Chapter 5

Genetics tools for the conservation of bats

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Abstract

Genetic tools are essential for uncovering several ecological aspects of biodiversity, particularly for groups as diverse as bats in terms of species and behaviors. Bats provide vital ecosystem services, including habitat restoration, seed dispersal, pollination, and pest control. In this chapter, we conducted a systematic review of studies that utilized genetic data to improve the understanding of bats, with a focus on the Neotropics, which host the greatest variety and concentration of bats globally. We highlighted the most significant patterns found in the studies, and discussed their contributions for bat conservation. Five families of bats (Vespertilionidae, Phyllostomidae, Rhinolophidae, Pteropodidae, and Molossidae) were the focus of 95% of the studies. The most prevalent

use of genetic data was for higher-level classification and taxonomy support. Climate fluctuations and the formation of geographical barriers during the late Pleistocene had a significant impact on the distribution of bats in Middle America. Although bats are relatively more mobile than other animal groups, geographical barriers and human-driven landscape alterations can still impact their genetic diversity to varying degrees. Genetic tools have contributed to bat management and conservation since before the 2000s, and recent advances in technologies - such as DNA metabarcoding and genome sequencing - have shown enormous potential for answering further questions.

Keywords: Chiroptera, literature review, molecular markers

5.1 Introduction

The order Chiroptera is the second most diverse group of mammals, representing 22% of all living mammals and displaying great physiological and ecological diversity (Hutson et al. 2001; MMD 2022). Bats can be found in every region of the world, except the Arctic and Antarctic, and certain isolated oceanic islands (Simmons 2005a, b; Fenton and Simmons 2015). With an evolutionary history of over 52 million years, bats have diversified into almost 1,450 species (Simmons et al. 2008; Kunz et al. 2011; MMD 2022). Their weight ranges from as little as 2g, in the case of the bumblebee bat (*Craseonycteris thonglongyai*), to over 1 kg in the golden-capped fruit bat (*Acerodon jubatus*) (Safi et al. 2013; Fenton and Simmons 2015). Some species of bats may form one of the largest aggregations of nonhuman mammals when measured in terms of individual numbers (Moratelli and Calisher 2015). They can be classified into several feeding strategies, including aerial insectivores (insects in flight), sanguivores (blood), nectarivores (flowers, nectar, and pollen), frugivores (fruits), omnivores (arthropods, flowers, fruits, and vertebrates), and animalivores (arthropods and small vertebrates) (Fauth 1996; Meyer and Kalko 2008; Kunz et al. 2011).

Frugivorous bats play a vital role in habitat revegetation because they carry the seeds of various plant species away from the mother plants, increasing the likelihood that these seeds will germinate and establish themselves in a new area (Lobova et al. 2009). In the Neotropics, neotropical fruit bats (Phyllostomidae) alone disperse seeds from at least 549 plant species belonging to 191 genera and 62 plant families (Ramirez-Francel

et al. 2022). Similarly, old-world bats disperse seeds of around 300 plant species, which produce at least 448 economically valuable products (Fujita and Tuttle 1991). Despite the significance of bats in seed dispersals, the impacts of land use changes on bats and seed dispersal services are still not fully understood (Regolin et al. 2020).

Pollination is another essential ecosystem service provided by bats, which therefore play vital roles in plant ecology and food production (Kunz et al. 2011). The neotropical subfamilies Glossophaginae and Lonchophyllinae are specialized nectar eaters (Tschapka et al. 2015). These bats are known to travel up to 3.8 km each night searching for flowers, combining short-range flights of up to 500 m with longer flights of 2 to 3 km that take them away from their core areas (Aguiar et al. 2014). In the Neotropics, bats pollinate approximately 549 plant species in 191 genera and 62 families, while old-world Pteropodid bats pollinate around 168 plant species in 100 genera and 41 families (Kunz et al. 2011). Bat-pollinated plants are a significant source of biomass in their habitats; columnar cacti, for example, are the dominant vegetation in neotropical xeric and semi-arid habitats (Zamora-Gutierrez et al. 2021). In Brazil, *Glossophaga soricina* and *Lonchophylla sp*. pollinate *Pilosocereus tuberculatus*, a tree-like cactus with a relict distribution restricted to a few localities in the xeric Caatinga, in the states of Pernambuco, Bahia, Sergipe and Piauí (Rocha et al. 2007). The flower-visiting niche of bats in the Neotropics is defined by a combination of shape, time, and space (Diniz and Aguiar 2022). Bat-pollinated plants are more dependent on their respective vertebrate pollinators than bird-pollinated plants; thus, the significance of bats as pollinators must be addressed, also due to the economic value of certain bat-pollinated crops (Ratto et al. 2018).

Bats have a diverse range of roosting sites, including foliage, caves, rock crevices, hollows in trees under peeling bark, and various man-made structures (Jones et al. 2009a; Garbino and Tavares 2018). In the Neotropics, guano produced by fruit-eating, hematophagous, and insect-eating bats is a key resource for cave-dwelling arthropods. Detritivores form the foundation of the food web in bat guano caves (Ferreira and Martins 1999). This diversity of guano types makes the food webs in neotropical caves more species-rich than those found elsewhere (Ferreira and Martins 1999; Fontanetti et al. 2002). In Thailand and Cambodia, guano is collected from bat shelters and used as a fertilizer for plants (Kunz et al. 2011), contributing to the local economy (Leelapaibul et al. 2005; Chhay 2012). In the early 2000s, guano extracted from Bracken Cave in Texas was sold for a price of $2.86 to $12.10 per kg (Tuttle and Moreno 2005).

In addition to the guano in caves, natural and urban shelters are crucial for preserving insectivorous species that provide insect pest control services. In the U.S., the species *Tadarida brasiliensis* saves US$741,000 annually by avoiding insecticide costs and preventing loss of cotton production (Cleveland et al. 2006). In Thailand, *Mops plicatus* prevents the loss of tons of rice due to arthropod infestations that damage crops (Wanger et al. 2014). Even artificial shelters in urban areas that house insectivorous bats contribute to the provision of pest control services. In Brazil, bats save US$94 per hectare of corn plantation, resulting in annual savings of US$390.6 million per harvest (Aguiar et al. 2021).

However, bats are also targeted by farmers because of their reputation as vectors of rabies. Current management practices aimed at controlling vampire bats have a devastating impact on bat populations, including both vampire bats and other species that coexist with vampire bat colonies, by destroying important roosts and indirectly affecting populations (Aguiar et al. 2010). The neotropical region has been greatly impacted by human-caused changes in land use and land cover, with some of the highest deforestation rates in the world. Brasileiro et al. (2022) recently found that, except for nectarivores, larger bats are generally more impacted by habitat loss and are disappearing faster than smaller bats, following a pattern of downsizing and its functional consequences that has been documented for other taxonomical groups (Dirzo et al. 2014; Galetti et al. 2015; Donoso et al. 2020). Furthermore, the region's fear, lack of awareness, and negative perception of bats continue to threaten bat populations (Rocha et al. 2020).

Due to the great diversity of species and habits, including the fact that bats are primarily nocturnal and therefore difficult to observe directly (Kalko et al. 1996), many questions about their taxonomy, genetics, evolution, ecology, and behavior remain unanswered. This is especially true in the Neotropics, where there is a high diversity of bats and their ecosystemic services, but also numerous gaps still exist in our understanding of their ecology, behavior, acoustics, and conservation status (Bernard et al. 2012). Molecular tools can be crucial in helping to answer questions about historical (e.g., range expansion, colonization events), contemporary (e.g., gene flow), and ecological processes by providing important genetic data (Dool 2020).

In this chapter, we reviewed the studies that have utilized genetic data to enhance our understanding of the Chiroptera order, with a particular focus on the Neotropics,

which harbor the greatest diversity and abundance of bats in the world (Burgin et al. 2018). Additionally, we discussed the significance of these data for the conservation of these iconic animals.

5.2 Literature review of genetic techniques applied in bat conservation

We conducted a literature survey of papers that used genetic tools to study the Chiroptera group in the following databases: Web of Science (http://www.webofscience.com) and Scopus (https://www.scopus.com). The survey was limited to English-written, peer-reviewed journal articles available until October 2020, and used search terms related to higher-level traditional bat taxa, including: Chiroptera* OR Megachiroptera OR Microchiroptera OR Craseonycteridae OR Emballonuridae OR Furipteridae OR Hipposideridae OR Megadermatidae OR Miniopteridae OR Molossidae OR Mormoopidae OR Mystacinidae OR Myzopodidae OR Natalidae OR Noctilionidae OR Nycteridae OR Phyllostomidae OR Pteropodidae OR Rhinolophidae OR Rhinopomatidae OR Thyropteridae OR Vespertilionidae AND genetic*. A total of 2028 papers were retrieved and screened, and papers that either did not focus on conservation issues in Chiroptera or were not bat-focused (e.g., papers focusing on diseases and health) were removed. After this screening process, 473 articles remained and were processed, selected, and screened using the software StArt (State of the Art through Systematic Review) v. 2.3.4 Beta (Zamboni et al. 2010), following the methodology of Berger-Tal et al. (2019). Information was manually extracted from each article, including: focal family and species; molecular markers used (e.g., mtDNA, microsatellites, SNPs, complete genome, mtDNA and nuDNA); biogeographic region according to the Slater-Wallace classification, as well as countries where the samples were collected; origin of animal samples (wild, captive, museum, online database); number of samples, capture method, and main conclusions observed.

The papers were categorized into 13 different topics based on their keywords and objectives. If the objectives were unclear, the paper was assigned to the topic that best reflected its analysis and conclusions. Papers under the category "Systematic Questions" aimed to assess taxonomy and species delimitation. The category "Effects of Fragmentation on Genetic Diversity" included works that analyzed fragmented landscapes and the resulting effects on genetic diversity distribution and population structure. "Impacts of Barriers on Gene Flow" covered studies that evaluated the impact

of specific barriers, such as the open sea, on gene flow between populations. "Bat Adaptations" referred to works that investigated the genetic adaptations of the group. "Impacts of Geographic Distance and/or Topology on Populations" encompassed studies that assessed, in general, how species populations distributed nearby are genetically affected by distance and topology. "Genetic Divergence among Populations" compared genetic diversity between two or more populations under similar ecological conditions. "Understanding Demographic History" included studies that aimed to comprehend the historical processes responsible for the geographical distribution of individuals. "Individual Identification" included papers whose goal was to identify the species of a sample under different conditions or in a new location, and methodological studies that described new molecular markers for a specific species (e.g., developing microsatellites). "Influence of Social Characteristics on Genetic Diversity Distribution" covered studies that evaluated the impact of mating systems or other social behaviors on genetic diversity. "Local Population Structure" referred to papers that evaluated or described the genetic diversity of a local population. "Genome Description" comprised the publication of complete mitogenomes and nuclear genomes. "Phylogeographic Hypotheses" included studies that tested pre-defined phylogeographic hypotheses. "Unclear" was a category for papers that did not fit into any of the above topics.

5.3 Issues and timeline overview

Overall, the main targets of studies within the Chiroptera group are systematics and understanding demographic history (Fig. 5.1), and there have been several advances in the methodologies applied to uncover phylogenetic relationships, detect significant evolutionary units, delimit species and their geographic ranges, and define taxonomic status and classification. These advances aid in the understanding of the diversity, evolution, and biogeography of the group, which are essential for their conservation. Out of the reviewed papers, only three were published in the 1980s, and they utilized chromosomal data and electrophoresis to infer genetic differentiation between species. For example, at the end of the decade, Bennett et al. (1988) used mtDNA markers to resolve an important phylogenetic question – that megabats (Pteropodidae family) are indeed bats, not flying primates, as previously suggested by Pettigrew (1986).

In the next decade (1990-1999), the number of papers published almost quintupled, totaling 14. The topics of systematics and demographic history accounted for

64% of the published studies, and the main methods used included mtDNA, microsatellites, and cytogenetics. Interestingly, the first paper using genomic data, specifically cosmid libraries, was produced during this decade, aiming to infer taxonomic levels from the new-world leaf-nosed bat, *Macrotus waterhousii* (Baker et al. 1997).

In the first decade of the 21st century (2000-2009), there was a significant increase in the number of publications about the Chiroptera group, with a total of 142 papers appearing in our survey. Systematics and demographic history accounted for 71.8% of the published studies, while 22 papers dealt with population genetics, two with landscape genetics, and two with genomic studies. The increase can be attributed to the advancements in genetic technologies, which made it possible to employ mtDNA studied either through direct sequencing or electrophoresis fragment analysis, as well as microsatellites, for population genetic studies. Concerning the most recent decade (2010-2020), we found 317 papers within our scope, repeating the trend of increased topics and publications observed in the previous decade. Of these papers, 68.1% (215) were about systematics and demographic history. There was also an increase in studies involving genomic data or complete genome descriptions, which likely reflects advances in molecular technologies, the development of several statistical approaches, and increased computing power (Beaumont and Rannala 2004; Pearse and Crandall 2004).

Neotropical studies followed the global trend, with few publications until the early 2000s, subsequently increasing in number and methods to support the resolution of various questions, particularly in terms of phylogenetic relationships. In the most recent year reviewed, the description of a new species, *Lasiurus arequipae*, endemic to Peru, was published (Málaga et al. 2020). Additionally, four systematic and taxonomy studies at the species level, using both mtDNA and nuDNA, were conducted for *Platyrrhinus chocoensis* (Palacios-Mosquera et al. 2020), *Tonatia saurophila* (Basantes et al. 2020), *Anoura caudifer*, and *Anoura geoffroyi* (Vargas-Arboleda et al. 2020). Also, next-generation sequencing was combined with morphological data to enhance knowledge of the phylogeny of the genus *Molossus* in the Neotropics (Loureiro et al. 2020).
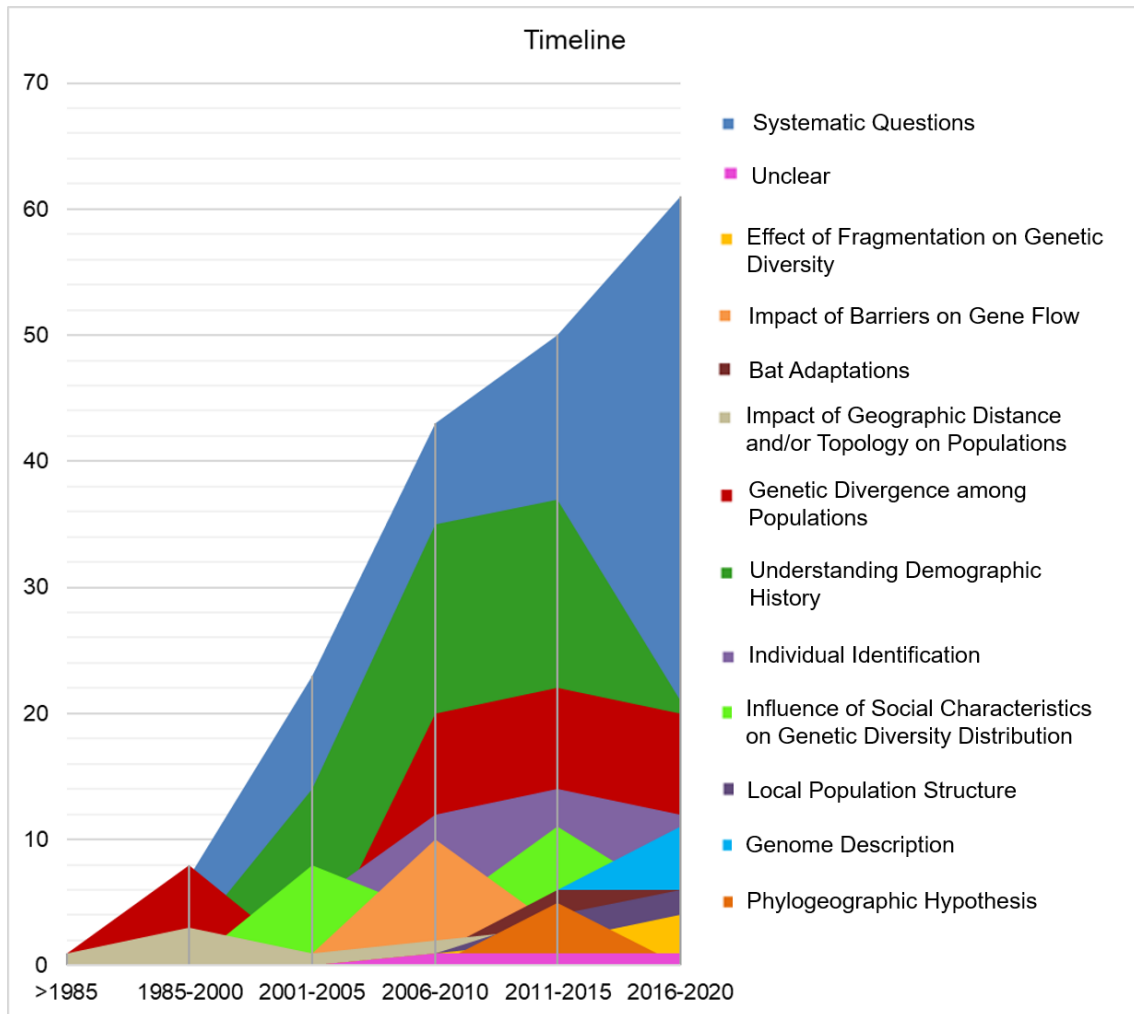
Figure 5.1 Timeline of studied topics based on a literature survey from 1982 to 2020.

Overall, we reviewed 473 articles on both the Yinpterochiroptera and Yangochiroptera suborders, from 1982 to October 2020. The studies were dominated (95%) by the five largest bat families, as reported by MMD (2022), with the Vespertilionidae (199) being the most studied, followed by Phyllostomidae (77), Rhinolophidae (66), Pteropodidae (62), and Molossidae (44). Most of the studies obtained samples from wild animals (345), followed by those using museum specimens (125) and sequences from public databases (61), with only a few using samples from captive animals (3). DNA was extracted from wing membranes or non-specified muscle/tissues.

The articles reviewed (as seen in Figure 5.2) covered a range of subjects, and the impact of these studies on the field of neotropical bats will be emphasized in the following sections.
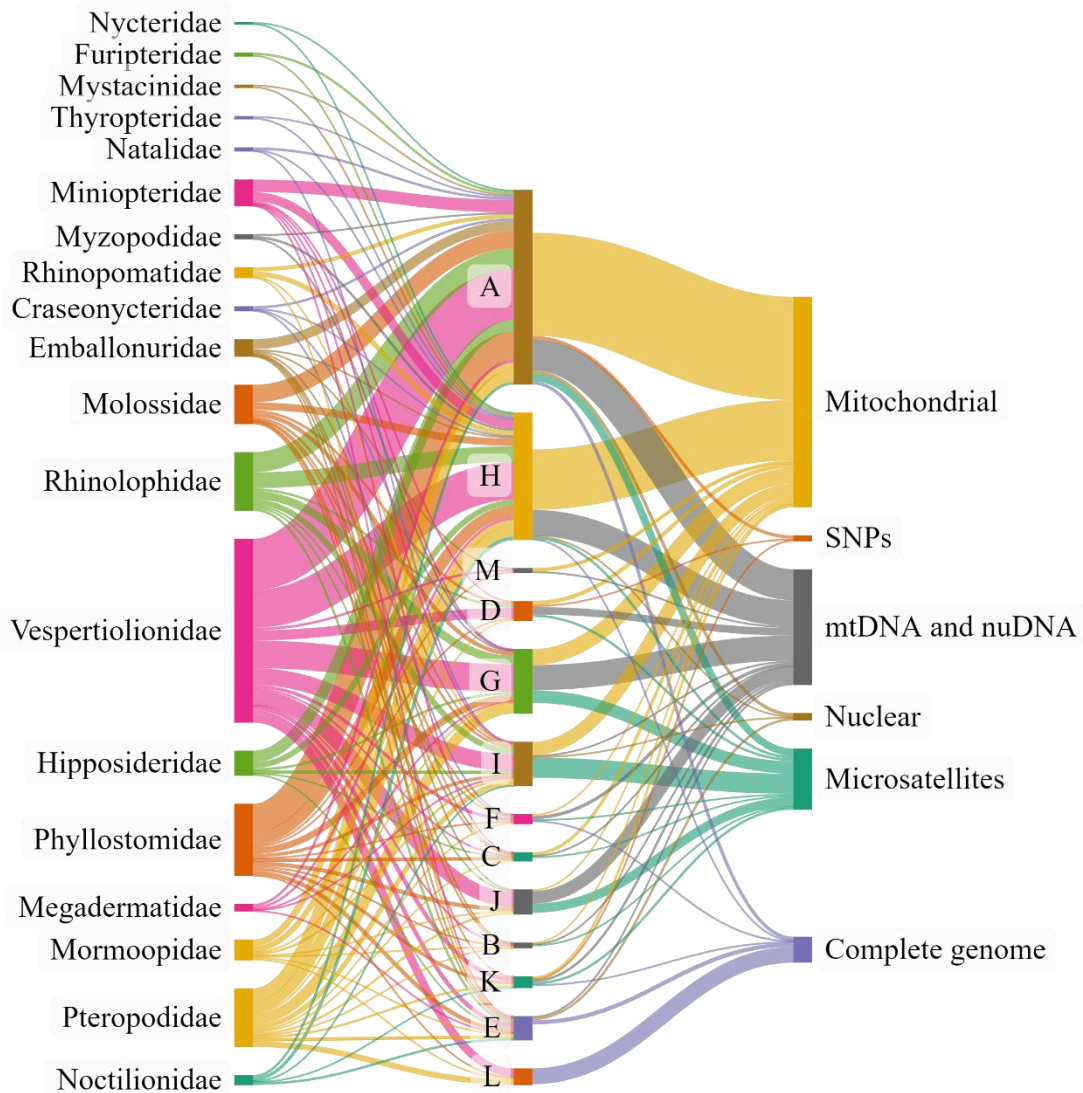
Figure 5.2 Flow of information from bat families, through the key research themes identified, to the molecular markers used in 473 studies on Chiroptera from 1982 to October 2020. The size of the bars and the thickness of the lines are proportional to the number of interactions for each theme. The themes include: [A] Systematic Questions; [B] Unclear themes; [C] Effects of Fragmentation on Genetic Diversity; [D] Impacts of Barriers on Gene Flow; [E] Bat Adaptations; [F] Impacts of Geographic Distance and/or Topology on Populations; [G] Genetic Divergence among Populations; [H] Understanding Demographic History; [I] Individual Identification; [J] Influence of Social Characteristics on Genetic Diversity Distribution; [K] Local Population Structure; [L] Genome Description; [M] Phylogeographic Hypotheses.

5.4 Molecular identification and taxonomy

Speciation events in bats have been documented to occur without significant morphological changes, leading to the identification of cryptic species across the entire order (Baker 1984; Barrat et al. 1995; Hoffmann and Baker 2003; Hulva et al. 2004). To address this challenge, the use of modern molecular techniques in conjunction with ecological and morphological data provides valuable information for species delimitation. The most frequently studied topic within the group of reviewed works was the use of genetic data to support taxonomy (n=230, Figure 5.2), with the aim of clarifying systematics and identifying cryptic species and new taxa. The most widely used methods for this purpose employed mitochondrial markers or a combination of mitochondrial and nuclear markers (including microsatellites). The advent of next-generation sequencing (NGS) also opened up new opportunities to improve species delimitation and evolutionary inferences (Pinto et al. 2019).

Thanks to NGS, the number of genetic markers available (e.g., Single Nucleotide Polymorphisms, or SNPs) increased, offering a greater amount of genetic information and enhancing phylogenetic resolution (Wagner et al. 2013). In Northeastern India, SNP data uncovered distinct geographic lineages within *Cynopterus sphinx*, as well as a previously undiscovered, cryptic cynopterine lineage that coexists with *C. sphinx* (Chattopadhyay et al. 2016).

In the Neotropics, studies aimed at supporting taxonomy accounted for 51.4% (56 studies), reflecting the ongoing challenge of taxonomy in this region due to its large geographical extent and high diversity of species, including areas with over 100 sympatric species (Ingala et al. 2021). Loureiro et al. (2020) utilized SNPs to address a taxonomic challenge in the *Molossus* genus (belonging to the Molossidae family), which is diverse and widely distributed in the Neotropics. With this approach, the number of species increased from 11 (Loureiro et al. 2019) to 14, with the discovery of two cryptic species within *M. rufus*, leading to the division of the group into two species (*M. currentium* and *M. bondae*).

Only five of the reviewed papers focused on species identification using guano as a source of genetic material, a non-invasive sample that does not require animal handling or sacrifice. These papers often resulted in the identification of new records, which

typically expanded the known distributional range of species. Two mitochondrial DNA (mtDNA) genes, Cytochrome b (Cyt-b) and Cytochrome c Oxidase subunit 1 (COI), have been commonly utilized for bat species identification.

5.5 Demographic history, life history and genetic diversity

Contemporary biodiversity patterns are the outcome of a prolonged and intricate evolutionary history, influenced by ecological processes and shaped by external environmental forces such as climatic changes, mountain uplifts, and sea level fluctuations (Rull 2011). To gain a deeper understanding of the evolution of species, it is important to examine the biogeographical regions and the historical processes that have acted within each region.

The late Pleistocene period was crucial for the population dynamics of vertebrates in Middle America, affected by both climate oscillations and the formation of geographical barriers. These processes led to population expansion in reptiles (Castoe et al. 2009), avians (Garcia-Moreno et al. 2004; Barber and Klicka 2010), and mammals (Guevara-Chumacero et al. 2010; Hurtado and D'Elía 2022).

The *Artibeus jamaicensis* complex is a significant group of bats in South, Central, and Middle America, whose mainland populations present higher genetic diversity compared to other *Artibeus* species and other bat species (as seen in Ruiz et al. 2013). Demographic history analysis suggests that there were at least two population expansion events during the Middle Pleistocene to the Late Pleistocene (Ruiz et al. 2013). The contraction and expansion of the tropical forest likely facilitated the northward movement of *Artibeus* lineages through corridors, enabling them to explore and colonize new habitats in South and Central America (Phillips et al. 1991).

Another important neotropical bat complex, the *Pteronotus* complex, which includes *Pteronotus personatus*, *Pteronotus parnellii*, and *Pteronotus davyi* (Rull 2011), had its demographic history outlined in the Early Pleistocene during a glacial event. Different lineages of *P. personatus* have been identified with the use of mtDNA markers, in localities such as: (i) the Gulf of Mexico, the Mexican Pacific coastal plain, and the Isthmus of Tehuantepec, (ii) Southeastern Mexico, (iii) Guatemala, (iv) Guyana and Suriname (using COI), and Guyana and Venezuela (using Cytb), and (v) Guyana, Suriname, French Guiana, and Brazil. Their vicariant events, which occurred roughly

1.624 million to 2.450 million years ago, had Central America as the center of two separate diversification processes - one towards Mexico and the other in South America (Zárate-Martínez et al. 2018).

In addition to environmental factors, aspects of behavior such as migration and philopatry can greatly impact gene flow patterns (Rodrigues et al. 2010). For example, the social system can result in asymmetrical gene flow, as seen in certain bat species (Kerth et al. 2002). This can have significant effects on population dynamics and dispersal, including the threats associated with such behaviors. Our review shows that the majority of bats display male-biased dispersal and female philopatry, as observed in species such as: *Desmodus rotundus* in the Neotropics (Huguin et al. 2018); *Eptesicus nilssonii, Miniopterus schreibersii, Nyctalus leisleri*, and *Hipposideros armiger* in the Palearctic (Xu et al. 2010; Nad'o et al. 2017; Gürün et al. 2019; Smirnov et al. 2020); *Chaerephon pumilus*, three species of *Scotonycteris*, and *Casinycteris argynnis* in the Afrotropics (Naidoo et al. 2016; Hassanin et al. 2015); and *Myotis vivesi* and *Antrozous pallidus* in the Neoartic (Floyd et al. 2010; Arnold and Wilkinson 2015). However, the male-biased dispersal seen in the mainland population of *A. jamaicensis* (Ortega and Arita 1999) may not hold true in the population on Cozumel Island, where individuals move among localities regardless of sex, indicating that – despite the species' polygynous nature – both males and females disperse across the island (Vázquez-Domínguez et al. 2013). It's worth noting that the common mating system in bats, particularly the case of female philopatry, is often cited as a cause for their high genetic structuring, substantial differentiation between colonies, and potential population bottlenecks (Xu et al. 2010; O'Donnell et al. 2016), but different patterns may be found.

Bats can be highly gregarious, forming large colonies and interacting with each other (Veith et al. 2004). In temperate zones, certain bat species visit underground sites for short periods during the transition from their summer colony to winter hibernation, a behavior known as "swarming". These sites are also used for mating and sperm transfer (Fenton 1969; Thomas et al. 1979; Helversen 1989), allowing for gene flow among colonies and the consequent relaxation of their genetic borders (Veith et al. 2004). There are no known records of swarming sites in neotropical bats, but they often form groups year-round (McCracken and Wilkinson 2000; Kunz and Lumsden 2003), which may represent an important mechanism to explain their genetic diversity in the region. Studies

of genetic structure and cryptic diversity in the Neotropics can aid in bat conservation by assessing species diversity and movements at a landscape level (Ito et al. 2022).

5.6 Lessons from population genetics

5.6.1 Impacts of geographical barriers and distance on gene flow

Topographical barriers can impact the genetic structure of animal populations (Andriollo et al. 2018). For bats, landscape features such as mountain ranges and large bodies of open water can act as major barriers to dispersal (Castella et al. 2000; Dàvalos 2005). However, the effect of these barriers may vary among bat species, with varying degrees of impact.

Studies have shown that bats have a significant capacity for dispersal, but ocean straits can act as barriers for some species, such as the *Myotis nattereri* complex in the Straits of Gibraltar (García-Mudarra et al. 2009), and *Tadaria brasiliensis* in the Northwest and Northeast Providence Channels (Speer et al. 2017). However, this is not always the case, and the ocean may not pose a strong barrier for other species, such as *Ardops nichollsi*, *Brachyphylla cavernarum*, and *A. jamaicensis* in the Lesser Antilles, in the Caribbean (Carstens et al. 2004). The Azorean bat (*Nyctalus azoreum)* (Thomas 1901), the only endemic mammal in the Azores Archipelago, was separated from its continental ancestor, the Leisler's bat (*N. leisleri)* (Kuhl 1917), during the late Pleistocene or early Holocene. Microsatellite analysis by Salgueiro (2010) showed a lack of contemporary gene flow between the Azorean bats and Leisler's bats (which is expected between different species), but also limited gene flow among the *N. azoreum* populations inhabiting all six Azorean islands, suggesting that open sea can act as an important barrier in isolating the Azorean bats. This highlights the need for attention to the conservation of this species (Salgueiro et al. 2008).

Other barriers have been identified between two frugivorous phyllostomid species, *Sturnira parvidens* and *Sturnira hondurensis*. Both are distributed throughout the Mesoamerican region and are separated by intervening highlands or lowland forests. The estimated divergence time between the two species is thought to have occurred during the early Pleistocene (circa 1.84 Ma) and Pliocene (circa 2.5 Ma), respectively. Despite both species having high genetic diversity, mitochondrial data and modeling indicate that isolation by landscape resistance (IBR) has played a role in separating *S. hondurensis*

populations. The highland habitat heterogeneity may have limited gene flow in regions such as the Sierra Madre Oriental, Sierra de Los Tuxtlas, Chiapas, and Guatemala (Torres-Morales et al. 2019).

Besides geographic barriers, distance also affects genetic diversity distribution. Although bats are capable of flight, increased geographic distances can result in decreased gene flow in various bat species (Rodrigues et al. 2010; Laine et al. 2013; Ripperger et al. 2013; Real-Monroy and Ortega 2017) due to isolation by distance (IBD). For example, in the mountainous region of the Gulf of Mexico, isolation by distance is a more significant factor than landscape features in the structuring of *S. parvidens* populations (Torres-Morales et al. 2019).

In bats, the level of IBD can vary based on the species' dispersal ability, and species with higher dispersal rates exhibit less genetic structuring compared to those with lower mobility (Meyer et al. 2009). For example, mitochondrial and microsatellite data has shown that the distances between the islands of the Mariana Islands, ranging from 5 to 100 km, do not serve as barriers for *P. mariannus* (Brown et al. 2011), as gene flow was maintained. The gene flow, verified with mtDNA, was also retained among *A. jamaicensis* populations in the Lesser Antilles, where the distances between islands exceed 100 km (Carstens et al. 2004). However, a much shorter distance of 14 km in the Strait of Gibraltar presents a barrier to the dispersal of *Myotis myotis*, even though high levels of gene flow were observed among continental colonies over large distances (up to 770 km) (Castella et al. 2000).

In addition to mobility, other factors can also impact the degree of isolation of a bat species, such as open water between islands (or between mainlands, or between island and the mainland), philopatry, whether a species is migrant or non-migrant, and dispersal abilities (Rodrigues et al. 2010; Frankham et al. 2002). It is important to note that a significant challenge for population genetic studies in bats, particularly in the Neotropics, is the lack of available molecular markers (microsatellites and SNPs) for effective population analysis. Currently, only 22 papers have aimed to develop microsatellites for bat species globally, four of which focusing specifically on neotropical species. The availability of more molecular markers should help improve our understanding of gene flows between populations (Loureiro et al. 2020), and the use of SNPs is expected to

enhance our understanding of how neotropical bat populations are structured (Loureiro et al. 2020).

5.6.2 Genetic structure and populations

Understanding and delineating population structure is a crucial step in comprehending the ecology and behavior of species, as well as in devising conservation management recommendations (Anderson et al. 2018). Both nuclear and mitochondrial markers are commonly used to achieve these goals, as demonstrated in a study of the endangered New Zealand long-tailed bat, *Chalinolobus tuberculatus*, which occurs in two valleys of Fiordland, New Zealand (O'Donnell et al. 2016). In this study, all nine colonies were analyzed and found to have high genetic diversity, with moderate signs of genetic bottlenecks and, although all colonies were still connected by gene flow, small-scale genetic divergence was detected within a valley, across distances of 1.5-30.0 km (O'Donnell et al. 2016).

In the Neotropics, Ferreira et al. (2014) used the Cytb gene to show that a South American endemic bat species, *Artibeus obscurus* (figure 5.3), shows a deep divergence between monophyletic clusters representing populations in different biomes (Atlantic Forest and Amazon). This divergence appears in the form of a clear division between east and west within South America, separated by the "diagonal dry belt" of the Cerrado biome in Brazil.

Figure 5.3 *Artibeus obscurus* species. Photo: Roberto Leonan M. Novaes.

Population genetic structuring was revealed in the Peruvian vampire bat (*D. rotundus*) through a DNA metabarcoding approach. Bohmann et al. (2018) found that populations of bats from the western coast of the Andes were distinct from those in the east, indicating that the Andes act as a barrier to dispersal for this species.

Ito et al. (2022) conducted a study of large colonies of *Pteronotus gymnonotus* in northeastern Brazil, ranging from 5,365 to 98,986 bats. They showed that the genetic distances among these colonies did not correlate with their geographical distances. *P. gymnonotus* is a relatively mobile species with the broadest latitudinal range among mormoopid bats, and it is found in various habitats. Low inbreeding coefficients were observed in all populations, indicating no genetic differentiation between colonies. The caves analyzed were separated by 700 km, but the large bat populations showed high gene flow despite the distance, demonstrating a weak pattern of isolation by distance.

5.6.3 Bats in islands

Bats are frequently the only native mammals on several isolated oceanic islands. Whereas about 60% of all bat species in the world live both on islands and mainland, 25% are endemic to islands (Jones et al. 2009), such as the endangered *P. mariannus* in the

Mariana Islands, a US territory of Guam (Brown et al. 2011). A review conducted by Welch and Leppanen (2017) indicated that most bat extinctions occurred on islands, and the impact of invasive species - such as domestic cats - is worse for island populations than mainland populations. Island populations tend to display reduced genetic variation, as reported for some insular mammals (Paetkau and Strobeck 1994; Eldridge et al. 1999; Hinten et al. 2003; Wang et al. 2005) and bats. For instance, Salgueiro et al. (2010) found that the genetic diversity of *N. azoreum*, a bat endemic to the Azores Archipelago, was lower than that of the mainland *N. leisleri*.

Endemic island populations are more vulnerable to stochastic events, such as typhoons and volcanic eruptions. Such events can represent a significant threat to the isolated populations of *P. mariannus* on the Mariana Islands (Wiles et al. 1989; 2004), and result in a drastic reduction in population size, thus increasing inbreeding and genetic drift, and potentially leading to a local extinction (Wiles et al. 1989; 2004; Soulè 1983).

Despite the threats faced by some populations, endemism levels on islands are high, particularly among the fox bats of the genus *Pteropus* from the Indian Ocean. Despite being able to fly over 50 km in one night (not limited by the absence of forested areas), and to traverse open water as well (Tidemann and Nelson 2004; Larsen et al. 2014), endemism remains high among these bats.

In the Neotropics, the Caribbean archipelago is comprised of numerous islands that differ in age, size, habitat, and level of isolation from other islands and from the mainland (Loureiro et al. 2020). The archipelago is divided into two main regions: the Lesser Antilles, located on the eastern margin of the Caribbean tectonic plate, and the Greater Antilles. Over 60 bat species inhabit these islands, including several endemic species (Dávalos, 2004; Loureiro et al. 2018). Loureiro et al. (2020) used the genotyping-by-sequencing (GBS) method to identify distinct patterns of population differentiation between *Molossus molossus* and *Molossus milleri* in the Caribbean, finding evidence that oceanic straits were acting as barriers to gene flow within *M. milleri* from the Greater Antilles, but not within *M. molossus* from the Lesser Antilles.

5.6.4 Effect of fragmentation and other anthropogenic effects on genetic diversity

Bats, being relatively mobile compared to other animal groups, have a certain level of resistance to the impacts of habitat fragmentation (Meyer et al. 2009). However, they are still susceptible to a wide range of environmental stressors, and respond to them in predictable ways. Concerning alterations to their natural habitats, bat populations can be negatively impacted by: Urbanization (lighting, traffic noise), deterioration of water quality (eutrophication), and increased agricultural activity (with the loss of habitat boundaries, and use of pesticides), among other changes (Jones et al. 2009). Many species of bats are forest dwellers, and the loss of forest cover, as well as the reduction in availability of foraging and roosting habitats, can lead to significant impacts (Hutson et al. 2001; Racey and Entwistle 2003). Thus, habitat fragmentation and destruction may contribute to substantial loss of genetic variation in bat populations.

Less mobile species, such as *Carollia perspicillata* in the Neotropics (Meyer et al. 2009), and the endemic and endangered New Zealand long-tailed bat, *Chalinolobus tuberculatus* (O'Donnell et al. 2016), are likely to be more severely impacted by human-driven landscape modification. On the other hand, bats with higher mobility may be less affected by habitat fragmentation. For example, *Carollia castanea* is relatively more resistant to habitat fragmentation than other low-mobility phyllostomid bat species, and its genetic diversity might take longer to respond to the effects of fragmentation (Ripperger et al. 2014). The authors emphasize the importance of considering not just mobility, but also the landscape composition and life history traits of a species, when assessing their response to habitat fragmentation.

In addition to habitat fragmentation, predation by domestic cats has been documented in several bat species, including *M. vivesi* (Vázquez-Domínguez et al. 2004), *Plecotus auratus* (Woods et al. 2003), and *Pipistrellus coromandra* (Virkar and Shrotriya 2013). Scrimgeour et al. (2012) used DNA samples to identify a single male cat as being responsible for killing 102 individuals of *Mystacina tuberculata* in just seven days, emphasizing the impact that domestic animals can have on bat populations.

Bats of the *Pteropodidae* family, as well as a few other species, face the threat of being hunted, which is mostly illegal and exacerbated in certain cases, such as *P.*

mariannus in the Mariana Islands (Brown et al. 2011), and *Pteropus rodricensis* on the Rodrigues Island, in the Indian Ocean (O'Brien et al. 2007). This hunting can be driven by cultural traditions where the bats are considered a delicacy food, or for commercial purposes (Wiles et al. 2010). Despite this threat, the genetic consequences of hunting on bat populations are still not well understood.

5.6.5 Bat adaptations

With around 70% of fossil data missing (Eiting and Gunnell 2009), the evolutionary history of bats is still a topic of debate, with many aspects of their phylogenetic reconstruction and unique adaptations yet to be understood (Teeling et al. 2018). This history likely involves introgressions, hybridizations, adaptations, and the incorporation of inherited genetic elements in different ways, such as through behavioral changes, which can then lead to rapid diversification and the evolution of associated mechanical and physical adaptations (Duckworth 2009; Mayr 1963). For feeding, animals must use a combination of foraging behavior and sensory perception, and changes in behavior can sometimes be mediated by the remodeling of sensory systems (Goldman-Huertas et al. 2015; Karageorgi et al. 2017). In a recent study, Davies et al. (2020) used transcriptome assembly to detect contrasting levels of positive selection in genes associated with the development, maintenance, and scope of visual function in bats. They traced these changes back to the origins of the superfamily noctilionoids, and of the family with the most dietary diversity, the neotropical leaf-nosed bats (Phyllostomidae), finding evidence of positive selection in vision genes during subsequent shifts to either nectarivory or frugivory. These changes likely reflect an effective preadaptation to the use of visual cues for identifying food and roosts, as well as for orientation.

An interesting case of feeding adaptation can be found in the greater bulldog bat (*Noctilio leporinus*). This species, which is relatively large, feeds on a variety of food sources including fish, flying insects, and aquatic invertebrates. The development of the piscivory habitat in this genus has been a recent occurrence (Pavan et al. 2013), and the most likely explanation is that it arose after the radiation of existing *Noctilio* species, without any significant modifications in morphology or physiology (Khan et al. 2014; Liem 1973). Overall, our understanding of the genetic basis of bat adaptations is still limited, but recent advancements in genomics hold promise for improving our knowledge in the future.

5.7 Final Considerations

Due to the high diversity of bats and our limited understanding of many aspects of their biology and species, we emphasize the importance of continued genetic research as to further our comprehension of their population dynamics, and of the impacts of human activities on their conservation. This is particularly crucial for species that are considered vulnerable and located in regions with high levels of diversity, such as the Neotropics, where new species are continuously being discovered, such as the endemic *Myotis barquezi*, which was described in December 2022 (Novaes et al. 2022).

The continued improvement of genetic techniques to obtain DNA, such as non-invasive (guano) and minimally-invasive (wing tissue, buccal samples) sampling methods, makes it possible to monitor individual bats and study their spatial and temporal patterns of dispersal and habitat use (Carroll et al. 2018). Furthermore, the collection of DNA from fecal samples and subsequent use of DNA metabarcoding to assess the diets of multitrophic assemblages has allowed for inferences of population structure (Bohmann et al. 2018; Ingala et al. 2021), making it a valuable tool for improving the understanding and conservation of bats.

The study of bats and their genomes holds the potential to impact a variety of scientific fields, such as healthy aging, disease resistance, ecosystem functioning, and the evolution of sensory perception (Teeling et al. 2018). Our review recovered 16 bat genome descriptions, including complete mitogenomes, transcriptomes, and cosmid libraries. So far, the complete mitochondrial genomes of the following bat species are available: *Myotis frater* (Chung et al. 2018), *Myotis rufoniger* (Bhak et al. 2017), *Myotis brandtii* (Jiang et al. 2016), *Myotis davidii* (Wang et al. 2016), *M. myotis* (Jebb et al. 2018; Huang et al. 2016), *Myotis muricola* (Yoon and Park 2015), *P. personatus* (López-Wilchis et al. 2017), *Pteropus alecto* (Gao et al. 2016), *Pteropus vampyrus* (Lu et al. 2016), *Hypsugo alaschanicus* (Kim and Park 2015), *Murina ussuriensis* (Yoon and Park 2015), *Pteropus scapulatus*, and *Pteropus poliocephalus* (Barragán et al. 2002). Additionally, the transcriptomes of *M. myotis* (Huang et al. 2016) and *Rousettus aegyptiacus* (Lee et al. 2015) have been determined, as well as a cosmid library of *M. waterhousii* (Baker et al. 1997). Despite the large number of bat species worldwide, the number of available genomes is still small, a trend also seen in other groups due to the high cost and specialized labor required.

Despite the increase in the number of genetic studies involving bats in the early 2000s, several families (such as *Nycteridae*, *Furipteridae*, *Mystacinidae*, *Thyropteridae*, and *Natalidae*) still require further investigation. There is also a need for a deeper understanding of the impact of barriers, fragmentation, and geographic distance on bat populations (Fig. 5.2). The continual advancement of new technologies has shown the potential of these tools in a wide range of genetic studies, and their contribution to the management and conservation of bats.

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