 cycles each of which consisted of denaturation at 94°C for one min, annealing at 46°C for one min and extension at 72°C for one min with a final strand elongation for one cycle at 72°C was done for an additional 5 min. The PCR products were analyzed in 1% agarose gel electrophoresis in TAE buffer (40 mm Tris, 40mM acetic acid and 1mM EDTA) with ethidium bromide staining. A 100-bp DNA ladder (Biolabs) was used as a molecular marker. Then PCR products were visualized under UV light and photographed. The PCR products which showed single and sharp bands were extracted directly without gel excising.

2.3. Sequencing

The purified PCR products were sequenced in an ABI PRISM 3730 µL sequencer (Applied BioSystems) and BigDyeTM Terminator Sequencing Kits with AmpliTaq-DNA polymerase (FS enzyme) (Applied Biosystems) following the protocols supplied by the manufacturer. After reading the targeted genes, the nucleotide sequences have been treated with different software programs of DNASIS, MacClade and PAUP [17] that enabled to detect genetic relatedness between different samples and breeds. The sequenced genes were tested by BLAST program to check their relatedness to the sequenced genes for genus streptobelia in the Genbank database.

3. Results and Discussion

The target fragment of the mitochondrial DNA of 12S rRNA gene was successfully amplified for the different dove samples. Unambiguous nucleotides from 12S rRNA gene were sequenced for 6 samples of the two dove species. In order to estimate the base composition and frequencies for the obtained sequences, the data were concatenated and the gab-containing sites were deleted so that 800 bp were left for analysis. The data showed base frequencies of A = 31.4%, C = 28.9%, G = 19.9% and T = 19.8%. The scarcity of G for the light strand is a common feature found in metazoan mtDNAs [18].

The electropherograms (Fig. 1) of the obtained sequence were compared from the two dove species in order to show an evidence for the base difference between them. The underlined peaks are an evidence for the base mutations occurred in this fragment between the two dove species. Of the 800 nucleotides, 781 were constant and 19 sites were variables. Nucleotide substitutions are generally considered in terms of changes within the two structural classes of nucleotides (purines and pyrimidines), that is, in terms of transitions and transversions [19]. All the substitutions occurred in the 12S rRNA of the two doves mitochondrial DNA was from transition type.

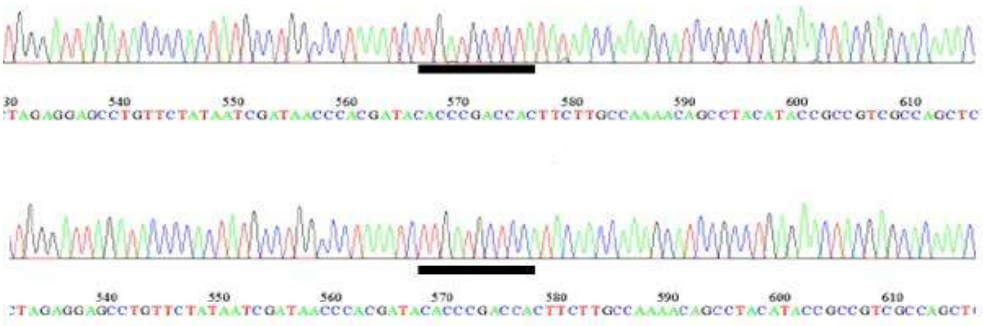

Fig. 1. *The chromatogram of a fragment of 12S rRNA gene for the two species studied. The above is for Taif sample and below is for Jazan sample. See the base difference above the black lines.*

As shown in Fig. 2, the two dove species showed difference in 19 sites along the sequenced fragment all of which were substitutions within purines or pyrimidines. Sixteen of these substitutions were between T and C, while 3 only were between G and A. As 12S rRNA gene is highly conserved among vertebrate species, it is reasonably to conclude that the recorded variablity could be considered interspecific. A supportive evidence for the previous conclusion is that these two species are considered conspecific or subspecies [20]. It can not be considered that the two taxa are two subspecies of *Streptopelia roseogrisea* since the availibity of *Streptopelia r. roseogrisea* in Arabia is immposible and the only subspecies inhabiting the Arabian continent is *Streptopelia roseogrisea arabica* [21].

```
                                                                      50
T     GACTCCTACA TCATAGGTAA AAGGAGCCGG CATCAGGCAC GCCTACAGCA
J     .......... C..C...C.. .......... .......... ..........

                                                                     100
T     GCCCAAGACG CCTTGCTTAG CCACACCCCC ACGGGTACTC AGCAGTAATT
J     .......... .......... .......... .......... ..........

                                                                     150
T     AACATTAAGC AATAAGTGTA AACTTGACTT AGTTATAGCG ACTCAGGGTT
J     .......... .......... .......... .......... ..........

                                                                     200
T     GGTAAATCTT GTGCCAGCCA CCGCGGCCAT ACAAGAGACC CAAACTAATC
J     .......... .......... ......T..C .......... ..........

                                                                     250
T     GTTCACGGCG TAAAGAGTGG ACTCATGCCT ATCACATTAA TTAGGGTCAA
J     .......... .......... .......... .......... ...A......

                                                                     300
T     AACGTAGCTG AGCTGTCATA AGCTTAAGGT ACGCTTAAAA CCACCCTAAA
J     .......... .......... ........AC ..A....... ..........

                                                                     350
T     GATGACCCTA ATCCATATGA CCTATTAAAC TCCACGAAAG CCAGGGCCCA
J     .......... .C.T...... .......... .......... ..........

                                                                     400
T     AACTGGGATT AGATACCCCA CTATGCCTGG CCCTAAATCT TGATGCTCCA
J     .......... .......... .......... .......... ........T.

                                                                     450
T     TACAACCAAA GCATCCGCCT GAGAACTACG AGCACAAACG CTTAAAACTC
J     ..T....... .......... .......... .......... ..........

                                                                     500
T     TAAGGACTTG GCGGTGCCCC AAACCCACCT AGAGGAGCCT GTTCTATAAT
J     .......... .......... .......... .......... ..........

                                                                     550
T     CGATAACCCA CGATACACCC GACCACTTCT TGCCAAAACA GCCTACATAC
J     .......... .......... .......... .......... ..........

                                                                     600
T     CGCCGTCGCC AGCTCACCTC TTCTGAGAGT ACCACAGTGA GCACAATCGC
J     .......... .......... .......... ..T....... ..........

                                                                     650
T     CCTAACCCCG CTAACAAGAC AGGTCAAGGT ATAGCTCATG AAGTGGAAGA
J     .......... .......... .......... .....C.... ..........

                                                                     700
T     AATGGGCTAC ATTTTCTAAC TTAGAAAACT CACGAAAGGG GGCATGAAAC
J     .......... .......... .........C .......... ..T......T

                                                                     750
T     AGCCCCTAGA AGGTGGATTT AGCAGTAAAG TGGGATAATA TAAGCCCCCT
J     ......C... .......... .......... .......... ..........

                                                                     800
T     TTAAGCTGGC TCTGAGGCAC GTACATACCG CCCGTCACCC TCCTCACAAG
J     ......C... .......... .......... .......... ..........
```

Fig. 2. *The aligned nucleotides of 12S rRNA gene sequenced in this study. T refers to Taif samples while J denotes Jazan population. Dotes means the identity of the two sequences.*

Little is known about the genetic relationships among the species of genus *Streptopelia* but none of these studies was tackling the genetic relationship between the two dove species of the current study. Khan and Arif [22] applied CO1 gene barcodes in investigating the phylogeny of the dove genera including *Streptopelia* without referring to the African *Streptopelia roseogrisea* in their study. Jhonson and Clayton [23] investigated the molecular phylogeny of doves that belong to genus Zenaida, while Jhonson et al. [24] investigated the molecular phylogeny of the genra *Streptopelia* and *Columba* and they reveald with strong statistical support, similar to this study, the sister relationship between *Streptopelia roseogrisea* and *Streptopelia decaocto*.

As the studied samples were collected from a local market in Taif and Jazan Proviences, it was difficult to identify these samples morphologically and also it was difficult ot relate these samples to their exact habitats. It was therefore resaonalbly to conduct Blast searching and constuct the phylogenetic tree. The Blast program was searched for the obtained sequence of this study and the obtained phylogentic tree (Fig. 3) obviously showed that the studied samples are belonging to *Streptopelia*. As *Streptopelia roseogrisea arabica* is mainly inhabing the west of Arabia [25] and it was considered as conseicific to *Streptopelia decaocto*, it is easily to consider the obtained molecular data successfully enough to identify the studied species.

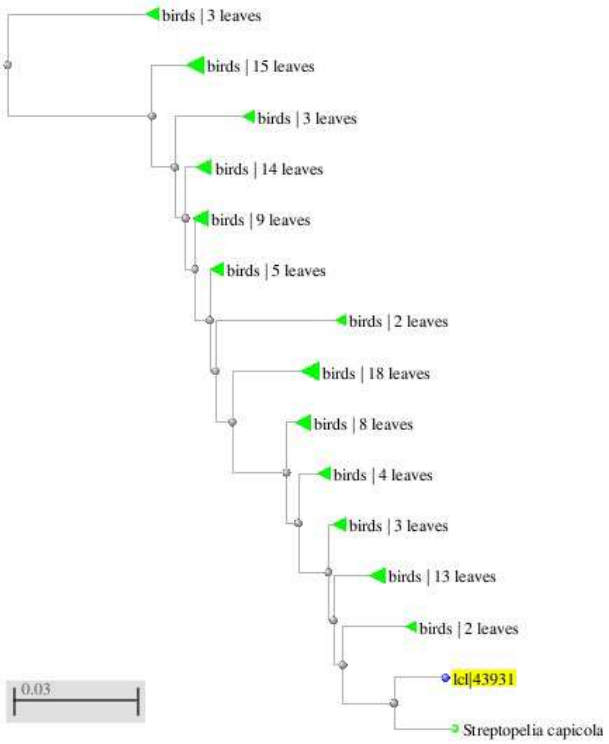

Fig. 3. *A phylogenetic tree obtained from Blast program.*

Finally, it could be concluded that the studied samples are belonging to the conspecific dove species *Streptopelia roseogrisea* and *Streptopelia decaocto* inhabiting the west of Saudi Arabia and the base difference that was recorded between them indicated their high degree of similarity.

4. Conclusion

There were few genetic differences between the two dove species along the sequenced fragment and there was no intra-specific difference. It is therefore noteworthy to conclude that the Eurasian dove is homogenous along the range of distribution in the western region of Saudi Arabia and the small differences between the two species could be attributed to their morphological similarity.

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