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Selection of mtDNA Marker Genes In The Study Of Bird Genetic Diversity: A Review

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***Abstract*—Mitochondrial DNA (mtDNA) has emerged as a crucial tool in understanding bird genetic diversity due to its unique characteristics, including maternal inheritance and high mutation rates. This review synthesizes the current understanding of various mtDNA marker genes, focusing on their applications in avian genetic research. The selection of mtDNA marker genes such as cytochrome c oxidase subunit I (COI), NADH dehydrogenase subunit 2 (ND2), cytochrome b (cyt b), and the D-loop region is evaluated for their advantages and limitations. The review highlights the significance of mtDNA markers in conservation genetics and phylogenetics, emphasizing their utility in resolving evolutionary relationships and assessing population structure.**

Keywords— Bird Diversity; Genetic; mtDNA

I. INTRODUCTION

Genetic diversity is fundamental for the survival and adaptability of species [1]. In birds, understanding genetic variation is essential for conservation strategies, especially in the face of habitat loss and climate change [2]. DNA markers serve as tools to quantify this diversity, enabling researchers to assess population structure, gene flow, and evolutionary history [3].

The selection of DNA markers is crucial in studies assessing the genetic diversity of bird populations. The choice of markers can significantly influence the outcomes of genetic analyses and the understanding of evolutionary relationships among species [4].

Mitochondrial DNA has been widely recognized as a valuable tool in molecular ecology and evolutionary biology. Its maternal inheritance, relatively high mutation rate, and compact genome make it particularly suitable for investigating genetic diversity, phylogenetics, and population structure in birds [5], [6], [7]. This review highlights the advantages and limitations of mtDNA as a marker, emphasizing its role in understanding avian diversity.

II. METHODS

This article was written using the literature review method. This method required article sources obtained from the Google Scholar website. The keywords used in searching for article sources were mtDNA and bird. The inclusion criteria of this article were articles on the topic of bird genetic diversity and using primary data. The articles used were research article, full text, and written in English.

III. RESULT AND DISCUSSION

Mitochondrial DNA (mtDNA) exhibits several distinct characteristics that make it a unique and valuable tool in genetic research:

- Small Circular Structure: mtDNA is approximately 16,569 base pairs long and is a small, circular double-stranded DNA molecule [8].
- Maternal Inheritance: mtDNA is inherited solely from the mother during fertilization, as mitochondria are typically passed from mother to offspring [8].
- High Mutation Rate: Due to the lack of protective histones and effective repair systems, mtDNA is more susceptible to mutations compared to nuclear DNA [8].
- Coding for Essential Proteins: mtDNA encodes for 13 protein-coding genes, which are essential for the proper functioning of mitochondria and the synthesis of ATP through oxidative phosphorylation [9].
- Presence of tRNA and rRNA Genes:

  Non-Coding Regions: In addition to protein-coding genes, mtDNA also contains genes for transfer RNA (tRNA) and ribosomal RNA (rRNA), which are crucial for protein synthesis within mitochondria [9].
- Dynamic Maintenance Mechanisms: Mitochondria have unique mechanisms to maintain mtDNA integrity, including degradation of excessively damaged genomes followed by replication of intact/repaired mtDNA. This process is not present in the nucleus and is enabled by multiple copies of mtDNA present in mitochondria [10].
- Correlation with Species-Specific Life-History Traits: The mtDNA mutational spectrum is sensitive to species-specific life-history traits. For example, long-lived species have an increased rate of A>G substitutions on the single-stranded heavy chain, which may be linked to their biased process of mutagenesis [11].
- Association with Non-B (Non-Canonical) DNA Structures: Deletion breakpoints frequently occur within or near regions showing non-canonical conformations, such as hairpins, cruciforms, and cloverleaf-like elements. This instability can contribute to the complex interplay between mtDNA and its associated mitochondrial disorders [12].

Understanding genetic diversity through the appropriate selection of DNA markers is vital for effective conservation strategies. For instance, identifying genetically distinct populations can inform management decisions, such as habitat protection and restoration efforts. Moreover, monitoring genetic diversity over time can help assess the effectiveness of conservation actions.

A. Considerations in Marker Selection

The choice of DNA marker depends on several factors [13], including:

- Research objectives: The specific goals of the study, such as assessing genetic diversity, population structure, or evolutionary relationships, will influence marker selection.
- Species characteristics: The life history traits of the species, such as migratory behavior and breeding systems, can affect genetic structure and should be considered when choosing markers.
- Technical resources: The availability of laboratory facilities and expertise in molecular techniques may limit the choice of markers.

B. Advantages of mtDNA Markers

mtDNA is inherited maternally, allowing researchers to trace lineages and study maternal ancestry without the complications of recombination found in nuclear DNA [3]. This characteristic simplifies the interpretation of genetic data, making it easier to identify evolutionary relationships among species [14]. The relatively high mutation rate of mtDNA facilitates the detection of genetic variation within and between populations [5]. This is crucial for assessing genetic diversity, especially in species with small population sizes or those that have undergone recent demographic changes [2]. mtDNA markers, such as cytochrome b (cyt b) and

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cytochrome c oxidase subunit I (COI), provide robust phylogenetic signals, enabling researchers to resolve evolutionary relationships among closely related species. This is particularly important in avian studies where cryptic species may exist [13]. Understanding genetic diversity through mtDNA can inform conservation strategies. By identifying genetically distinct populations, conservationists can prioritize efforts to protect these groups, ensuring the preservation of genetic variability essential for adaptation and survival [15].

C. Limitations of mtDNA Markers

While mtDNA is effective for resolving deeper evolutionary relationships, it may lack the resolution needed to distinguish between very recently diverged populations. This limitation can lead to underestimations of diversity in species that have undergone rapid evolutionary changes. mtDNA has a smaller effective population size compared to nuclear markers, making it more susceptible to genetic drift [16]. This can result in reduced genetic diversity in populations that have experienced bottlenecks or isolation, potentially skewing interpretations of genetic health [13]. The assumption that mtDNA evolves neutrally is often challenged. Selection may act on mitochondrial genes, leading to patterns of variation that do not accurately reflect demographic history. This complicates the interpretation of mtDNA data in the context of population genetics.

D. Advantages and Limitations of Specific mtDNA Genes

The use of specific mtDNA genes has provided valuable insights into avian phylogenetics and population genetics. However, each gene comes with its own set of advantages and limitations [Tabel 1].

1. Cytochrome c oxidase subunit I (COI)

   COI is known for its high level of genetic variation, making it an effective marker for distinguishing between closely related species and assessing population structure. This gene is widely used in DNA barcoding, which aids in species identification [17]. The availability of universal primers for COI facilitates its amplification across diverse taxa, enhancing its utility in large-scale biodiversity studies. While COI is effective for distinguishing between species, it may not provide sufficient resolution for very closely related populations that have diverged recently [18]. High levels of variation can lead to saturation in the substitution rate, complicating phylogenetic analyses and potentially obscuring true evolutionary relationships.

2. Cytochrome b (cyt b)

   Cyt b provides robust phylogenetic signals, allowing researchers to resolve evolutionary relationships among avian species effectively. Its relatively high mutation rate contributes to its effectiveness in studying recent divergences [16]. As a single-copy gene, cyt b simplifies data interpretation compared to multi-copy genes, reducing the risk of co-amplification issues associated with nuclear markers. Compared to COI, cyt b has a lower mutation rate, which may limit its effectiveness in studies requiring fine-scale resolution of genetic diversity [13].

3. ND2

   The ND2 gene is a protein-coding gene located in the mitochondrial genome and has been extensively used in avian diversity studies. ND2 has a relatively high substitution rate, approximately twice that of the commonly used cytochrome c oxidase subunit I (COI) gene, making it suitable for resolving relationships among closely related species [4]. ND2 provides robust phylogenetic signals, enabling researchers to resolve evolutionary relationships among avian species effectively. ND2 can be amplified across a wide variety of avian taxa using a small set of primers, making it a versatile marker [19][20]. However, the ND2 gene also has some limitations. The substitution rate of ND2 can vary among different avian lineages, potentially affecting the accuracy of phylogenetic inferences [21].

4. D-loop

   The D-loop, also known as the control region, is the most variable region of the mitochondrial genome and has been widely used in avian diversity research [7]. The D-loop exhibits a high level of sequence variation, making it suitable for

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studying population-level genetic diversity and recent evolutionary events. The D-loop can be easily amplified using universal primers, facilitating its use in a wide range of avian species. However, the D-loop also has some limitations. The high mutation rate of the D-loop can lead to saturation in the substitution rate, complicating phylogenetic analyses and potentially obscuring true evolutionary relationships. The presence of repetitive sequences and indels in the D-loop can make sequence alignment challenging, especially when comparing distantly related species [22].

TABLE I. ADVANTAGES AND LIMITATIONS OF SPECIFIC MTDNA GENES

mtDNA Gene	Advantages	Limitations
COI	High variation, universal primers [17]	Limited resolution for recent divergences, potential for saturation [18]
Cyt-b	Phylogenetic resolution, single copy [16]	Lower mutation rate [13]
ND2	High substitution rate, phylogenetic resolution, broad applicability [4]	Variation in substitution rates [21]
D-loop	High variability, ease of amplification [7]	Potential for saturation, difficulty in alignment [22]

E. Applications in Avian Diversity Research

- Phylogenetic analysis:

  Genes like cytochrome b (cyt b) and NADH dehydrogenase subunit 2 (ND2) provide robust phylogenetic signals, enabling researchers to resolve evolutionary relationships among closely related bird species [22][4]. The use of mtDNA markers, particularly COI, has been successful in species identification across a wide array of taxa, including birds [23].

- Population genetics:

  Studies have shown that mtDNA can reveal dramatic population-level differentiation undetected with other methods. For example, a study on six common North American bird species found ample mtDNA genetic variation but not strongly partitioned among geographic or subspecific populations [24]. The genetic diversity of avian populations can be quantified using mtDNA markers. For instance, a study found that diversity in the mitochondrial genome is correlated with range size, indicating that larger ranges often have higher genetic diversity [14].

- Conservation biology

  DNA barcoding using COI has been successful in identifying bird species, particularly useful for species discovery and conservation efforts. The use of mtDNA markers like ND2 has helped identify cryptic species in avian populations, such as those found in the Philippine Islands where nearly half of the studied species showed possible species-level divergences [25].

- Taxonomic revisions

  Integrating genetic and phenotypic data using mtDNA markers has led to substantial taxonomic revisions among avian species. For example, a study on Philippine birds revealed high genetic endemism among sampled islands and suggested the need for taxonomic revisions [25].

- Conservation implications

  Analyzing mtDNA genetic diversity can provide insights into population history and demographic changes. For instance, a study on the red kite (*Milvus milvus*) highlighted the importance of quantifying and characterizing avian genetic diversity for conservation efforts [26].

IV. Conclusion

Mitochondrial DNA serves as a powerful marker for studying genetic diversity in birds, offering insights into evolutionary relationships and informing conservation strategies. The choice of mitochondrial DNA genes as markers in avian diversity research presents both advantages and disadvantages. A comprehensive understanding of these markers' strengths and weaknesses is essential for effectively studying avian genetic diversity and informing conservation strategies.

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