CHARACTERIZATION OF THE GENETIC SIGNIFICANCE OF BIRD SPECIES WINTERING IN THE CASPIAN SEA

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Abstract: The Caspian Sea represents a critical aquatic ecosystem, serving as a significant migratory corridor and wintering habitat for numerous avian species throughout the year. The taxonomic diversity of bird populations wintering along the Caspian coastline, their physiological adaptations, and resilience to climatic fluctuations have garnered considerable scientific interest. Genetic diversity is a fundamental determinant in evolutionary selection and ecological adaptation mechanisms. Molecular analyses of the DNA of avian species overwintering in the Caspian region reveal a remarkably high degree of genetic variation. This genetic heterogeneity plays a pivotal role in facilitating adaptation to diverse climatic conditions and enhancing ecological plasticity. This study aims to investigate the genetic diversity of wintering bird species in the Caspian Sea region and assess the genetic significance of molecular markers in avian population dynamics.
Keywords: birds, genetic diversity, DNA, molecular markers, SSR, ISSR

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Introduction:

The class *Aves* originated approximately 80–100 million years ago, undergoing extensive evolutionary diversification. The high basal metabolic rate of birds, coupled with the locomotor versatility of many species-enabling both flight and aquatic adaptation-has facilitated their extensive biogeographical distribution. Avian species exhibit pronounced migratory behavior in response to adverse climatic conditions, allowing them to exploit seasonally favorable habitats for survival and reproduction. (I. Babayev, F. Asgarov, F. Ahmadov, *Biological Diversity: Waterfowl of the Azerbaijani Part of the Caspian*).

The Caspian Sea represents the largest enclosed inland water body globally, bordered by Russia, Kazakhstan, Turkmenistan, Iran, and Azerbaijan. This distinctive hydrological basin is characterized by both marine and lacustrine features, with its ecological system being influenced by factors such as salinity gradients, climatic conditions, and adjacent terrestrial biomes. Spanning an area of approximately 371,000 square kilometers, the Caspian Sea reaches depths exceeding 1,000 meters in certain regions. Its endorheic nature—lacking a natural outflow—results in variable salinity levels, which play a crucial role in shaping the basin's biological diversity (Kosarev & Yablonskaya, 1994).

The Caspian Sea is a vestige of the ancient Paratethys Sea, and its distinctive hydrochemical properties have facilitated the evolutionary processes of various endemic species. The water salinity exhibits a significant gradient, transitioning from nearly freshwater conditions in the northern regions to more saline waters in the central and southern areas. These dynamic salinity variations regulate the distribution and abundance of aquatic organisms, thereby influencing the ecological dynamics and foraging behaviors of

avian species that depend on this ecosystem (Zonn et al., 2010).

The climate surrounding the Caspian Sea exhibits a gradient from temperate to arid, with winter temperatures being comparatively milder than those at higher latitudes. The wetlands, deltas, and shallow coastal zones are ecologically rich, providing abundant food resources such as fish, mollusks, and plant matter. These ecological conditions facilitate foraging, resting, and nesting for numerous avian species. Notably, the extensive reed beds and wetlands in the Volga Delta and other coastal regions serve as critical refuges for migratory birds, offering shelter from both predation and adverse climatic conditions (Cramp & Simmons, 1977).

Wintering Bird Species of the Caspian Sea:

Wintering birds are migratory species that relocate to more temperate regions during the winter months to avoid extreme climatic conditions and food shortages in their breeding habitats. The primary objective of these birds is to mitigate the challenges posed by adverse environmental factors, such as low temperatures and limited food availability, thereby enhancing their chances of survival during harsh seasonal conditions (Greenlight Environmental Consultancy LTD).

The wintering period for bird species begins in October and lasts until March (www.arthian.com). In Azerbaijan, however, the arrival of birds migrating to the Caspian Sea coast for wintering coincides with the month of January.

A number of avian species utilize the Caspian Sea as a wintering habitat. The most frequently observed species in this region include the following:

Dalmatian Pelican (*Pelecanus crispus*) – This large pelagic bird species is characterized by its distinctive curved cervical plumage and a pronounced preference for wetland ecosystems. It primarily exhibits piscivorous feeding behavior, foraging in shallow aquatic environments. Dalmatian pelicans are highly social, forming small, cohesive colonies and typically establishing nests in floating macrophytes or on remote islands. The loss of suitable habitat, primarily due to anthropogenic influences, has resulted in a significant decline in their population, highlighting the necessity for targeted conservation strategies to prevent further population decline (BirdLife International, 2018).

Common Pochard (*Aythya ferina*) – This diving duck species is frequently encountered in the shallow waters of the Caspian Sea. It preferentially inhabits freshwater lakes, wetlands, and estuarine areas with abundant aquatic vegetation. Males are distinguishable by their chestnut-colored head and red eyes, while females exhibit a more cryptic coloration. In terms of foraging, *Aythya ferina* is omnivorous, consuming a diet consisting primarily of plant material, small invertebrates, and seeds, thus demonstrating ecological plasticity and the ability to adapt to varying environmental conditions (Fox et al., 2016).

Greater Flamingo (*Phoenicopterus roseus*) – This species is primarily observed in the southern parts of the Caspian Sea and feeds predominantly on small invertebrates and algae. It employs a specialized filter-feeding mechanism using its bill, enabling it to extract food from shallow waters, a process facilitated by its elongated legs and neck. *Phoenicopterus roseus* forms large colonies, which provide enhanced protection from predators. The species' characteristic pink coloration is derived from carotenoid pigments, which are bioaccumulated through its diet, particularly from crustaceans and plankton (Johnson & Cézilly, 2007).

Red-breasted Goose (*Branta ruficollis*) – This critically endangered species utilizes the Caspian Sea as a primary wintering site. It is characterized by its distinctive plumage, which includes red, black, and white patterns. The species primarily feeds on grasses, roots, and seeds, foraging in coastal meadows and wetland habitats. Habitat degradation and climate change have significantly impacted its population dynamics, necessitating the implementation of conservation strategies to ensure its persistence

(Wetlands International, 2019).

Percentage distribution of wintering bird species

Azerbaijan, due to its diverse regional characteristics, serves as an advantageous site for studying the distribution of Caucasian endemics. It is also a critical location for monitoring avian migration patterns. In particular, the Beşbarmaq Mountain gorge offers a vantage point for observing migratory bird species. Along the coastline, the Kura Delta and the extensive lagoons of Qızılağac provide wintering grounds for various bird species, with a notable concentration of waterfowl, making these areas of significant ornithological interest (Ornithological Society of the Middle East, the Caucasus, and Central Asia).

In the 2022 winter bird census conducted in Azerbaijan, a total of 820,086 individuals from 118 species were recorded. Subsequent research on January 25, 2023, indicated an increase to approximately 960,000 individuals across 157 species. A significant proportion of the recorded avifauna consists of Anatidae, which represent 76% of the total count. Within this group, 51% are dabbling ducks, 5% remain unidentified, and 20% are diving ducks. Among the species, *Anas penelope* (Wigeon) dominates with 20% of the total, followed by *Anas crecca* (Common Teal) at 14%, and *Aythya ferina* (Pochard) at 3%. The census data reveals that 95% of the recorded birds are waterfowl, 35% are grassland species, 2.3% are flamingos, and 0.3% are cormorants (Sultanov et al., 2023).

Nine species have been included in the IUCN Red List and the Red Book of the Republic of Azerbaijan. The smallest species in Group I include the bustards (*Otis tarda*) with 29,770 individuals, the black-tailed godwit (*Limosa limosa*) with 370 individuals, the ferruginous duck (*Aythya nyroca*) with 353 individuals, and the Dalmatian pelican (*Pelecanus crispus*) with 347 individuals. In Group II, notable species include the flamingo (*Phoenicopterus roseus*) with 21,932 individuals, the mute swan (*Cygnus olor*) with 268 individuals, and tundra birds (*Calidris alpina*) with 190 individuals. In Gobustan National Park, the total number of bird species is approximately 0.5 million. Furthermore, 152,137 individuals were recorded in the Absheron National Park, with additional species counts observed in the Pirallahi Island aquatic zone. In the Alat and Gobustan bays, as well as Ağgöl AES, the bird populations ranged between 40,000-60,000 individuals, while approximately 30,000 individuals were recorded near the Baku Deepwater Jackets Plant, and more than 10,000 birds were reported in other regions, typically through monitoring via leased territories (Sultanov et al., 2023).

Materials and Methods

The diversity and abundance of bird species wintering in the Caspian Sea have garnered significant interest, not only from other countries but also from Azerbaijan. DNA analysis and genetic passporting of bird species play a pivotal role in understanding their systematics and phylogeny. The discovery of the DNA structure has heralded a new era in ornithology. The application of DNA analysis to determine the familial relationships and classification of avian species, as well as the specific role of genetic markers, is crucial for advancing the understanding of avian taxonomy and evolutionary processes (Wink, M. *DNA Analyses Have Revolutionized Studies on the Taxonomy and Evolution of Birds*).

The Role of SSR and ISSR Markers

Genetic markers are pivotal in genetic mapping, playing an essential role in identifying the loci of closely linked alleles on chromosomes (The Editors of Encyclopaedia Britannica, Article History). The application of molecular markers has expanded significantly in research due to their ability to localize quantitative trait loci (QTLs), which in turn facilitates the identification of genes, making them invaluable tools for genetic enhancement studies (SSR and ISSR markers in assessing genetic diversity in *Gallus gallus domesticus*: A quantitative analysis of scientific production).

SSR (Simple Sequence Repeats) and ISSR (Inter-Simple Sequence Repeats) markers are extensively employed in molecular genetics and genomics, offering comprehensive insights into the genetic diversity, population structure, phylogenetic relationships, and evolutionary processes of avian species (Zhan et al., 2010; Dawson et al., 2013). These markers are instrumental in evaluating both the quantitative and qualitative aspects of genetic variation, as well as in assessing gene flow and genetic differentiation driven by natural selection (O'Brien et al., 2006).

SSR markers consist of short tandem repeat sequences that are widely distributed within the genome, exhibit high mutation rates, and can be amplified using specific primers (Primmer et al., 2005). Their codominant inheritance model enables accurate genotyping of both heterozygous and homozygous individuals. The primary applications of SSR markers in genetic studies of avian populations include the following:

Analysis of population genetics and genetic structuring: SSR markers enable the assessment of genetic variation within and between populations, the calculation of genetic differentiation indices (such as Fst and AMOVA), and the evaluation of gene flow direction, providing valuable insights into the evolutionary dynamics of species (Küpper et al., 2012).

Evaluation of phylogenetic and evolutionary relationships: The high degree of polymorphism and extensive genomic distribution of SSR markers facilitate their application in reconstructing phylogenetic relationships within species and among closely related species. These markers provide valuable insights into evolutionary processes, genetic divergence, and the identification of distinct evolutionary lineages (Bensch & Åkesson, 2005).

Parentage analysis and kinship determination: SSR markers, characterized by a codominant inheritance model, facilitate the accurate genotyping of individuals, thereby providing a reliable method for investigating parent-offspring relationships and analyzing pedigree structures (Jensen et al., 2003).

Conservation genetics and protection of endangered species: Through the use of SSR markers, the assessment of genetic diversity loss, population bottlenecks, and inbreeding levels can be conducted, providing critical insights for the development of effective conservation strategies (Oyler-McCance et al., 2010).

ISSR markers amplify intermicrosatellite regions within the genome using primers based on non-specific simple sequence repeats (Bornet & Branchard, 2001). These markers exhibit a dominant inheritance model and serve as an efficient and cost-effective tool for analyzing genetic polymorphism. The applications of ISSR markers in avian species include:

Assessment of Genetic Variation and Population Structure: ISSR markers enable the quantification of genetic diversity indices (He, Na, Ne), facilitating the evaluation of genetic variation within and among populations (Hale et al., 2012).

Evolutionary and Phylogeographic Analyses: Through the examination of genetic clustering patterns and allele distribution, ISSR markers provide insights into avian evolutionary trajectories and migratory routes (Zink & Barrowclough, 2008).

Investigation of Hybridization and Speciation Processes: ISSR markers serve as a robust molecular tool for detecting hybrid individuals and assessing interspecific gene flow, thereby contributing to the elucidation of speciation mechanisms (Mosaad et al., 2019).

Leg Bands in Birds and Their Role in Scientific Research: Leg bands constitute a fundamental methodological approach in ornithological studies, facilitating biometric assessments and ecological analyses (Sutherland et al., 2004). When systematically applied, this technique serves as a critical tool in population dynamics research, conservation biology, and climate change modeling (Canadian Wildlife Service, 2018). Leg bands, also referred to as identification rings, represent one of the most extensively utilized marking techniques in avian research. This method enables the precise identification of individual birds, thereby contributing to longitudinal ecological and

behavioral investigations.

The selection of band material, design, and application strategy is contingent upon the target species, research objectives, and prevailing environmental conditions.

The primary classifications of leg bands are as follows:

Taxonomic and Structural Differentiation of Leg Bands: Metal leg bands are provided by national and international bird banding programs, with each band assigned a unique identification code for individual recognition (USGS Bird Banding Laboratory, 2020).

Stainless steel bands, which demonstrate high resistance to corrosion, are typically favored for long-term monitoring studies due to their durability (Bairlein, 2001).

Aluminum bands, characterized by their aerodynamic lightness, are predominantly utilized in smaller passerine species, offering minimal weight impact on the birds (Baillie & Peach, 1992).

Colored Plastic Bands: Colored plastic bands are encoded using various color combination systems to expedite the process of individual identification (Sutherland et al., 2004).

Composed of lightweight polymer composites, they are specifically designed for small to medium-sized avian species (Canadian Wildlife Service, 2018).

High-visibility spectral colors are employed to generate optical contrast, enhancing visual recognition (Newton, 2010).

Anodized or Protective Coated Metal Bands: These bands are fabricated using advanced anodization technology, which provides superior resistance to both chemical and mechanical abrasion (USGS Bird Banding Laboratory, 2020).

They are particularly suited for use in oceanic bird species and environments characterized by elevated salinity levels (Bairlein, 2001).

Biometrically Designed Locking and Pinning Bands: These bands are engineered with a specialized mechanical fixation mechanism to prevent detachment, especially in raptorial and large avian species with powerful beaks (Canadian Wildlife Service, 2018).

They are utilized in long-term monitoring protocols, with a design that minimizes the risk of unintended opening (Baillie & Peach, 1992).

Application of Leg Bands in Biometric and Ecological Analyses

Migration and Navigation Analysis: The recapture and observation of banded individuals enable the study of their migration trajectories, direction changes, and adaptation mechanisms in response to ecological barriers (Newton, 2010).

When combined with geo-mechanical tracking technologies, migration modifications related to ecotone boundaries and climatic changes can be analyzed with precision (Bairlein, 2001).

Demographic and Population Dynamics Studies: Long-term monitoring of banded individuals allows for the quantitative analysis of changes in population density, dispersion patterns, and mortality rates (Baillie & Peach, 1992). Reproductive success and generation growth rates can be accurately measured through the individual tracking of banded individuals (Sutherland et al., 2004).

Ecological and Ethological Studies: The color-coding systems of leg bands facilitate the identification of social structure, dominance hierarchies, and inter-individual interactions within bird flocks (Canadian Wildlife Service, 2018). Leg bands are applied as an effective visual marking method for studying mating strategies, territorial behaviors, and reproductive selection mechanisms (Newton, 2010).

Application of Leg Bands and Safety Standards. Methodology of the Banding Process: Birds are meticulously handled to minimize stress, with biometric measurements, including wing length, body mass, and tarsus size, recorded for subsequent analysis (USGS Bird Banding Laboratory, 2020).

The size of the band is selected according to the specific species to avoid any restriction in movement or potential tissue damage (Baillie & Peach, 1992).

The band is applied to the tarsus or metatarsus using specialized, certified banding tools to ensure secure placement and minimize

injury (Sutherland et al., 2004).

Potential Risks and Their Minimization

Band Loss or Deformation: Bands with inadequate durability may suffer mechanical damage or detachment, leading to the loss of valuable data (Bairlein, 2001).

Mechanical Injuries: Improperly sized bands may lead to tissue compression, resulting in hematomas or necrosis (USGS Bird Banding Laboratory, 2020).

Results and Discussion

The Caspian Sea serves as a critical wintering habitat for numerous avian species, providing essential resources such as food, shelter, and favorable climatic conditions. The genetic adaptations of these birds enable them to thrive in this environment, thereby underscoring the importance of implementing effective conservation strategies to ensure their long-term survival. Given the ongoing impacts of climate change and anthropogenic activities on the Caspian Sea ecosystem, investigating the genetic and ecological dimensions of wintering bird populations is crucial for their conservation.

SSR and ISSR markers represent powerful molecular tools for assessing genetic diversity, lineage relationships, ecological interactions, and selection processes in wildlife populations. SSR and ISSR markers play an integral role in avian genetic studies. The co-dominant inheritance pattern of SSR markers enhances their utility in precise genetic mapping, population structure analysis, and parentage determination (Garza & Freimer, 1996, Sevin Teoman Duran, Saltanat Aghayeva). In contrast, ISSR markers, which do not require genomic resources and function as dominant markers, are particularly advantageous for detecting widespread genetic polymorphisms, making them invaluable in phylogenetic and population genetic research (Bhargava; Fuentes, 2010). Thus, the selection of an appropriate marker type is contingent upon the specific objectives of the research, the desired accuracy, and the availability of resources.

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