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IMPORTANCE OF DNA SAMPLING METHODS FOR ASSESSING GENETIC DIVERSITY IN BIRDS - A BRIEF REVIEW

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Abstract

In studies involving various bird species, DNA analysis is crucial due to the valuable data it can provide. Experiments on genetic diversity have become common in many areas of biodiversity science. Although obtaining high-quality DNA from samples is critical, surprisingly few reviews focus on effective field sampling techniques to preserve DNA before laboratory extraction. Research on wild bird populations often relies on DNA samples collected from easy sources such as blood, saliva, or cloacal swabs. DNA provides a wealth of information for researchers. Analyzing it can yield various insights, ranging from the identity of individuals (such as determining their sex and parental relationships) as well as gathering data on entire populations (like estimating population sizes and understanding the significance of gene flow between them). This paper aims to evaluate the specialized scientific articles and highlight the methods used to produce less stress on populations.

Key words*: DNA extraction, DNA methods, sampling techniques, wild bird.*

INTRODUCTION

Every individual has a unique genetic composition, shaped by their hereditary material, namely the DNA (Hoy, 2013). The vast diversity of genes within a population enables it to withstand the stress induced by environmental factors. Genetic diversity is a key driver of evolution and serves as a primary criterion for natural selection, leading to the survival of the most powerful individuals. "Genetic diversity is defined as genetic variability present within species." Genetic diversity arises from the recombination of genetic material during inheritance and varies over time and across different environments (Stewart et al., 2019).

Sexual reproduction plays an important role in preserving genetic diversity by producing unique offspring through the combination of genes from both parents. Additionally, factors such as gene mutations, genetic drift, and gene flow contribute to genetic diversity (Stewart et al., 2019).

Research on wild birds' ecology, behaviour, health and genetics, benefits from the ongoing development of innovative sampling techniques. Traditional approaches for gathering biological material often require capturing and handling individual birds in order to draw blood, collect tissue samples, or pluck feathers. However, these techniques can disturb or stress the birds, potentially leading to decreased survival rates among those captured, negatively impacting populations (Baus et al., 2019). Hot and humid weather in tropical and subtropical regions serves as a critical risk factor, significantly increasing the likelihood of wound infections that can occur during capture or invasive sampling (Sheldon et al., 2008). The harmful effects of invasive sampling methods can differ significantly based on the studied species and the specific research objectives (Lefort et al., 2015). Therefore, it is crucial to minimize unexpected outcomes and unwanted side effects during sample collection, especially when working with endangered or elusive bird species (Baus et al., 2019).

In this context, the purpose of this literature review was to identify the methods used to produce less stress on bird populations for our study. Therefore, we present a methodical bibliographic synthesis pointing by what means non-invasive collecting of genetic samples has been used to research the genetic diversity on wild birds. In order to define the tendencies and classify the sample categories used, the studied species and the research questions addressed, we have conducted a search in international free online databases regarding the scientific publications on this matter.

MATERIALS AND METHODS

The paper is a brief review of the literature on the application of DNA sampling methods for assessing genetic diversity in birds, therefore the method used was the critical analysis of the literature. The search engines for articles were Google Academic and Research Gate. Research papers with free access were used or in other cases, where the scientific paper was not free, the work was requested straight from the authors.

RESULTS AND DISCUSSIONS

As DNA sequencing technology advances, studies on host organism microbial communities (microbiota) are becoming more common and affordable (Abughazaleh et al., 2021). Evaluating these microbial communities requires a successful extraction of microbial DNA (Feinstein et al., 2009).

In recent years, there has been a growing interest in animal microbiomes (Russell et al., 2024, Abughazaleh et al., 2021, Bahrndorff et al., 2016; Barko et al., 2018, Bodawatta, et al., 2021, Esser et al., 2019, Gilroy, 2021, Hasan & Yang, 2019; Kau et al., 2011, Kogut & Fernandez-Miyakawa, 2022, Kohl, 2012; Loftus et al., 2021). Many migratory bird species have high-energy consumption demands that depend on available resources, which fluctuate and are reflected in the gut microbiome and immune functions (Song et al. 2022). Due to the significant diversity in wild birds regarding their life history, habitat, migratory patterns, and lifespan, there is considerable variation in the microbiota that makes up an individual’s microbiome (Ottinger, 2024). Natural microbiome on a host individual, positively influence health, pathogen resistance, digestion, and other bodily functions (Russell et al., 2024, Bodawatta et al., 2021; Broom & Kogut, 2018; Davidson et al., 2020; Drobniak et al., 2022; Trevelline & Kohl, 2022). Microbiome research can enhance our understanding of non-native species invasions, host responses to pathogens and contaminants, human-induced environmental changes, and the effects of climate change, all of which may decrease gut microbiome in wild animal populations (Bahrndor et al., 2016,).

The environment, dietary choices, and host health, have an effect on the diversity and abundance of bacterial types in a host organism (Jensen et al., 2007). Birds can be found in nearly every corner of the globe, and they are crucial players in maintaining the balance of ecosystems. Their diverse roles enrich the environment, from pollinating plants to controlling insect/pest populations (Şekercioğlu et al., 2004, Wenny et al., 2011, Whelan et al., 2008). While there is significant potential for microbiome research to have an effect on host evolution and ecology regarding animal well-being and conservation, studies on the wildlife microbiome remain an emerging area of research (Simmons et al., 2018, Gillingham, 2017).

Genetic diversity is indispensable for species survival and resilience. It fosters a range of physical traits that enable individuals to adapt to stress, combat diseases, and thrive in challenging environments (Minias et al., 2015). As our world rapidly changes, natural selection becomes crucial. Genetic diversity acts as a safeguard, allowing the most adaptable individuals to survive while vulnerable varieties are eliminated (Stewart et al., 2019).

By promoting genetic diversity, we preserve essential gene varieties that may provide resistance to pests and diseases. Crossbreeding diverse genetic variants leads to new species varieties with desired traits, such as increased disease resistance and tolerance to environmental stress (Gebhardt et al., 2008). Additionally, genetic diversity decreases the spread of harmful hereditary traits and guarantees that some individuals of a certain specie will persist, enabling renewal and survival. Prioritizing genetic diversity is essential for our ecosystems' health and our planet's sustainability (Baus et al., 2019).

Utilizing mitochondrial DNA sequences for molecular phylogeny unlocks fascinating insights that can significantly enhance our wildlife conservation efforts (Arif et al., 2010). This tool not only helps us trace evolutionary relationships but also makes strategies to protect endangered species and preserve biodiversity (Khan & Arif, 2013).

High-quality genomic DNA is crucial for effective molecular analyses in wildlife conservation (Hoglund et al., 2007). While blood and tissue samples are frequently used for DNA extraction, it is imperative to depend on non-invasive sources such as feces, hair, feathers, and buccal cells - especially in studies where traditional sampling methods are impractical. This approach is essential for conducting thorough research on wild animals (Khan & Arif, 2013).

Research on wild birds' ecology, behaviour, population strength and genetics, benefits from the continuous advancement of innovative sampling techniques. Traditional methods for collecting biological material typically includes capturing and handling the birds to draw blood, scrape tissue, or pluck feathers (Baus et al., 2019; Taberlet et al., 1999). Though, these techniques can disturb or stress the individuals, potentially leading to lower survival rates among captured birds (Brown & Brown, 2009; Owen, 2011). This could have negative effects on their populations (Baus et al., 2019).

The advancement and implementation of non-destructive sampling methods for collecting genetic material from bird species are essential for scientists to diminish disturbance while acquiring critical data about populations and individual birds (Egloff et al., 2009; Quinn et al., 1987; Queller et al., 1993; Dunn & Lifjeld, 1994; Jarne & Lagoda, 1996). Genetic data plays a pivotal role in deepening our knowledge regarding numerous aspects of birds ecology and are crucial for resolving questions that direct observation alone cannot answer (Queller et al., 1993; Dunn & Lifjeld, 1994; Jarne & Lagoda, 1996). Furthermore, genetic data provides invaluable insights into mating systems, parentage assignments, kinship analysis, species evolution, and gene flow (Egloff et al., 2009; Quinn et al., 1987; Queller et al., 1993; Dunn & Lifjeld, 1994; Jarne & Lagoda, 1996).

Microorganisms research has a significant potential to influence avifauna evolution and ecology in relation to animal wealth and conservation; however, the research on wildlife microbiomes is still evolving. We will illustrate a few budget-friendly, non-invasive methods for DNA sampling and examination as found in the literature.

Non-invasive genetic sampling (NGS) involves collecting DNA from sources left behind by animals, such as hair, feces, urine, eggshells, saliva and shed skin, without the need to capture them (Horváth et al., 2005; Waits & Paetkau, 2015). This method requires less effort, expertise, and lower costs compared to invasive techniques (Miño & del Lama, 2009; Wheat et al., 2016; Kirol et al., 2018; Ferreira et al., 2018). Therefore, NGS is a valuable alternative for studying elusive or endangered species and for conservation research (Piggott & Taylor, 2003; Khan & Arif, 2013).

In 2009, Egloff and his team, created a non-destructive and non-invasive technique of sampling avian maternal DNA from the surface of eggshells. The method needed to be suitable for field use and capable of high-throughput collecting. Additionally, it was aimed to develop a method that was not reliant on the timing of sampling and was resistant to contamination from paternal or embryonic sources of DNA (Egloff et al., 2009). The study aimed to develop a non-destructive technique for sampling maternal DNA without needing blood from the mother. They isolated genomic DNA from avian eggshell powder obtained by filing the outer shell. By comparing microsatellite profiles from the eggshell DNA to those of the parents, they confirmed the presence of maternal DNA in all gull nests assessed (Egloff et al., 2009). Additionally, the profiles were undistinguishable among eggs from the same clutch. The method enables rapid, non-invasive DNA collection from eggshells, useful for various genetic studies. One of the applications is evaluating the fertilization grade of nonviable herring gull eggs. The microsatellite profiles of the eggshell powder showed no match with those of the fertilized embryonic contents, allowing differentiation between unfertilized eggs and embryos that had aborted early. This could provide valuable insights into avian reproductive health (Egloff et al., 2009).

Other authors such as Knutie et al., 2018 describe a portable, inexpensive kit for non-invasive fecal collection from small birds, suitable for field studies in remote areas. The collection kit consists of a flat-bottomed paper bag, a large tray, a grate, a clothespin, and a 10% bleach solution for sterilization.

The tray is placed under the grate in the bag to prevent contamination. After capturing the bird and keeping it in the bag for 3–5 minutes, it defecates, allowing for the collection of the sample, which is then moved to a tube and preserved or frozen. The procedure associates two previously published approaches as follows:

(1) the bird bag for wild birds (Craven et al., 2000; Grond et al., 2017);
(2) a grated cage for captive birds (Graczyk et al., 1998; Hancock et al., 1998).

The team used gloves when collecting fecal samples together with sterile swabs (Bokulich et al., 2019) and 2 mL microcentrifuge tubes. Then used a tray as a funnel to direct feces into the tube. The sterile swab was used to transfer the feces, placing the swab in the tube being the best option for further DNA extraction as it contains fecal bacteria. Further, the tube was placed on wet ice before freezing it at -20 or -80°C for long-term storage and DNA extraction (Knutie & Gotanda, 2018).

Another non-invasive method for collecting DNA samples described by Borrelli et al., in 2020 presents a similar approach as the one described above: using a box for collecting stool samples from wild birds or small animals which includes a plastic storage container, a plastic tray, a vinyl-coated hardware fabric, and a 10% bleach solution. The tray in the plastic container is placed under the raised grate to minimize fecal sample contamination. Unlike the previously published sampling technique for small wild birds, the procedure was modified for average and large wild avians, fluctuating from one hundred grams to one kilogram of body mass and up to fifty centimeters in height. The plastic container it can be reused after careful sanitization, using a grill brush to avoid cross-contamination. The authors argue that it is more durable than a paper sack, particularly for prey birds that have sharp talons and a strong beak. Moreover, it might be beneficial to handle the birds as well (Borrelli et al., 2020).

Other researchers established an efficient and cost-effective protocol for collecting samples from wild birds using three types of inexpensive buccal swabs. Their study found that foam-tipped swabs yielded higher DNA than cotton-tipped swabs, with extraction and amplification success rates of 100% and 97.2%, respectively. By omitting a drying step and storing swabs in Longmire buffer, they have improved the field efficiency while obtaining enough DNA for population genetic research. This protocol is ideal for sampling juveniles or small birds, reducing stress compared to blood draws (Vilstrup et al., 2018).

A methodical bibliographic synthesis explored the non-invasive sampling of genetic material in studying avian populations in the Neotropics (Baus et al., 2019). An analysis of 21 articles from 2007 to 2017 revealed that shed feathers were the most used samples (66.7%), followed by remains (14.2%), eggs (9.5%), and non-invasive blood samples (4.8%). The primary research topics included population genetics (38.1%), species identification (28.6%), phylogenetics (14.3%), molecular sexing (9.5%), and parentage examination (9.5%). Brazil accounted for nearly half (47.6%) of the studies. While interest in non-invasive sampling is growing, its application remains concentrated in developed countries and is limited to specific research questions. Expanding this method could enhance the understanding of various characteristics of Neotropical birds, including the anthropological impact on wildlife (Baus et al., 2019).

CONCLUSIONS

Advancements in DNA sequencing technology are significantly enhancing our comprehension of microbial communities, particularly within animal microbiomes. These microbiomes are crucial for the overall health of hosts and their ability to fend off pathogens. Notably, the composition and functionality of these communities are shaped by a variety of factors, including diet, environmental conditions, and the ecological niche of the host species.

Researchers are increasingly employing non-invasive sampling techniques to gather vital genetic data while minimizing stress and disruption to wild birds. Techniques such as collecting feces, feathers, and hair provide a wealth of information without causing harm or significant interference with the birds' natural behavior. For instances where more precise genetic material is needed, minimally invasive methods - such as blood sampling, feather sampling, and skin biopsies - can be employed, though these require a higher level of interaction and expertise from researchers.

The selection of an appropriate sampling method is contingent upon several factors: the specific species being studied, the overarching research objectives, and the ethical considerations involved in handling wildlife. Researchers must follow ethical guidelines and legal regulations to ensure animal welfare and safety.

This review aims to elucidate the various non-invasive methods currently in use for DNA data collection, emphasizing the significant strides made in this rapidly evolving field. It serves as an important resource for scientists seeking to understand the complex interactions within animal microbiomes while prioritizing the well-being of their subjects.

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