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MITOCHONDRIAL EVOLUTION IN BATS: A CRITICAL REVIEW OF BIOENERGETICS, LIFE HISTORY, AND THE LIMITS OF MITOCHONDRIAL DNA AS AN EVOLUTIONARY MARKER

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RESUMO

Este artigo teve como objetivo analisar criticamente a evolução mitocondrial em morcegos, integrando evidências sobre bioenergética, história de vida, seleção molecular, filogenia e limitações metodológicas do DNA mitocondrial. Trata-se de uma revisão integrativa crítica, de natureza teórico-analítica, baseada em literatura científica sobre evolução molecular, mitogenômica, fisiologia comparada e métodos filogenéticos. A análise indicou que a variação das taxas evolutivas mitocondriais em Chiroptera não pode ser explicada por um único fator, como metabolismo, tamanho corporal ou longevidade, mas pela interação entre dimensões moleculares, fisiológicas, ecológicas, filogenéticas e metodológicas. Propõe-se um modelo conceitual multifatorial para interpretar essa variação. O estudo reforça que o mtDNA deve ser compreendido não apenas como marcador evolutivo, mas como genoma funcional e historicamente condicionado.

Palavras-chave: Chiroptera; mitogenômica; seleção molecular; filogenia; longevidade.

ABSTRACT

This article aimed to critically analyze mitochondrial evolution in bats by integrating evidence on bioenergetics, life history, molecular selection, phylogeny, and methodological limitations of mitochondrial DNA. It is a critical integrative review with a theoretical-analytical approach, based on scientific literature on molecular evolution, mitogenomics, comparative physiology, and phylogenetic methods. The analysis indicated that variation in mitochondrial evolutionary rates in Chiroptera cannot be explained by a single factor, such as metabolism, body size, or longevity, but rather by the interaction among molecular, physiological, ecological, phylogenetic, and methodological dimensions. A multifactorial conceptual model is proposed to interpret this variation. The study reinforces that mtDNA should be understood not only as an evolutionary marker, but also as a functional and historically conditioned genome.

Keywords: Chiroptera; mitogenomics; molecular selection; phylogeny; longevity.

1. INTRODUCTION

Mitochondrial DNA has been widely used in evolutionary, phylogenetic, and phylogeographic studies because of its compact genomic organization, relative ease of recovery, broad availability in public databases, and high substitution rate compared with many nuclear markers. Across different animal groups, this genome has become an important tool for inferring evolutionary relationships, estimating lineage divergence, and investigating patterns of molecular diversity. However, although mtDNA is frequently employed as an evolutionary marker, its dynamics should not be interpreted as a simple reflection of a uniform molecular clock, since its evolutionary rates may vary substantially among species, lineages, and major

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taxonomic groups (Gissi; Iannelli; Pesole, 2000; Galtier et al., 2009; Allio et al., 2017).

Variation in mitochondrial evolutionary rates represents a central issue in evolutionary biology, as it challenges interpretations based on the temporal constancy of molecular substitutions. Comparative studies indicate that factors such as body size, metabolism, generation time, longevity, effective population size, efficiency of molecular repair mechanisms, and the intensity of natural selection may influence the rate at which mutations and substitutions accumulate in the mitochondrial genome. Thus, mtDNA should not be understood solely as a neutral tool for phylogenetic reconstruction, but also as a genomic component subject to its own evolutionary pressures (Martin; Palumbi, 1993; Nabholz; Glémin; Galtier, 2008; Lynch, 2010; Bromham, 2011).

This complexity becomes particularly relevant in mammals, a group in which variation in molecular rates has been associated with differences in life-history traits and physiological strategies. Species with smaller body size, higher metabolic rates, or shorter generation times may exhibit distinct patterns of molecular evolution compared with larger, longer-lived species or those with more conservative metabolic strategies. However, these relationships are neither simple nor universal, since metabolism, longevity, reproduction, and population size may act in combination, or even in opposition, in shaping the evolution of the mitochondrial genome (Martin; Palumbi, 1993; Nabholz; Glémin; Galtier, 2008; Bromham, 2011).

In this context, bats constitute a particularly relevant group for the analysis of mitochondrial evolution. The order Chiroptera is characterized by high taxonomic diversity, broad ecological variation, distinct feeding strategies, wide geographic distribution, and a physiological feature that is unique among mammals: powered flight. Flight imposes intense respiratory, muscular, and metabolic demands, making the mitochondrion a central element for understanding the physiology and evolution of the group. For this reason, bats provide a promising model for discussing how bioenergetic demands may influence molecular evolution (Maina, 2000; Shen et al., 2010).

Nevertheless, bats also exhibit characteristics that challenge linear explanations based solely on elevated metabolism. Despite the intense energetic demand associated with flight, many species show exceptional longevity relative to body size, as well as physiological strategies for energy conservation, such as torpor, seasonal metabolic variation, and episodes of extreme bradycardia in specific ecological contexts. This combination produces an apparent evolutionary paradox: bats combine high metabolic demands with physiological mechanisms potentially associated with cellular maintenance and reduced molecular damage

(Seim et al., 2013; O'Mara; Wikelski; Dechmann, 2017).

In addition to physiological and life-history traits, mitochondrial evolution in bats must also be examined in light of molecular selection and bioenergetic adaptation. Because the mitochondrial genome encodes genes directly involved in oxidative phosphorylation, changes in this genome may be related to specific energetic demands, ecological specializations, and selective pressures associated with flight, diet, and metabolism. Studies on the adaptive evolution of the mitochondrial genome indicate that mitochondrial genes may show signatures of positive selection and lineage-specific evolutionary patterns, reinforcing the need to interpret variation in mitochondrial rates beyond purely neutral processes (Fonseca et al., 2008; Gershoni; Templeton; Mishmar, 2009; Shen et al., 2010; Botero-Castro et al., 2018).

The use of complete mitogenomes in phylogenetic studies of bats has expanded the ability to investigate evolutionary relationships within the order Chiroptera, especially in groups for which isolated markers provide limited resolution. However, increasing the amount of mitochondrial information does not eliminate the inherent limitations of the marker itself. mtDNA represents a specific hereditary history that may differ from the evolutionary history of species due to processes such as selection, introgression, compositional bias, incomplete lineage sorting, and conflicts between mitochondrial and nuclear genomes. Therefore, even well-supported mitochondrial trees must be interpreted critically, particularly in diverse groups with complex evolutionary radiations (Yu et al., 2016; Platt et al., 2018; Camacho et al., 2022).

This discussion also has important methodological implications. Because species share ancestry, comparative analyses investigating relationships between evolutionary rates and phenotypic, ecological, or physiological traits should not treat each species as a fully independent observation. Phylogenetic comparative methods are essential for distinguishing patterns associated with biological variables from those resulting from shared evolutionary history. Accordingly, any robust interpretation of mitochondrial evolution in bats must simultaneously consider molecular variation, the biological attributes of the group, phylogenetic structure, and the methodological limitations of the marker being used (Martins; Hansen, 1997; Freckleton; Harvey; Pagel, 2002; Revell, 2010; Blomberg et al., 2012).

Against this background, this article aims to critically analyze the literature on mitochondrial evolution in bats, integrating evidence on bioenergetics, life history, molecular selection, phylogeny, and the methodological limitations of mitochondrial DNA. It argues that variation in mitochondrial evolutionary rates in Chiroptera should not be explained by a single factor, such as metabolism or body size, but rather by a multifactorial model in which flight, longevity, selection, shared ancestry, and the limitations of mtDNA itself interact to produce heterogeneous patterns of molecular evolution (Bromham, 2011; Allio et al., 2017; Botero-Castro et al., 2018; Liu et al., 2024).

2. METHODOLOGY

This study is characterized as a critical integrative review with a theoretical-analytical approach, focused on the scientific literature on mitochondrial evolution, molecular evolutionary rates, bioenergetics, life history, phylogeny, and bat mitogenomics. This type of review was chosen because the topic requires the integration of evidence from different fields, including molecular evolution, comparative physiology, evolutionary genomics, phylogenetic systematics, and comparative methods, in order to build a broad and critically grounded interpretation of variation in mitochondrial evolutionary rates in Chiroptera.

To construct the bibliographic corpus, scientific publications available in databases such as PubMed, Web of Science, Scopus, Google Scholar, and ScienceDirect were considered, in addition to papers published in specialized journals in molecular evolution, phylogeny, genomics, and evolutionary biology. Searches were guided by combinations of English descriptors, including: *mitochondrial evolution*, *mitochondrial DNA*, *molecular evolutionary rate*, *mitogenome*, *bats*, *Chiroptera*, *flight metabolism*, *life history*, *longevity*, *phylogenetic comparative methods*, *PGLS*, *mitonuclear conflict*, *long-read sequencing*, *Oxford Nanopore Technologies*, and *PacBio*. The search included both classic studies and recent publications, without restricting the selection to a closed time interval, since the aim was to combine well-established theoretical foundations with contemporary advances related to mitogenomics, phylogeny, and sequencing technologies.

Studies were prioritized when they contributed directly to understanding mitochondrial evolution in animals, mammals, and bats, especially those addressing variation in molecular rates, metabolism, body size, generation time, longevity, molecular selection, bioenergetics, Chiroptera phylogeny, mitonuclear conflicts, and phylogenetic comparative methods. Studies on relevant methodological tools and approaches were also considered, including work on complete mitogenomes, nuclear data, long-read sequencing, and phylogenetically informed analyses.

Studies were included when they addressed at least one of the following aspects: I) variation in mitochondrial evolutionary rates; II) hypotheses related to metabolism, body size, generation time, or longevity; III) molecular evolution, physiology, and genomics of bats; IV) the use of mitogenomes in phylogeny; V) conflicts between mitochondrial and nuclear genomes; VI) phylogenetic comparative methods; and VII) technological advances applied to mitogenome assembly and interpretation. Studies with no direct relationship to mitochondrial evolution, exclusively clinical studies without connection to evolutionary dynamics, non-peer-reviewed publications when equivalent scientific literature was available, and studies whose taxonomic or methodological focus did not contribute to the central question of this article were excluded.

Unlike a systematic review, this study did not aim to quantify the scientific literature on the topic, perform a meta-analysis, or exhaustively enumerate all available publications. Instead, it adopted a critically oriented and theoretically guided selection of the literature, with the purpose of integrating relevant evidence and building a conceptual interpretation of mitochondrial evolution in bats. Thus, the studies were not treated merely as descriptive units, but as sources of arguments, hypotheses, evidence, and limitations to be critically compared.

The selected literature was organized into five main analytical axes. The first axis addressed variation in mitochondrial evolutionary rates and the limits of interpreting mtDNA as a molecular marker, bringing together studies on the molecular clock, substitution rates, mitochondrial mutation, and differences between mitochondrial and nuclear genomes. The second axis examined the relationships among life history, metabolism, body size, generation time, and longevity, considering classical hypotheses proposed to explain differences in molecular rates among species. The third axis focused on bioenergetics and physiological adaptations in bats, especially in relation to powered flight, metabolic demand, torpor, and longevity. The fourth axis addressed mitogenomic evolution, molecular selection, and mitonuclear conflicts, with attention to the limitations of relying exclusively on mitochondrial DNA in evolutionary inference. Finally, the fifth axis discussed phylogenetic and methodological implications for comparative studies, including shared ancestry, phylogenetic correction, and the integration of mitochondrial and nuclear data.

The analysis of the studies was guided by three main criteria: theoretical contribution, applicability to bats, and conceptual or methodological limitations. Theoretical contribution was assessed by considering how each study helps explain variation in mitochondrial evolutionary rates. Applicability to bats was examined according to the relevance of each hypothesis to a group characterized by powered flight, high energetic demand, ecological diversity, and exceptional longevity relative to body size. Conceptual or methodological limitations were analyzed by considering issues such as overgeneralization, exclusive reliance on mtDNA, lack of phylogenetic control, possible conflicts between mitochondrial and nuclear histories, uneven sampling across clades, and the difficulty of distinguishing neutral from adaptive processes.

The synthesis of the literature was conducted in a comparative and interpretative manner. Initially, the main explanatory hypotheses for variation in mitochondrial rates were identified, including metabolism, body size, generation time, longevity, molecular selection, compositional bias, and effective population size. These hypotheses were then confronted with the biological particularities of bats, especially the paradox between the high energetic demand associated with flight and prolonged longevity. Finally, the phylogenetic, methodological, and technological implications of using complete mitogenomes in Chiroptera were analyzed, considering that mitochondrial trees may not fully represent species evolutionary history when introgression, selection, compositional bias, mitonuclear conflict, or limitations related to sampling and genome assembly are involved.

This review also considered methodological studies that are fundamental for interpreting comparative data across species. Because related species share evolutionary history, the literature on phylogenetic comparative methods was used to support the need to interpret relationships between biological traits and molecular rates in light of common ancestry. Accordingly, studies on phylogenetic methods, comparative regression, phylogenetic signal, and the equivalence between independent contrasts and PGLS were incorporated as a conceptual basis for discussing why future analyses of mitochondrial evolution in bats should avoid inferences based on species treated as statistically independent units (Martins; Hansen, 1997; Freckleton; Harvey; Pagel, 2002; Revell, 2010; Blomberg et al., 2012).

As a final step, the evidence analyzed was synthesized into an integrative conceptual model. This model was designed to organize, analytically, the main factors associated with variation in mitochondrial evolutionary rates in bats, considering molecular, physiological, ecological, phylogenetic, and methodological dimensions. Its purpose is not to quantify the relative contribution of each dimension, but to provide an interpretative framework for future studies on mitochondrial evolution in Chiroptera. In this way, the adopted methodology transformed the selected literature into a structured critical analysis, avoiding a sequential description of studies and favoring a synthesis aimed at a multifactorial understanding of mitochondrial evolution in bats.

3. MITOCHONDRIAL DNA BETWEEN EVOLUTIONARY

MARKER AND OBJECT OF EVOLUTION

Mitochondrial DNA occupies a central position in evolutionary studies because it combines features that favor its use as a molecular marker, including compact genomic organization, relative ease of sequencing, broad availability in public databases, and a high substitution rate compared with many nuclear markers. For these reasons, mtDNA has been widely employed in phylogenetic inference, phylogeographic studies, analyses of genetic diversity, and estimates of lineage divergence. However, its widespread use does not eliminate the need to critically evaluate the processes that shape its own evolution, since the mitochondrial genome does not behave as a neutral and uniform marker across all animal groups (Galtier et al., 2009; Allio et al., 2017).

The notion that mitochondrial DNA evolves at a relatively predictable rate has gradually been challenged by studies demonstrating strong heterogeneity among lineages. In mammals, for example, there is evidence of lineage-specific variation in mitochondrial evolutionary rates, indicating that different groups may accumulate substitutions at different rates over time. This pattern makes the simplified application of molecular-clock models problematic, especially when biological differences among organisms are not considered in the interpretation of estimated rates (Gissi; Iannelli; Pesole, 2000; Martin; Palumbi, 1993).

Variation in mitochondrial rates may be associated with multiple factors, including metabolism, body size, generation time, longevity, effective population size, natural selection, molecular repair efficiency, and differences in mutational dynamics between mitochondrial and nuclear genomes. This multiplicity of factors indicates that mtDNA evolution cannot be reduced to a purely stochastic or neutral process. Rather, the mitochondrial genome should be understood as an active component of the evolutionary history of organisms, subject to functional constraints and selective pressures related to energy production and cellular maintenance (Lynch, 2010; Bromham, 2011).

This perspective is particularly important because mtDNA is often used to infer relationships among species, although it may itself be influenced by processes that distort or complicate evolutionary interpretation. Natural selection, compositional bias, introgression, and differences between mitochondrial history and nuclear history may produce patterns that do not directly correspond to the history of species. Thus, the use of mitochondrial DNA in evolutionary studies requires a dual reading: it is both a methodological tool and an object of biological investigation (Galtier et al., 2009; Allio et al., 2017).

In this sense, the main limitation of a simplified interpretation of mtDNA lies in treating it merely as a technical marker, without considering that its evolution may respond to physiological, ecological, and historical characteristics of organisms. In groups with pronounced metabolic particularities, such as bats, this caution becomes even more necessary. Mitochondrial evolution in Chiroptera should therefore be analyzed not only in terms of the observed substitution rate, but also in relation to the biological processes that may explain why certain lineages exhibit acceleration, deceleration, or distinctive patterns of molecular evolution.

4. LIFE HISTORY, METABOLISM, AND LONGEVITY: COMPETING HYPOTHESES

Variation in molecular evolutionary rates has often been associated with life-history traits, especially body size, metabolism, generation time, and longevity. In general, smaller species with faster metabolism and shorter reproductive cycles would be expected to accumulate substitutions at a higher rate than larger, longer-lived species or those with longer generation times. This interpretation has been used to explain differences in molecular rates across animal groups, including mammals, but it should not be applied in a linear or universal manner (Martin; Palumbi, 1993; Bromham, 2011).

The metabolic hypothesis suggests that organisms with higher metabolic rates may produce more reactive oxygen species and, consequently, have a greater potential for DNA damage. Because mitochondria are directly involved in cellular energy production, it would be plausible to expect a relationship between elevated metabolism and increased mitochondrial mutation rates. However, this association is complex, since organisms exposed to high energetic demands may also evolve more efficient mechanisms of protection, molecular repair, and cellular maintenance (Martin; Palumbi, 1993; Lynch, 2010).

The generation-time hypothesis proposes that species with shorter generations accumulate substitutions more rapidly over evolutionary time because they undergo a greater number of reproductive events per unit of time. This explanation may be relevant for understanding differences among lineages, but it has limitations when analyzed in isolation, since generation time, body size, metabolism, and longevity are often correlated (Bromham, 2011).

Longevity adds another layer of complexity to the interpretation of mitochondrial evolution. Longer-lived species may possess more efficient genome-maintenance mechanisms, reducing the accumulation of mutations throughout life. In mammals, the longevity hypothesis has been proposed as a relevant explanation for variation in mitochondrial mutation rates, suggesting that lifespan and the efficiency of cellular repair systems may directly influence mtDNA evolutionary dynamics (Nabholz; Glémin; Galtier, 2008).

These hypotheses, however, do not operate independently. Body size, metabolism, generation time, and longevity interact with one another and may generate convergent or contradictory predictions. In some groups, a trait associated with increased evolutionary rates may be offset by physiological mechanisms that reduce molecular damage. Therefore, interpreting mitochondrial evolution requires understanding life history as an integrated set of attributes rather than as isolated variables (Bromham, 2011; Lynch, 2010).

In bats, this tension is particularly evident. The order Chiroptera includes species that are generally small, have high energetic demands associated with flight, and yet display relatively high longevity in relation to body size. Thus, the group challenges simple explanations: while powered flight suggests increased metabolic pressure on the mitochondrial genome, longevity indicates the presence of mechanisms capable of modulating or reducing molecular damage. Accordingly, bats do not fit well into explanatory models based solely on a direct relationship between elevated metabolism and increased evolutionary rate.

5. BATS AS A PARADOXICAL MODEL OF MITOCHONDRIAL EVOLUTION

Bats represent a particularly relevant group for understanding the relationship between bioenergetics and molecular evolution in mammals. The main evolutionary innovation of the order Chiroptera is powered flight, a trait that imposes intense demands on the respiratory, circulatory, muscular, and energetic systems. Because flight requires high ATP production, mitochondrial function occupies a central position in the physiological performance of these animals (Maina, 2000; Shen et al., 2010).

The origin of flight in bats has been associated with changes in genes related to energy metabolism, indicating that the evolution of this ability involved molecular adaptations linked to the production and efficient use of energy. Because the mitochondrial genome encodes key components of oxidative phosphorylation, changes in these genes may have played an important role in the bioenergetic adaptation of the group. Thus, mitochondrial evolution in bats should be understood within a functional context, in which molecular changes may reflect pressures associated with flight and energetic demand (Shen et al., 2010).

However, bats cannot be interpreted merely as mammals with elevated metabolism. Many species exhibit exceptional longevity compared with other mammals of similar body size, challenging the expectation that small, metabolically active organisms should accumulate molecular damage rapidly and have short lifespans. Genomic studies of *Myotis brandtii*, for example, indicate mechanisms associated with longevity and the distinctive physiology of bats, reinforcing the idea that the group possesses molecular and physiological adaptations capable of modulating the effects of elevated metabolism (Seim et al., 2013).

In addition to longevity, bats exhibit physiological strategies for energy conservation, such as torpor, seasonal variation in metabolism, and episodes of extreme bradycardia in specific ecological contexts. These strategies may reduce energetic expenditure during particular periods and modify the expected relationship between metabolic activity and molecular damage. In frugivorous bats, cyclic bouts of extreme bradycardia have been interpreted as mechanisms capable of counteracting high metabolic costs, showing that bat metabolism is dynamic and physiologically regulated (O'Mara; Wikelski; Dechmann, 2017).

Taken together, these characteristics make bats a paradoxical model of mitochondrial evolution. On the one hand, powered flight implies intense energetic demand and could favor changes in the mitochondrial genome. On the other hand, longevity, torpor, and energy-saving mechanisms suggest the existence of adaptations that reduce or modulate the molecular costs of metabolism. Thus, mitochondrial evolution in Chiroptera should be interpreted as the result of a balance among energetic demand, physiological regulation, and cellular maintenance.

The ecological diversity of bats further increases this complexity. The order includes insectivorous, frugivorous, nectarivorous, hematophagous, carnivorous, and omnivorous species, as well as lineages that occupy different environments, altitudes, and climatic regimes. This ecological diversity may impose distinct selective pressures on energy metabolism and, consequently, on mitochondrial genes. Therefore, variation in mitochondrial evolutionary rates in bats may reflect not only flight as a general trait of the group, but also specific adaptations associated with diet, environment, and the evolutionary history of different lineages.

6. MITOCHONDRIAL SELECTION, BIOENERGETICS, AND ADAPTATION

Interpreting mitochondrial evolution requires distinguishing neutral processes from adaptive processes. Although mtDNA has often been used as a relatively neutral molecular marker, accumulated evidence indicates that mitochondrial genes may be subject to natural selection. This possibility is especially relevant because the mitochondrial genome encodes proteins directly involved in oxidative phosphorylation, a central process for ATP production. Thus, changes in mitochondrial genes may have important functional consequences for energy metabolism (Fonseca et al., 2008; Gershoni; Templeton; Mishmar, 2009).

Mitochondrial bioenergetics may act as an important force in evolutionary processes, since differences in energy-production efficiency can affect performance, survival, reproduction, and adaptation to specific environments. In organisms subjected to intense energetic demands, such as bats, changes in mitochondrial function may have important evolutionary implications. For this reason, variation in mitochondrial rates should not be interpreted automatically as simple differences in mutation rate, since it may also reflect positive selection, functional constraints, or changes associated with energetic adaptation (Gershoni; Templeton; Mishmar, 2009; Shen et al., 2010).

In bats, selection on genes related to energy metabolism is particularly relevant because of the origin of powered flight. The ability to fly requires high energetic performance, and studies indicate that metabolism-related genes may have undergone adaptive evolution during the origin and diversification of the group. This suggests that mitochondria may have played an important role in the evolutionary transition that allowed bats to occupy the aerial niche among mammals (Shen et al., 2010).

The analysis of specific lineages reinforces this interpretation. In vampire bats, patterns of high mitochondrial evolutionary rate associated with compositional bias and positive selection have been identified, indicating that mitogenomic acceleration may result from the interaction between molecular processes and ecological specializations. This case is particularly important because it demonstrates that mitochondrial evolution in bats can vary strongly among lineages and that extreme patterns should not be generalized to the entire order without critical analysis (Botero-Castro et al., 2018).

Recent comparative genomic studies also indicate that bats show relevant signals of molecular adaptation, complex demography, and modifications associated with their evolutionary history. These findings reinforce the view that the evolution of the group involves multiple processes, including adaptations related to metabolism, longevity, immunity, and ecology. In the context of mitochondrial evolution, this strengthens the hypothesis that rate heterogeneity does not result only from neutral fluctuations, but also from selective pressures and functional constraints associated with the bat way of life (Liu et al., 2024).

Therefore, mitochondrial evolution in Chiroptera should be analyzed as a phenomenon in which mutation, selection, energetic function, and ecology interact. Acceleration in a given lineage may reflect an increase in mutation rate, but it may also indicate adaptive selection, compositional bias, or effects related to demographic history. Robust interpretation therefore depends on avoiding single-factor explanations and recognizing that mtDNA simultaneously carries signals of history, function, and method.

7. PHYLOGENY, COMPLETE MITOGENOMES, AND MITONUCLEAR CONFLICTS

The use of complete mitogenomes has expanded the ability to investigate phylogenetic relationships in bats. Compared with isolated mitochondrial markers, complete mitochondrial genomes provide a larger number of characters and may improve the resolution of evolutionary relationships, especially in taxonomically complex groups. Studies of Vespertilionidae and Phyllostomidae show that mitogenomic data can contribute to clarifying internal relationships and revising classificatory hypotheses within Chiroptera (Yu et al., 2016; Camacho et al., 2022).

Despite these advantages, complete mitogenomes still represent a single hereditary history: the history of the mitochondrial genome. This means that increasing the number of mitochondrial genes does not amount to incorporating independent genomic sources of information. Because mitochondrial genes are inherited as a linked unit, they may produce statistically well-supported trees that nevertheless remain discordant with nuclear history or species history. Therefore, the statistical robustness of a mitochondrial tree does not, by itself, ensure that it fully represents the evolution of the group under analysis.

This issue is evident in studies identifying conflicts between mitochondrial and nuclear genomes. In New World bats of the genus *Myotis*, conflicting evolutionary histories have been observed between the two genomes, indicating that inferences based only on mtDNA may lead to incomplete or biased interpretations. Such conflicts may result from mitochondrial introgression, incomplete lineage sorting, selection, or differences in population dynamics among markers. This type of evidence reinforces the need for caution when relying exclusively on mitochondrial data in groups with complex diversification histories (Platt et al., 2018).

Recent advances in long-read sequencing technologies have contributed to reducing some historical limitations of mitogenomic analysis. Long-read approaches, such as nanopore-based sequencing, facilitate the recovery of complete mitogenomes, support the identification of mitochondrial variants, and may expand the capacity to analyze features such as heteroplasmy and structural variants. Nevertheless, these technologies do not eliminate the need to integrate nuclear data and adopt careful phylogenetic interpretation, especially when the aim is to reconstruct the evolutionary history of species rather than only the history of the mitochondrial genome (Dhorne-Pollet; Barrey; Pollet, 2020). The cited study presents an approach for sequencing complete animal mitochondrial genomes using nanopore long-read sequencing and applying it to the identification of variants in mitochondrial DNA.

The order Chiroptera has an evolutionary history marked by great diversity, broad geographic distribution, and extensive radiations. Studies on the speciation dynamics of bats show that diversification within the group occurred in a complex manner, with differences among lineages and clades. In this context, inferences based on a single genomic source may capture part of the evolutionary history, but not necessarily all dimensions of the group's diversification (Shi; Rabosky, 2015).

Broad mammalian phylogenies also show that the evolutionary position of bats and their diversification should be interpreted within a wider macroevolutionary framework. Deep historical events, such as diversifications associated with environmental changes and extinctions, may influence the structure of extant lineages. Thus, mitochondrial evolution in Chiroptera must be situated both within the internal context of the order and within the broader context of placental mammal evolution (Meredith et al., 2011; Upham; Esselstyn; Jetz, 2019).

Thus, complete mitogenomes are valuable tools, but they are not sufficient to resolve all phylogenetic and evolutionary problems in bats on their own. Their use must be accompanied by critical interpretation of mitonuclear conflicts, taxonomic sampling, tree choice, temporal calibration, nucleotide composition, selection effects, and the technologies used in genome assembly. In a critical review of mitochondrial evolution, this point is essential: mtDNA provides relevant evolutionary information, but this information must be interpreted within the biological, technological, and methodological limits of the marker.

8. METHODOLOGICAL IMPLICATIONS FOR COMPARATIVE STUDIES IN BATS

The investigation of mitochondrial evolution in bats requires attention to the methodological implications of comparative studies. When relationships between biological traits and evolutionary rates are analyzed, species cannot be treated as statistically independent observations, because they share ancestry. Closely related species tend to be similar not only due to current ecological pressures, but also because of common inheritance. Ignoring this dependence may lead to the identification of spurious associations between biological variables and molecular patterns (Martins; Hansen, 1997; Freckleton; Harvey; Pagel, 2002).

Phylogenetic comparative methods were developed precisely to address this problem. They allow researchers to incorporate the structure of the evolutionary tree into analyses, distinguishing patterns associated with shared history from those related to ecological or physiological variables. In studies of mitochondrial rates, this is particularly important because specific clades may exhibit high or low rates for historical reasons, rather than necessarily because of an ecological trait measured in the present (Freckleton; Harvey; Pagel, 2002; Revell, 2010).

The presence of phylogenetic signal in comparative data indicates that related species tend to show more similar values for a given trait. In the case of mitochondrial evolutionary rates, evaluating phylogenetic structure is essential to determine whether observed variation is randomly distributed among species or concentrated in particular clades. This distinction is crucial for avoiding simplistic interpretations, especially in groups such as bats, in which some families or genera may be more represented in available molecular databases (Revell, 2010).

The conceptual equivalence between phylogenetically independent contrasts and phylogenetic regression models, such as PGLS, further reinforces the importance of incorporating shared ancestry into analyses. Although the present article does not conduct original empirical analyses, the methodological literature indicates that future studies on mitochondrial evolution in Chiroptera should use phylogenetically informed approaches when testing associations among molecular rate, body mass, longevity, ecology, altitude, geographic range, or diet (Blomberg et al., 2012).

In addition to phylogenetic correction, other methodological precautions are necessary. Tree choice, temporal calibration, selection of mitochondrial genes, inclusion or exclusion of particular clades, sequence quality, and taxonomic representativeness may strongly influence conclusions. In bats, this issue is particularly relevant because some groups, such as *Myotis*, Phyllostomidae, and Vespertilionidae, tend to be more represented in molecular databases than others. This sampling asymmetry may affect perceptions of general patterns in mitochondrial evolution.

A methodologically robust approach to mitochondrial evolution in bats should integrate four levels of caution: molecular caution, by recognizing that mtDNA may be subject to selection and compositional bias; phylogenetic caution, by considering shared ancestry and mitonuclear conflict; statistical caution, by avoiding the treatment of species as independent data points; and biological caution, by interpreting molecular rates in light of the physiology, ecology, and life history of the group. This integration allows the available literature to be transformed into critical analysis, avoiding a fragmented description of isolated results.

9. INTEGRATIVE MODEL FOR MITOCHONDRIAL EVOLUTION IN BATS

The synthesis of the literature indicates that mitochondrial evolution in bats should be understood through a multifactorial model. Explanations based exclusively on metabolism, body size, generation time, or longevity are insufficient to capture the complexity of the group. Bats combine powered flight, high energetic demand, extended longevity, ecological diversity, physiological strategies for energy conservation, and a complex phylogenetic history. Therefore, variation in mitochondrial rates in Chiroptera should be interpreted as the product of interactions among molecular, physiological, ecological, phylogenetic, and methodological dimensions.

Based on this synthesis, an integrative conceptual model is proposed to guide the interpretation of mitochondrial evolution in bats. The model organizes variation in mitochondrial evolutionary rates into five interdependent dimensions: molecular, physiological, ecological, phylogenetic, and methodological. These dimensions do not act in isolation, but overlap in producing the patterns observed across different lineages of Chiroptera. Table 1 summarizes the main components of each dimension and their contribution to the interpretation of mitochondrial evolution in the group.

Table 1 – Integrative conceptual model of mitochondrial evolution in bats

Dimension	Main components	Role in the interpretation of mitochondrial evolution in bats
Molecular	Mutation rate; natural selection; compositional bias; molecular repair; oxidative phosphorylation	Indicates that mtDNA should not be treated merely as a neutral marker, as it may reflect mutational, selective, and functional processes linked to energy production.
Physiological	Powered flight; metabolism; torpor; bradycardia; longevity; energetic demand	Explains the bat paradox: high energetic demand associated with flight, combined with physiological mechanisms that may modulate molecular damage.
Ecological	Diet; altitude; climate; geographic distribution; habitat use	Shows that different lifestyles may impose distinct selective pressures on energy metabolism and mitochondrial genes.
Phylogenetic	Shared ancestry; evolutionary radiations; differences among clades; mitonuclear conflict	Indicates that mitochondrial rate patterns may reflect shared evolutionary history, rather than only independent responses to ecological or physiological factors.
Methodological	Use of mtDNA and nuclear DNA; tree choice; temporal calibration; sequencing; comparative methods	Reinforces that part of the observed patterns may depend on methodological decisions, sampling, genome assembly, and the inference models used.

Source: *Prepared by the authors based on the critical synthesis of the literature.*
Note: *The model interprets variation in mitochondrial evolutionary rates in Chiroptera as the result of interactions among molecular, physiological, ecological, phylogenetic, and methodological dimensions.*

The first dimension of the model is molecular. It includes mutation rate, natural selection, compositional bias, molecular repair efficiency, and the function of genes involved in oxidative phosphorylation. This dimension recognizes that the mitochondrial genome is not merely a neutral marker, but a functional system directly linked to energy production. Thus, changes in mitochondrial genes may reflect both mutational processes and selective pressures associated with bioenergetic performance (Fonseca et al., 2008; Gershoni; Templeton; Mishmar, 2009; Lynch, 2010).

The second dimension is physiological. It involves flight, metabolism, torpor, bradycardia, longevity, and energetic demand. In bats, this dimension is central because powered flight requires high energy production, but this demand is modulated by physiological strategies that may reduce metabolic costs and cellular damage. The extended longevity observed in many species suggests that molecular maintenance mechanisms may play an important role in the evolution of the group, making it inappropriate to assume that higher metabolism necessarily results in higher evolutionary rates (Maina, 2000; Seim et al., 2013; O'Mara; Wikelski; Dechmann, 2017).

The third dimension is ecological. Dietary diversity, geographic range, habitat use, altitudinal variation, and climatic conditions may influence energetic demand and molecular adaptation across different lineages. Hematophagous, frugivorous, insectivorous, nectarivorous, and carnivorous bats face distinct ecological challenges, which may be reflected in different selective pressures on genes related to metabolism. Thus, ecology should not be treated as a peripheral element, but as part of the conditions that shape mitochondrial evolution (Botero-Castro et al., 2018; Liu et al., 2024).

The fourth dimension is phylogenetic. Species share ancestry, and this common history structures the distribution of biological and molecular traits. Certain families or lineages may show similar patterns of mitochondrial rate variation not because they independently respond to the same ecological factor, but because they inherited traits from common ancestors. For this reason, phylogeny should be incorporated both into theoretical interpretation and into future empirical analyses of mitochondrial evolution in bats (Martins; Hansen, 1997; Freckleton; Harvey; Pagel, 2002; Revell, 2010).

The fifth dimension is methodological. It involves the type of marker used, the exclusive or combined use of mtDNA and nuclear DNA, phylogenetic tree choice, temporal calibration, rate-estimation methods, sequencing technologies, and statistical models. This dimension recognizes that part of the observed patterns may arise not only from bat biology, but also from methodological decisions made by researchers. Therefore, any synthesis of mitochondrial evolution must distinguish between genuine biological signals and potential artifacts produced by marker choice, sampling, genome assembly, or analytical method.

The proposed integrative model is based on the premise that none of these dimensions acts in isolation. The mitochondrial evolutionary rate observed in a bat lineage may result simultaneously from selective pressures on energy-related genes, physiological adaptations to flight, longevity strategies, lineage-specific ecological traits, shared phylogenetic history, and methodological limitations of mtDNA-based inference. This approach makes it possible to move beyond simplistic explanations and build a more robust interpretation of mitochondrial evolution in Chiroptera.

The main contribution of this critical review is to propose that mitochondrial evolution in bats should be understood as a phenomenon shaped by the interaction among function, history, and method. mtDNA should not be viewed merely as a sequence used to reconstruct trees, but as a functionally relevant, historically inherited, and methodologically complex genome. In bats, this complexity is amplified by the unique combination of flight, longevity, ecological diversity, and evolutionary radiation. Therefore, understanding mitochondrial rates in this group requires an integrative approach capable of articulating molecular, physiological, ecological, phylogenetic, technological, and methodological evidence.

10. CONCLUSIONS

The central question of this study was to understand how bioenergetics, life history, molecular selection, phylogeny, and the methodological limitations of mitochondrial DNA help explain variation in mitochondrial evolutionary rates in bats. The analysis indicates that this variation cannot be attributed to a single factor, such as elevated metabolism, body size, or generation time, but rather to the interaction among different biological and methodological dimensions.

Bats stand out as an evolutionary model because they combine powered flight, high energetic demand, ecological diversity, and extended longevity relative to body size. This combination makes a linear interpretation of mitochondrial evolution inadequate, since increased metabolic demand may be modulated by physiological mechanisms of energy conservation, cellular maintenance, and metabolic regulation.

The review also showed that mtDNA evolution in Chiroptera involves both neutral and adaptive processes. Molecular selection, compositional bias, ecological specializations, and conflicts between mitochondrial and nuclear genomes may influence the observed patterns, requiring caution in the use of mitochondrial DNA as an evolutionary marker.

Thus, the objectives of this study were achieved, since the literature was critically analyzed and integrated into a multifactorial conceptual model. This model allows mitochondrial evolution in bats to be interpreted as the result of interactions among molecular, physiological, ecological, phylogenetic, and methodological dimensions, providing a more robust theoretical basis for future studies on evolutionary rates in Chiroptera.

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