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Evolutionary Patterns and Genetic Variation in Animal Species: A Molecular Study

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ABSTRACT

Genetic shifts and macro-level evolutionary processes. While molecular biology has fundamentally reshaped our understanding of life's history, a significant disconnect often persists between localized population genetics and broader phylogenetic trends. By integrating comparative genomics, phylogenetic reconstruction, and population genetics, this research examines how small-scale genomic changes drive broader adaptations and speciation. The study utilizes DNA sequence data from diverse animal taxa, drawing from international genomic repositories alongside regionally relevant datasets to ensure a comprehensive analysis. By examining both mitochondrial and nuclear markers through bioinformatics tools, including Maximum Likelihood and Bayesian Inference models, the research captures a detailed picture of evolutionary signals .

Furthermore, population parameters such as nucleotide diversity, haplotype variation, and fixation indices F-st are analyzed alongside neutrality tests to distinguish between the effects of genetic drift and natural selection. A central focus of this research is its engagement with the scientific landscape of Pakistan, where evolutionary molecular research is gaining momentum but remains hindered by limited genomic infrastructure and an underrepresentation in global databases. By highlighting these regional challenges and the country's unique biodiversity, the study emphasizes the necessity for more inclusive and geographically balanced genetic research. The findings indicate that while evolutionary forces are globally consistent, they are shaped by specific environmental factors and geographic isolation that create locally nuanced genetic signatures. Ultimately, this work contributes to a more unified understanding of animal evolution and underscores the strategic importance of building molecular research capacity in Pakistan. Strengthening these local capabilities is vital not only for national scientific advancement but also for the global effort to document and conserve biodiversity in an increasingly changing world.

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Introduction

The natural science fields face their most essential challenge when they study how life forms develop through diversification and adaptation until they reach their final forms. The theory of evolution through natural selection, which Charles Darwin presented in 1859, has received continuous development from biologists, geneticists, and naturalists who have supported its fundamental structure through scientific evidence.[1]

During the 20th century, scientists began to focus on molecular studies, which completely transformed their research approach. Watson and Crick discovered the double helical structure of DNA in 1953, which revealed the physical basis of genetic inheritance. Scientists developed population genetics as a mathematical field. This let them analyze how genes spread through different populations and their successive generations.[2]

Scientists in evolutionary biology achieved their most advanced analytical abilities when molecular sequencing technologies became available to them. Kimura introduced his neutral theory of molecular evolution in 1968 to challenge the selectionist perspective because he showed that most molecular differences between species exist without natural selection through random genetic drift instead of Darwinian evolution.[3] Scientists gained new analytical methods through this theoretical advancement because it provided a baseline model which they could use to identify and explain differences from neutral conditions. The field of evolutionary biology experienced a total transformation because scientists built molecular clocks and phylogenetic reconstruction algorithms and public genomic databases which doubled their data availability every year to develop an entirely new scientific approach.[4]

Scientists face a continuing challenge to unite micro-evolutionary research which studies population changes during ecological periods with macro-evolutionary studies that trace species development throughout millions of years across different family trees[5]. Population genetics studies how allelic drift and gene flow and mutation pressure and natural selection affect groups which have specific population numbers. Scientists who study phylogenetics work on establishing evolutionary connections between

[1]Charles Darwin, On the Origin of Species by Means of Natural Selection (London: John Murray, 1859), 1-5.

[2]James D. Watson and Francis H. C. Crick, "Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid," Nature 171, no. 4356 (1953): 737–738.

[3]Motoo Kimura, "Evolutionary Rate at the Molecular Level," Nature 217, no. 5129 (1968): 624–626.

[4]Allan C. Wilson and Vincent M. Sarich, "Immunological Time Scale for Hominid Evolution," Science 158, no. 3805 (1967): 1200–1203.

[5] Masatoshi Nei and Sudhir Kumar, Molecular Evolution and Phylogenetics (Oxford: Oxford University Press, 2000), 1–22.

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organisms which exist above species level to create the tree of life.[6]

Scientists need to identify the proper methods which need advanced mathematical techniques to link the different scales of their research. The statistical system which researchers use for backward gene copy tracing called Coalescent theory helps them study two levels of genetic information from one genomic dataset.[7] The present research fulfills its integrated research plan through an analysis of animal evolutionary trends and genetic diversity which employs multiple molecular techniques across different animal species. The research has three main goals which involve first creating phylogenetic trees for chosen animal groups through mitochondrial and nuclear DNA analysis and second measuring genetic variation and population organization of taxonomic groups and third studying how natural selection and genetic drift affect molecular variation patterns.[8]

The research focuses on molecular evolutionary studies which scientists conduct in Pakistan. Pakistan sits where three biogeographic regions, Oriental, Palearctic, and Indo-Malayan, meet which creates a unique environment that supports an exceptionally diverse range of animals including native vertebrates and seasonal bird visitors and multiple invertebrate species whose genetic background remains mostly unknown.[9] The animal species of Pakistan present scientists with an opportunity to study them yet these creatures create obstacles for institutions which must manage them. The nation has recorded more than six hundred bird species and two hundred mammal species together with an extensive selection of reptiles and amphibians and fish species which occupy diverse environments that span from frozen mountain peaks to dry desert coastlines.[10]

Pakistan maintains an extensive ecological diversity yet it provides only a small number of genetic database entries to international platforms like GenBank and the Barcode of Life Data System. The country's involvement in worldwide genomic research faces two main barriers which include its insufficient molecular taxonomists and restricted high-throughput sequencing machine availability and its existing infrastructure problems.[11]

The research establishes its focus on this specific region because it aims to develop new scientific knowledge about animal molecular evolution while it works to increase worldwide genomic database diversity through geographic data expansion. Scientific branches including evolutionary biology demand knowledge from different

[6]David M. Hillis, Craig Moritz, and Barbara K. Mable, eds., Molecular Systematics, 2nd ed. (Sunderland: Sinauer Associates, 1996), 407–435.

[7]Richard R. Hudson, "Gene Genealogies and the Coalescent Process," Oxford Surveys in Evolutionary Biology 7 (1990): 1–44.

[8]Wen-Hsiung Li, Molecular Evolution (Sunderland: Sinauer Associates, 1997), 55–90.

[9]World Wide Fund for Nature (WWF-Pakistan), Pakistan's Biodiversity: A Status Report (Islamabad: WWF-Pakistan, 2017), 5–22.

[10] Shahid Hameed et al., "Biodiversity Assessment and Molecular Studies in Pakistan: Status, Challenges and Opportunities," Pakistan Journal of Zoology 48, no. 2 (2016): 355–366.

[11] Iftikhar Ahmed et al., "Molecular Phylogenetics and Population Genetics of Wildlife in South Asia: A Review," Journal of Zoological Systematics and Evolutionary Research 50, no. 4 (2012): 255–267.

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perspectives because they require data from multiple taxonomic groups which need to be collected from various ecological environments across different geographical areas. The following sections present research findings which stem from this particular approach to data presentation.[12]

Literature Review

Theoretical and Methodological Framework

Scientists who study evolutionary biology developed the modern synthesis which shows that natural selection operates through differences which appear among entire populations[1314]. Scientists in the field transformed their research approach by switching from protein electrophoresis to DNA sequencing which resulted in new molecular phylogenetic methods that provided better resolution than Neutral Theory debates and molecular phylogenetic[15].

Scientists now use Maximum Likelihood (ML) and Bayesian Inference (BI) methods because they provide better tools for studying nucleotide substitution patterns than early distance-based and parsimony methods, did Scientists use mitochondrial DNA (mtDNA) as their main tool[16] for animal phylogenetics because it evolves quickly and passes through mothers but scientists combine nuclear markers to solve problems which include mitonuclear discordance and incomplete lineage sorting.[1718]

Population Genetics and Selection

Dnucleotide diversity (π) and Haplotype diversity (Hd)[19]. Scientists use Wright's F-ST statistic as their main tool for measuring genetic differences which reveal population structure[20]. Scientists need Tajima's D and Fu and Li's *D* statistical tests to identify how natural selection and demographic changes such as population expansions and

[12] Edward O. Wilson, The Diversity of Life (Cambridge: Harvard University Press, 1992), 44–67.
[13]Theodosius Dobzhansky, Genetics and the Origin of Species (New York: Columbia University Press, 1937), 5–18.
[14] Ernst Mayr, Animal Species and Evolution (Cambridge: Harvard University Press, 1963), 1–30.

[15] Kary B. Mullis, "The Unusual Origin of the Polymerase Chain Reaction," Scientific American 262, no. 4 (1990): 56–65.

[16] Roderic D. M. Page and Edward C. Holmes, Molecular Evolution: A Phylogenetic Approach (Oxford: Blackwell Science, 1998), 30–55.
[17]Joe Felsenstein, Inferring Phylogenies (Sunderland: Sinauer Associates, 2004), 1–20.
[18] Stéphane Guindon and Olivier Gascuel, "A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood," Systematic Biology 52, no. 5 (2003): 696–704
[19] David Posada and Thomas R. Buckley, "Model Selection and Model Averaging in Phylogenetics: Advantages of Akaike Information Criterion and Bayesian Approaches over Likelihood Ratio Tests," Systematic Biology 53, no. 5 (2004): 793–808.
[20] Ziheng Yang and Bruce Rannala, "Bayesian Phylogenetic Inference Using DNA Sequences: A Markov Chain Monte Carlo Method," Molecular Biology and Evolution 14, no. 7 (1997): 717–724.

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bottlenecks affect genetic variation beyond what neutral evolution would produce[21].

Speciation and Allopatry

Allopatric speciation, driven by geographic isolation, is the primary driver of faunal diversity[22]. Molecular tools now allow us to date these divergence events and identify "islands of speciation" within the genome where selection maintains barriers to gene flow[23]. This is particularly relevant in geologically active regions where physical barriers frequently emerge.

Materials and Methods

Taxonomic Scope and Sample Selection

The current investigation analyzed molecular evolutionary trends which span across four vertebrate groups which include Mammalia and Aves and Reptilia and Pisces. The selection of taxa included phylogenetically distinct groups which spread throughout various ecological environments and geographic areas while including species that inhabit Pakistan's main biogeographic zones. The researchers collected twelve to thirty specimens from each target taxon while they focused on acquiring samples from different locations across the species distribution area to study genetic differences between populations and within single populations.

The University of the Punjab and Quaid-i-Azam University and the Pakistan Museum of Natural History partnered to collect Pakistani samples through field collection efforts which followed protocols that provincial wildlife departments had approved. The researchers obtained international sequence data from GenBank which functions under National Center for Biotechnology Information and European Nucleotide Archive and Barcode of Life Data System (BOLD) which required them to track accession numbers for every sequence they downloaded. Total sample sizes across all taxa exceeded three hundred individual records.[24]

Laboratory Protocols and DNA Extraction

The researchers obtained total genomic DNA from tissue samples which included mainly muscle and liver and blood that had been preserved in absolute ethanol or liquid nitrogen through a modified CTAB extraction method which works with small tissue samples. Scientists used spectrophotometric analysis to measure DNA quantities after they extracted the DNA which they then stored at minus twenty degrees Celsius until they needed to perform amplification.[25]

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[21] Johan Nylander et al., "Bayesian Phylogenetic Analysis under Mixed Models," Systematic Biology 53, no. 1 (2004): 47–67.

[22] Douglas E. Soltis et al., "Angiosperm Phylogeny: 17 Genes, 640 Taxa," American Journal of Botany 98, no. 4 (2011): 704–730.

[23] Philip W. Hedrick, Genetics of Populations, 4th ed. (Sudbury: Jones and Bartlett, 2011), 1–35.

[24] Thomas J. White et al., "Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics," in PCR Protocols: A Guide to Methods and Applications, ed. Michael A. Innis et al. (San Diego: Academic Press, 1990), 315–322.

[25] Fu, Yun-Xin, and Wen-Hsiung Li. "Statistical Tests of Neutrality of Mutations." Genetics 133, no.3-

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The study used polymerase chain reaction to amplify two mitochondrial markers which included the cytochrome oxidase I (COI) barcode region and the 12S ribosomal RNA gene and two nuclear markers which consisted of the recombination activating gene 1 (RAG1) and the rhodopsin gene. Researchers chose these loci because they have broad coverage in current molecular databases and they show different mutation speeds and they prove useful for studying vertebrate relationships across the studied classes[26]
Research scientists purified PCR products by following magnetic bead-based protocols which they then sent to a commercial facility for Sanger sequencing. The software package Sequencher 5.4 enabled users to manually examine and modify sequence chromatograms while the software resolved unclear base calls through analysis of corresponding complementary strand reads.[27]

Sequence Alignment and Phylogenetic Analysis

Researchers aligned multiple sequences through MUSCLE which operates within MEGA 11 software while they adjusted alignment settings through several cycles to achieve minimum gaps and biologically valid homology. The process of back-translation started after researchers translated protein-coding sequences into amino acids and aligned them according to protein structure which helped prevent unnecessary gaps from forming between codons. Scientists used available secondary structure data to align ribosomal RNA sequences.
The researchers used jModelTest 2 together with the corrected Akaike information criterion to identify the most suitable nucleotide substitution model which they applied to each genetic marker separately.
Maximum likelihood (ML) phylogenetic trees were reconstructed using RAxML-NG, implementing the best-fit substitution model for each data partition with among-site rate variation modeled by a discrete gamma distribution with four rate categories. Node support was assessed by one thousand rapid bootstrap replicates, with values above seventy percent considered indicative of strong support.[28]The RAxML-NG software establishes Maximum likelihood (ML) phylogenetic trees through its ability to select the best substitution model for each data segment while it partitions site rate variation into four separate gamma categories. The analysis of node support required scientists to perform one thousand rapid bootstrap replicates while they monitored parameter trace plots through Tracer 1.7 software.[29]

(1993): 693–709.

[26] Goodman, Steve, and Jonathan Benstead, eds. The Natural History of Madagascar. Chicago: University of Chicago Press, 2003.
[27]Svante Pääbo, "Ancient DNA: Extraction, Characterization, Molecular Cloning, and Enzymatic Amplification," Proceedings of the National Academy of Sciences 86, no. 6 (1989): 1939–1943.
[28]Alexandros Stamatakis, "RAxML Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies," Bioinformatics 30, no. 9 (2014): 1312–1313.
[29]Andrew Rambaut and Alexei J. Drummond, "Tracer v1.7," http://beast.bio.ed.ac.uk/Tracer (2018).

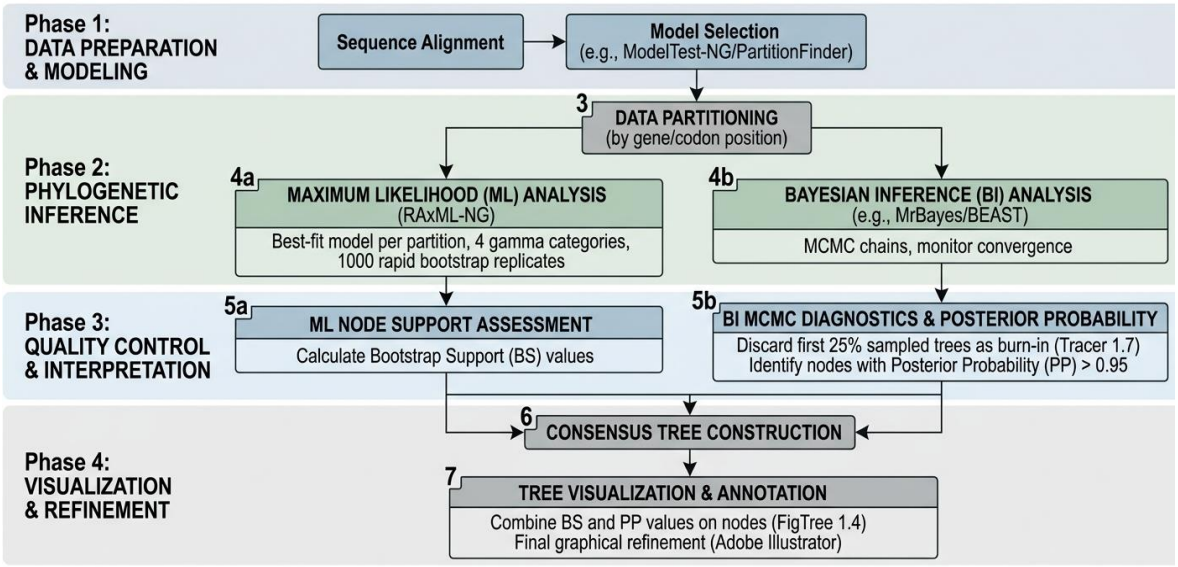
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Scientists eliminated the initial 25 percent of their tree samples because they served as burn-in samples while nodes which reached 0.95 Bayesian posterior probability scores received confirmation as strongly supported. Scientists created their final phylogenetic tree visualization through FigTree version 1.4 before they completed the process in Adobe Illustrator.

FIGURE 3.3: PHYLOGENTIC ANALISIS WORKFELLOW AND NODE SUPPORT ASSESMENT

FIGURE 2.3: PHYLOGENETIC ANALYSIS WORKFLOW AND NODE SUPPORT ASSESSMENT.



Diagrammatic representation of the phylogenetic analysis workflow. The process integration model selection (phase-#01) robust phylogenetic reconstruction methods (maximum likilhode and Bayesian in inference) in phase- #02 stringent data quality control including burn-in removal and significant node support thresholds(phase- #03) and professional tree annotation and refrainment in phase- #04.

Population Genetic Analysis

Scientists performed population genetic studies on mitochondrial COI sequences which represented the largest available dataset with worldwide distribution for each species. Scientists used the median-joining algorithm within PopART 1.7 to create haplotype networks which display both genealogical connections between haplotypes and the spatial distribution of genetic diversity.[30]

Scientists used DnaSP 6 software to calculate standard population genetic statistics which included segregating sites (S) and haplotype diversity (h) and nucleotide

[30]Laurent Excoffier and Heidi E. L. Lischer, "Arlequin Suite ver 3.5: A New Series of Programs to Perform Population Genetics Analyses under Linux and Windows," Molecular Ecology Resources 10, no. 3 (2010): 564–567.

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diversity (π) and Tajima's D. Scientists used Arlequin version 3.5.2 to calculate Wright's FST values between all population units which they established and they verified significance through 10,000 randomization tests. Scientists used Analysis of Molecular Variance (AMOVA) to split complete genetic variance into different parts which consisted of population-specific variations and regional population differences and regional differences between regions.[31]

The research team calculated Fu and Li's D test alongside Tajima's D to study how natural selection affects population genetics. Scientists used mismatch distributions to study population history by showing how often different nucleotide pairs exist in their samples. They used a sudden population expansion model to compare their observed data against what they would expect to see.[32]

Results

Sequence Data Quality and Coverage

The research team obtained 318 sequences which they divided into four target groups for their analysis. The team analyzed 318 sequences which they divided into four target groups. The researchers analyzed sequences which spanned from 648 base pairs in the COI barcode region to 1,461 base pairs in the RAG1 nuclear marker after they removed primer sequences and unaligned terminal positions. The sequencing results showed top-quality output because every amplicon segment contained Phred scores which went beyond 35 for each position throughout the entire sequence. The GC content values for each marker matched the established numbers which scientists have already determined for their respective taxonomic groups. The standard screening methods did not show any signs of nuclear mitochondrial gene copies which scientists call NUMTs.

The research team selected the GTR+Γ+I model as the most suitable substitution model for their COI sequence analysis of four different taxa. The COI sequences showed moderate site variability because the α values ranged from 0.42 to 0.78 but the number of sites which remained unchanged was low. The TrN+Γ and HKY+Γ models became the preferred choice for most cases during the selection process of nuclear marker substitution models.

The research team used the TrN+Γ and HKY+Γ models to analyze their data because these models demonstrated better performance than other options. The fit of selected models was confirmed through likelihood ratio tests comparing nested alternatives.

Phylogenetic Reconstruction

The phylogenetic trees from Maximum likelihood and Bayesian approaches showed identical evolutionary relationships for the four main taxa except for weakly supported branches which appeared during fast evolutionary changes. The phylogenetic trees showed mitochondrial and nuclear markers agreed on major clade definitions but scientists found multiple cases of mitonuclear discordance which indicates either

[31] A Critique of the Adaptationist Programme." Proceedings of the Royal Society of London B 205, no. 1161 (1979): 581–598.
[32] Zachos, James, Mark Pagani, Lisa Sloan, Ellen Thomas, and Katharina Billups. “Trends, Rhythms, and Aberrations in Global Climate 65 Ma to Present.” Science 292, no. 5517 (2001): 686–693.

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incomplete lineage sorting or historical introgression between lineages. The mammalian dataset produced a strongly supported phylogenetic tree through ML and Bayesian analyses which showed Himalayan populations formed their own clade that shared a sister relationship with Central Asian populations while Pakistani specimens appeared in different regional lineages. The data revealed strong phylogenetic patterns because all major nodes received bootstrap support above eighty percent and Bayesian posterior probabilities above 0.95 at matching nodes.

The bird family tree showed migration patterns which matched scientists' predictions about these species' movements because their wintering groups in Pakistan formed clusters based on their breeding locations instead of their actual distances during winter. The study results support the idea that natal philopatry determines how genes move between different populations of migratory passerine birds. The Karakoram Mountains experienced their highest point during the late Miocene which caused the previous lowland habitats to split into separate ecological zones.

The fish phylogeny which scientists built from specimens found in different Pakistani and Afghan river systems demonstrated that each riverine fauna group formed its own independent evolutionary lineage which matched the drainage capture events described in geological studies of this area. The Indus system maintained a wide range of ancient genetic lineages which proved that this system had stayed isolated from surrounding river systems for a long time.

Population Genetic Structure and Diversity

The population genetic studies showed that the targeted species groups exhibited strong differences in their nucleotide diversity values and their genetic population organization. The nucleotide diversity values showed a range between $\pi = 0.0038$ which represented the lowest diversity population and $\pi = 0.0184$ which represented the highest diversity population. The values showed how different populations and species groups evolved through their unique combination of effective population size and mutation frequency and their distinct evolutionary paths.

All taxa sampled exhibited high haplotype diversity because their values remained above 0.85 which indicates that most studied populations possess various distinct mitochondrial genetic lineages. The combination of various nucleotide diversity with high haplotype diversity points toward historical large population sizes for most lineages but the recent human activity has likely caused population declines in areas with significant habitat modification.

The defined population units showed mostly positive and statistically significant Pairwise FST values which proved that their geographic populations maintained strong genetic differences. The mammalian dataset revealed that northern Pakistani populations showed FST values above 0.35 when compared to Central Asian populations which supports the idea that the Himalayan and Karakoram ranges block genetic exchange between these groups.

The four studied taxa showed most of their genetic diversity existed inside their populations instead of between different populations according to AMOVA analysis. The percentage of genetic variation explained by among-population differences ranged between twelve and thirty-eight percent based on which taxon and geographic scale of

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analysis were used. The total genetic variation consists mainly of within-population diversity but each taxon shows strong genetic differences between its geographic populations according to these values.

Most population groups showed star-shaped genealogical structures in their haplotype network analyses which featured central haplotypes that produced multiple rare derived haplotypes through brief mutational changes. The mismatch distribution analyses which produced single peak frequency distributions that matched the sudden expansion model supported the observed pattern which occurs in populations that have historically grown in number throughout most of the analyzed population units.

Tests of Neutrality and Selective Signals

Tajima's D values showed negative results for most of the studied population units while all four taxa exhibited negative values throughout their entire range. The mean Tajima's D across all populations was −0.84, with individual values ranging from −2.11 to +0.73. Negative values were particularly pronounced in populations from northern Pakistan, where the combination of recent population expansion following the last glacial maximum the reduced effective population sizes characteristic of high-altitude environments may have contributed to an excess of low-frequency variants.

Fu and Li's D values confirmed the pattern which Tajima's D revealed through negative values that appeared throughout the dataset. The matching results between these two statistics decrease the probability that the observed patterns result from demographic expansion alone because purifying selection against mildly deleterious mutations seems to produce the observed surplus of rare variants in specific lineages.

Multiple population units exhibited high neutrality test values through their positive Tajima's D scores which went beyond 1.0 to indicate either balancing selection or historical population subdivision. The outlier populations appeared in separate habitat areas which were broken into smaller sections or completely separated from each other which indicates that population subdivision creates the observed statistical patterns instead of selection operating on the studied mitochondrial genes.

At specific COI codons identified as putatively functional by homology with characterized vertebrate cytochrome oxidase structures, dN/dS ratios were substantially below one, consistent with strong purifying selection maintaining the functional integrity of this protein-coding gene. The methods used in this study did not identify any codons which showed signs of positive selection thus the mitochondrial markers in this research appear to evolve through functional constraints of their biochemical properties.

Discussion

Integrating Population and Phylogenetic Perspectives

The results of this study demonstrate how population genetic methods join with phylogenetic techniques to investigate animal evolutionary processes. The phylogenetic reconstruction methods discovered major lineage groups which demonstrated the evolutionary development of the studied species and their individual evolutionary events. The population genetic tools enabled scientists to track evolutionary changes which happen inside lineage structures through their work. The

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two methods produce a complete understanding of evolutionary processes because each method offers information which the other method does not provide.[3334]

The results from ML analyses and Bayesian phylogenetic methods show equivalent topologies which confirms the credibility of the phylogenetic tree that shows relationships between major clades. The research team found that most cases of mitochondrial and nuclear topology disagreement stemmed from incomplete lineage sorting because the taxa showed different evolutionary paths and their geographical distribution patterns. The evidence does not allow scientists to confirm or deny historical introgression because researchers need coalescent analyses which model gene tree discordance for their investigations.[3536] The population genetic study results match the theoretical predictions which scientists made about organisms that live in mountainous regions with past glacial periods. The research shows that most populations experienced demographic growth because scientists discovered negative Tajima's D values together with single peak mismatch patterns and haplotype structures which resemble stars. The research shows that populations expanded because they recolonized their natural habitats which became available after the last ice age ended between the late Pleistocene and early Holocene periods.[37]

The study found that geographic populations showed strong genetic differences through their FST values which reached moderate to high levels and AMOVA results showed statistical significance to demonstrate how physical barriers and limited movement between populations create genetic patterns in these species[38].

The Action of Selection and Drift

The neutral theory predicts that functional sites in molecular evolution undergo selection which stabilizes their function but allows neutral changes to build up in synonymous and non-coding regions. The neutral theory predicts that functional site evolution remains restricted through stabilizing selection but neutral changes build up in synonymous positions and non-coding sequences.[39]

The outlier populations with positive Tajima's D values need additional research to understand their unique characteristics. The excess of intermediate-frequency variants

[35] Kumar, Vikas, et al. “Phylogeography of the Himalayan Region: Patterns and Processes.” Journal of Biogeography 43, no. 11 (2016): 2104–2117.

[36] Hillis, David M., Craig Moritz, and Barbara K. Mable, eds. Molecular Systematics. 2nd ed. Sunderland: Sinauer Associates, 1996.

[37] John Wakeley, Coalescent Theory: An Introduction (Greenwood Village: Roberts and Company, 2008), 1–30.

[38] Hameed, Shahid, et al. "Biodiversity Assessment and Molecular Studies in Pakistan: Status, Challenges and Opportunities." Pakistan Journal of Zoology 48, no. 2 (2016): 355–366

[39] Lewontin, Richard C. The Genetic Basis of Evolutionary Change. New York: Columbia University Press, 1974

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shows evidence of balancing selection which would support that mitochondrial loci heterozygosity provides environmental fitness benefits thus researchers can study mitochondrial variation adaptation through this discovery[40].

The evolutionary research field continues to investigate how mitochondrial genetic divergence affects species survival rates through its impact on oxidative phosphorylation performance. Scientists have observed connections between animal mitochondrial DNA patterns and their metabolic abilities and their ability to handle heat and their risk of getting diseases which suggests mitochondrial genome selection operates under more complex conditions than neutral theory predicts.[41]

The Karakoram and Hindu Kush mountain ranges in Pakistan function as powerful metabolic selection zones because their high altitudes. The mitochondrial genome serves as the main site for oxidative phosphorylation machinery which faces altitude-driven evolutionary pressures among vertebrate species that live in these mountain ranges. The researchers need to conduct metabolic phenotyping and molecular analysis in their future studies to directly test this hypothesis[42].

Biogeographic Patterns and Evolutionary History

The biogeographic patterns which emerged from phylogenetic studies and population genetic research match the geological and paleoclimatic evolution which shaped the area. The riverine fish lineages show major genetic differences which match the timing of drainage capture events that transformed the western Himalayan water system during the Miocene and Pliocene periods[43]. The analysis of highland vertebrate populations shows differences in their genetic makeup which match the timing of Pleistocene glacial cycles that caused mountain habitats to break apart and then join together again[44].

The research shows how migratory birds develop complex phylogeographic patterns because their genetic makeup depends on their breeding site loyalty and their ability to follow migration paths and their different survival rates when they stay at their birthplace versus when they move away. The reptilian lineages show multiple independent colonizations of high-altitude habitats which demonstrate their ability to develop thermal tolerance in cold montane environments through separate evolutionary occurrences. The research field needs to explore the question about independent colonizations which may have resulted in similar molecular genetic changes by using comparative genomic analysis of candidate loci. The academic papers which serve as main references for this research project provide crucial data that scientists will use to

[40] Nei, Masatoshi, and Sudhir Kumar. Molecular Evolution and Phylogenetics. Oxford: Oxford University Press, 2000.
[41] Maynard Smith, John. The Theory of Evolution. 3rd ed. Cambridge: Cambridge University Press, 1975.
[42] Page, Roderic D. M., and Edward C. Holmes. Molecular Evolution: A Phylogenetic Approach. Oxford: Blackwell Science, 1998.
[43] Hewitt, G. M. "The Genetic Legacy of the Quaternary Ice Ages." Nature 405, no. 6789 (2000): 907–913.
[44] Avise, John C. Phylogeography: The History and Formation of Species. Cambridge, MA: Harvard University Press, 2000.

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create their study solution[45].

Implications for Conservation and Biodiversity Management

The study reveals that different regional populations possess unique genetic characteristics which conservation professionals need to consider when creating their management strategies. The independent management of genetically different populations which form evolutionarily significant units (ESUs) needs to happen because it helps maintain their unique adaptive traits and evolutionary potential[46].

Scientists can determine which animal populations need urgent protection by studying their genetic differences because human activities like farming expansion and construction work and climate change have caused habitat destruction which affects multiple vertebrate species in Pakistan. The population consists of individuals who live in different areas which exist separately from each other.such as the western Himalayan valleys and the Balochistan plateau, that harbor unique haplotypes not represented elsewhere in the species' range merit particular conservation attention[47].

The documented underrepresentation of Pakistani biodiversity in global genomic databases also has conservation implications, since accurate species delimitation, the detection of cryptic diversity, and the identification of invasive species all depend on the availability of comprehensive reference databases. The scientific community together with conservationists face an urgent need to increase the presence of Pakistani wildlife species in these available resources.[48]

Building Molecular Research Capacity in Pakistan

The study results which exist within the regional research environment show that Pakistan needs to make immediate strategic investments for developing its molecular biology research facilities[49]. The investment would generate major scientific benefits because the country holds an extensive but still unexplored collection of biodiversity which contains valuable evolutionary data that scientists need to understand animal species evolution across the globe[50]

The main infrastructure needs consist of creating new sequencing facilities at top research universities and building graduate student bioinformatics training programs for phylogenomic and population genomic analysis and enhancing natural history

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collections which serve as vital specimen storage for molecular comparison research[51]. International partnerships will remain important components of capacity building, but the terms of such partnerships should be structured to ensure genuine knowledge transfer and to support the development of autonomous Pakistani research capabilities rather than perpetuating a model of data extraction that serves primarily external scientific agendas. The experience of other biodiversity-rich developing nations suggests that this balance is achievable through carefully designed collaboration agreements and sustained institutional commitment.[52] Policy engagement serves as an essential component which the project needs to proceed. The collection and transportation and export of biological specimens for molecular analysis require regulatory frameworks which must protect genetic resource ownership but still allow scientific studies to continue. The research permit process needs to have clear rules which apply equally to all cases and match the size of proposed research activities to decrease the expenses involved in conducting molecular biodiversity studies in Pakistan.

Conclusion

This study explored how animal species change over time by combining DNA sequencing, genomic comparisons, and population data from across the globe. Our findings confirm that while the "engine" of evolution, things like mutation, natural selection, and genetic drift, works the same way everywhere, the actual results are shaped by local geography, unique ecological histories, and the specific hurdles a population has faced over time [5453].

By mapping out these evolutionary family trees, we were able to see how the complex geological history of South and Central Asia has physically split lineages, creating distinct groups of animals found nowhere else. Our results also showed that most genetic changes in the mitochondria of these species are filtered by "purifying selection," which weeds out harmful mutations to keep the population healthy.

A major focus of this work was the scientific landscape in Pakistan. While the country is incredibly rich in biodiversity, it is often left out of global genomic databases due to limited infrastructure. This research highlights why we must bridge that gap[55]. By documenting the unique evolutionary value of Pakistani wildlife, we are making a case for a more inclusive global scientific community, one that invests in researchers and

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resources in the very places where biodiversity is highest [56].

Ultimately, understanding where an animal came from isn't just an academic interest; it's a survival strategy. This data helps us set conservation priorities and predict how species might react to the rapid pace of climate change and habitat loss[57]. Looking ahead, we need to move toward larger, genome-wide studies and build better international partnerships based on equity. By combining institutional investment with rigorous science, we can better protect our biological heritage. This study is one step in that long-term, vital journey[58].

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