Genetic Diversity and Population Structure of tufted deer (*Elaphodus cephalophus*) in Chongqing, China

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Article

Genetic Diversity and Population Structure of tufted deer (*Elaphodus cephalophus*) in Chongqing, China

Fuli Wang [1], Chengzhong Yang [2], Yalin Xiong [2], Qian Xiang [2], Xiaojuan Cui [1,*] and Jianjun Peng [1,*]

[1] School of Life and Health Sciences, Hunan University of Science and Technology, Xiangtan 411201, China

[2] College of Life Sciences, Chongqing Normal University, Chongqing 401331, China

* Correspondence: xjcui@hnust.edu.cn (X.C.); jjpeng321@hnust.edu.cn (J.P.); Tel: +86-18873229329 (X.C.); +86-18203032060 (J.P.)

Simple Summary: The tufted deer (*Elaphodus cephalophus*) is a rare and endangered animal that lives only in Southeast Asia, and protecting it requires understanding how healthy and connected its populations are. This study looked at three groups of tufted deer living in different mountains in China to see how genetically diverse they are and how they exchange genes with each other. By analyzing specific parts of their DNA, researchers found that one area, Simian Mountain, has the most genetic diversity, making it very important for the species' survival and gene flow. The study also revealed that one group, Jinfo Mountain, is more isolated, likely due to its unique terrain, which could hinder its ability to mingle with other groups. Evidence suggests that the overall population has recently expanded, but some groups remain separated. These findings highlight the importance of conserving and managing these deer populations carefully, especially by protecting key areas like Simian Mountain and creating ways for the animals to move between habitats. The results will help scientists and conservationists develop effective strategies to safeguard the tufted deer and ensure the future of this unique species.

Abstract: Effective conservation and management of the endangered tufted deer (*Elaphodus cephalophus*) necessitate genetic diversity research. This study systematically assessed the genetic diversity, population structure, gene flow, and demographic history of three representative tufted deer populations in Chongqing, China (Jinfo Mountain, Simian Mountain, Northeastern Mountainous area) using combined mitochondrial Cytochrome b (Cyt b) gene and D-loop region analysis. Our findings reveal the D-loop region demonstrates significantly higher genetic variability and a faster mutation rate than Cyt b. The Simian Mountain population consistently displayed the highest haplotype diversity, suggesting its role as a genetic diversity hotspot and crucial hub for regional gene flow. Haplotype network analysis showed frequent gene exchange between Simian Mountain and Northeastern Mountainous populations, while the Jinfo Mountain population exhibits significant genetic isolation, likely due to unique topographical features. Historical demographic analyses suggest a likely slow expansion, with evidence for recent population expansion despite concurrent regional genetic isolation. This study underscores the critical importance of multi-scale genetic markers for deciphering species' evolutionary histories and provides essential groundwork for tufted deer conservation. We recommend prioritizing Simian Mountain conservation, enhancing Jinfo Mountain genetic management, and maintaining regional population connectivity through ecological corridors and continuous monitoring.

Keywords: tufted deer; mitochondrial DNA; genetic diversity; Chongqing

1. Introduction

The tufted deer (*Elaphodus cephalophus*), the sole species within the genus *Elaphodus* of the Cervidae family, is classified as Near Threatened (NT) by the International Union for Conservation

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of Nature (IUCN) Red List of Threatened Species [1]. Characterized by its yellowish-brown coat, prominent brown frontal tuft, and short, unbranched antlers [2]. the tufted deer possesses unique morphological features that underscore its phylogenetic significance. Endemic to Southeast Asia, this species primarily inhabits mountainous forests in southeastern China [1,2]. Chongqing, situated on the southeastern rim of the Sichuan Basin, features a landscape dominated by mountains and hills [3]. This distinctive environment provides an ideal habitat for the tufted deer, facilitating the establishment of stable populations within the region.

The unique genetic background of tufted deer makes the study of its genetic diversity highly valuable for developing conservation strategies, understanding population differentiation, and exploring adaptive evolution. Analyzing microsatellite markers, mitochondrial DNA, or whole-genome data allows for the assessment of population genetic structure, demographic history, and environmental adaptability [4–7]. This information is crucial for comprehending current population status and formulating precise conservation strategies aimed at promoting gene flow and preserving genetic resources. From an ecological and evolutionary perspective, genetic diversity reflects a species' health, adaptation mechanisms, and evolutionary history, helping to decipher the resilience of tufted deer to environmental changes [8–10] Furthermore, high levels of genetic diversity enhance a species' environmental adaptability and disease resistance, providing vital indicators for species classification and ecosystem health monitoring [11–13].

Mitochondrial DNA (mtDNA) exists in multiple copies per cell, enabling its efficient amplification via Polymerase Chain Reaction (PCR) even from samples with low DNA concentration or significant degradation [14]. Furthermore, mtDNA is predominantly maternally inherited and lacks sexual recombination, conferring unique advantages for investigating population genetic structure, phylogeny, and demographic history [9,15,16]. Specifically, the cytochrome b (Cyt b) gene, a protein-coding region, evolves at a moderate rate [9]. This allows it to retain sufficient variability for detecting genetic differentiation and long-term divergence among populations while remaining relatively conserved [17]. Conversely, the D-loop region, a highly variable non-coding control region, is instrumental in revealing fine-scale genetic structure, haplotype distribution, and recent population dynamics within populations [18–20]. The combined analysis of these two markers leverages their complementary strengths, enhancing the reliability of genetic diversity assessments and yielding a more comprehensive and accurate understanding of population history, structure, and dynamic changes [21–23].

Despite the tufted deer being an endemic Southeast Asian species with a widespread distribution and unique genetic background, systematic research into its genetic diversity and population structure remains limited. A 2016 study utilized mtDNA control region (CR) and nuclear microsatellite markers to analyze the genetic structure and gene flow between two tufted deer populations in Bashan and Wuling Mountains, separated by the natural barrier of the Yangtze River [24]. However, the overall genetic landscape of the species has yet to be fully elucidated. This study, for the first time, integrates mtDNA Cyt b gene and D-loop region analyses to systematically assess the genetic diversity, population structure, gene flow, and evolutionary dynamics of tufted deer populations in Chongqing, China, thereby addressing a critical research gap and providing a scientific basis for species conservation and management.

2. Materials and Methods

2.1. Sample Collection and DNA Extraction

To minimize disturbance to the target species, this study employed a non-invasive fecal sampling approach to obtain genetic material from tufted deer. Host DNA was extracted from collected tufted deer fecal samples [25–28]. Between 2022 and 2023, a total of 46 tufted deer fecal samples were randomly collected from distinct forest regions within Chongqing, China: Jinfo Mountain National Nature Reserve (JF, n=13); Simian Mountain National Nature Reserve (SM, n=21);

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and the northeastern mountainous areas of Chongqing (NEM, n=12) (Figure 1). During sampling, personnel wore sterile disposable gloves, and collected samples were immediately stored at -80°C.

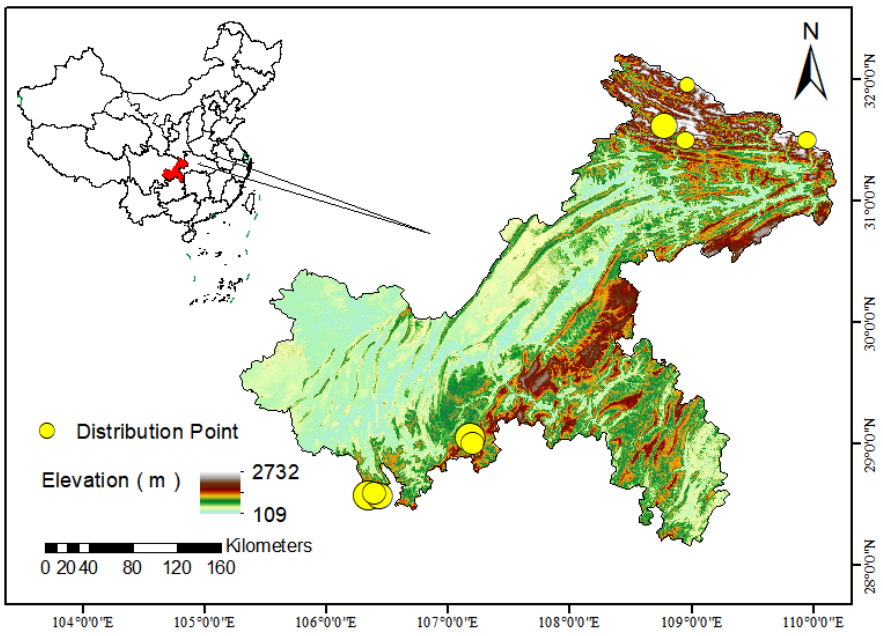

Figure 1. Depicting tufted deer fecal sample collection sites.

Host DNA was extracted from fecal samples using the QIAamp DNA Stool Mini Kit. Subsequently, DNA concentration and purity were measured with a micro-volume spectrophotometer (AuSine/Nano-400A) before storage at -20°C for later use.

2.2. PCR Amplification, Gene Cloning, and Sequencing

For the amplification of mtDNA, Polymerase Chain Reaction (PCR) was employed with specific primer pairs. The forward primer for the Cyt b gene was 5′-CAAACGGAGCATCAATGTT-3′, and the reverse primer was 5′-TGTCTCGTGGAGAAAGAGT-3′. Additionally, the D-loop region was targeted with the forward primer 5′-TAAGTCAAATCAGTCCTCGTCAA-3′ and the reverse primer 5′-GTTAAGTCCAGCTACAATTCATG-3′. PCR reactions were performed using TIANGEN's 2× Taq PCR MasterMix II. The thermocycling program consisted of an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds (denaturation), 57°C for 30 seconds (annealing), and 72°C for 1 minute (extension). A final extension step was carried out at 72°C for 5 minutes.

PCR products were purified using the Biospin Gel Extraction Kit and subsequently cloned into the pTOPO-TA/Blunt Cloning Kit (Aidlab). Individual colonies were inoculated into 400 μl of Luria-Bertani (LB) medium containing 100 μg/ml ampicillin (Amp) and incubated at 37°C with shaking for 2 hours. Colony PCR was then performed to identify positive clones, and sent to Bioscience Company for sequencing(Changsha, China).

2.3. Data Analysis

After obtaining sequencing results, bases were manually verified against electropherograms to ensure accuracy. Sequences were then compared with entries in NCBI BLAST [29] to confirm their derivation from tufted deer. MEGA11 software was used for multiple sequence alignment and trimming, as well as for calculating haplotype genetic distances and nucleotide composition [30]. To analyze population genetic structure, DnaSP6 was employed to compute nucleotide diversity (Pi), number of haplotypes (H), haplotype diversity (Hd), and average nucleotide differences (K), along

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with mismatch distribution analysis [31]. Analysis of molecular variance (AMOVA) and genetic differentiation indices (F_{st}) were obtained using Arlequin 3.5 [32], Neutrality tests, including Tajima's D and Fu's FS, were also conducted. Gene flow (N_m was calculated using the formula $N_m = 0.25(1 - F_{st})/ F_{st}$ [33]. A median-joining haplotype network was constructed with PopART 1.7, and neighbor-joining (NJ) trees were generated using MEGA11 to visualize relationships among haplotypes [34,35].

3. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.1. Sequence Characteristics and Genetic Diversity

Mitochondrial DNA analysis of three muntjac populations (JF/SM/NEM) from Chongqing, China, revealed distinct sequence characteristics. The Cyt b gene exhibited a notable AT bias (AT = 58.4%, GC = 41.6%) (Table 1). In contrast, the D-loop region displayed a more balanced nucleotide composition (AT = 51.7%, GC = 48.3%) (Table 1). This relatively higher GC content in the non-coding D-loop region suggests potential differential selective pressures, indicating distinct adaptive evolutionary patterns. The AT-rich patterns observed across both sequences align with the typical evolutionary trajectory of mammalian mitochondrial DNA, consistent with established mitochondrial gene characteristics.

Table 1. Nucleotide Composition of Cyt b Gene and D-loop Region in Muntjac Populations.

Items	Base composition (%)						
	T(U)	C	A	G	AT(U)	GC	Total
Cyt b	30.7	24.5	27.7	17.1	58.4	41.6	327
D-loop	28.1	27.5	23.6	20.8	51.7	48.3	334

Genetic variation in the D-loop region was markedly higher than in the Cyt b gene, as evidenced by three key indices: (1) a greater number of polymorphic sites (D-loop S=43 vs. Cyt b S=17); (2) higher nucleotide diversity (π=0.02127 vs. 0.01424); and (3) richer haplotype diversity (Hd=0.97295 vs. 0.96522) (Table 2). These results are consistent with the inherently high variability of the D-loop, indicating that this region is especially sensitive to recent mutational events and can effectively reflect dynamic evolutionary processes such as population expansion and gene flow.

Population-specific analyses revealed the following patterns: (1) For the Cyt b gene, the NEM population exhibited the highest genetic diversity (Hd=0.96970; π=0.01602), suggesting a historically large effective population size or greater demographic stability; (2) For the D-loop region, the SM population displayed the highest diversity (Hd=0.97143; π=0.01982), implying either more extensive gene flow or enhanced habitat connectivity; and (3) The JF population showed the lowest diversity at both markers (Cyt b Hd=0.87179; D-loop Hd=0.91026), which may indicate genetic isolation or historical bottleneck events.

Table 2. Genetic diversity indices of the Cyt b gene and D-loop region in tufted deer.

Genetic Marker	Group	Polymorphism Site Analysis (S)	Number of Haplotypes (H)	Haplotype Diversity (Hd)	Average Number of Nucleotide Differences (K)	Nucleotide Diversity (π)
Cyt b	JF	8	6	0.87179	2.74359	0.00839
	SM	12	12	0.90952	4.04762	0.01238

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	NEM	13	10	0.96970	5.16667	0.01602
	Total Data Estimates	17	25	0.96522	4.65700	0.01424
	JF	18	9	0.91026	3.92308	0.01178
	SM	25	16	0.97143	6.60000	0.01982
D-loop	NEM	20	10	0.95455	7.96970	0.02393
	Total Data Estimates	43	30	0.97295	7.08213	0.02127

3.2. Haplotype Distribution and Genetic Structure Analysis

Analysis of the Cyt b gene region revealed 25 distinct haplotypes (JF=6, SM=12, NEM=10), with Hap_11 identified as the dominant haplotype. In contrast, the D-loop region exhibited a higher number of haplotypes (30 in total, JF=9, SM=16, NEM=10), where Hap_4 emerged as a predominant haplotype shared across populations (Table 3). Notably, the SM population demonstrated the highest haplotype diversity in both genetic markers, indicating substantial genetic variation within this group. This high diversity not only suggests a potentially enhanced adaptive capacity but also implies a unique evolutionary history or ecological niche advantage for the SM population.

Table 3. Haplotype distribution of tufted deer in the Cyt b gene and D-loop regions.

Items	Haplotypes	Individual No.	Number
Cyt b	Hap_1	JF-01, JF-02, JF-03, JF-11	4
	Hap_2	JF-04, JF-05	2
	Hap_3	JF-06, JF-13	2
	Hap_4	JF-07	1
	Hap_5	JF-08, JF-12	2
	Hap_6	JF-09, JF-10	2
	Hap_7	SM-01, SM-21	2
	Hap_8	SM-02, SM-16	2
	Hap_9	SM-03, SM-17	2
	Hap_10	SM-04	1
	Hap_11	SM-05, SM-06, SM-07, SM-09, SM-13, SM-19	6
	Hap_12	SM-08	1
Items	**Haplotypes**	**Individual No.**	**Number**
	Hap_13	SM-10, SM-20, NEM-11	3
	Hap_14	SM-11, NEM-08, NEM-10	3
	Hap_15	SM-12	1
	Hap_16	SM-14, NEM-01	2
	Hap_17	SM-15	1
	Hap_18	SM-18	1
	Hap_19	NEM-02	1
	Hap_20	NEM-03	1
	Hap_21	NEM-04, NEM-06	2
	Hap_22	NEM-05	1
	Hap_23	NEM-07	1
	Hap_24	NEM-09	1
	Hap_25	NEM-12	1
D-loop	Hap_1	JF-01, JF-09, SM-10, SM-12	4
	Hap_2	JF-02, SM-20	1
	Hap_3	JF-03	1
	Hap_4	JF-04, JF-06, JF-10, JF-11, SM-15	5
	Hap_5	JF-05	1

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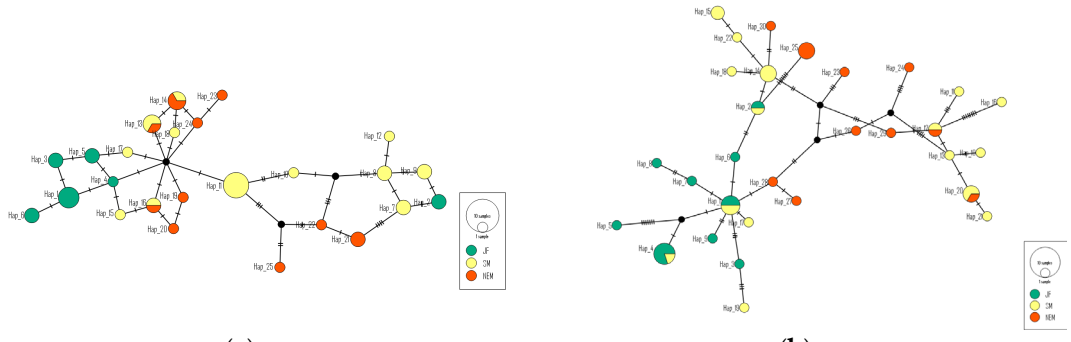
Hap_6	JF-07	1
Hap_7	JF-08	1
Hap_8	JF-12	1
Hap_9	JF-13	1
Hap_10	SM-01	1
Hap_11	SM-02	1
Hap_12	SM-03, NEM-10	2
Hap_13	SM-04	1
Hap_14	SM-05, SM-07, SM-19	3
Hap_15	SM-06, SM-09	2
Hap_16	SM-08	1
Hap_17	SM-11	1
Hap_18	SM-13	1
Hap_19	SM-14	1
Hap_20	SM-16, SM-21, NEM-01	3
Hap_21	SM-17	1
Hap_22	SM-18	1
Hap_23	NEM-02	1
Hap_24	NEM-03	1
Hap_25	NEM-04, NEM-05, NEM-06	3
Hap_26	NEM-07	1
Hap_27	NEM-08	1
Hap_28	NEM-09	1
Hap_29	NEM-11	1
Hap_30	NEM-12	1

Mitochondrial haplotype networks (Figure 2) and Neighbor-Joining (NJ) phylogenetic analyses (Figure 3) collectively elucidated the complex and rich genetic structure and evolutionary relationships of tufted deer populations. Within the Cyt b network, two high-frequency central haplotypes (H13/H14) were broadly shared between the SM and NEM populations, suggesting historical gene flow. The D-loop network, however, displayed greater diversity (30 haplotypes) and a multi-centered structure. Specifically, the SM population occupied a pivotal hub position within this network, connecting various haplotypes and populations, thereby acting as a crucial nexus for genetic exchange and migration. In the NJ tree analysis, both Cyt b and D-loop regions presented a multi-branching structure, without clear evidence of region-exclusive haplotypes, indicating frequent inter-regional migration and gene flow. While dominant haplotypes (Clod I) were widely distributed across populations, some regions still exhibited strong haplotype specificity, reflecting the influence of distinct evolutionary histories or geographical isolation.



(**a**) (**b**)

Figure 2. Haplotype networks of tufted deer based on mitochondrial Cyt b gene (a) and D-loop region (b).

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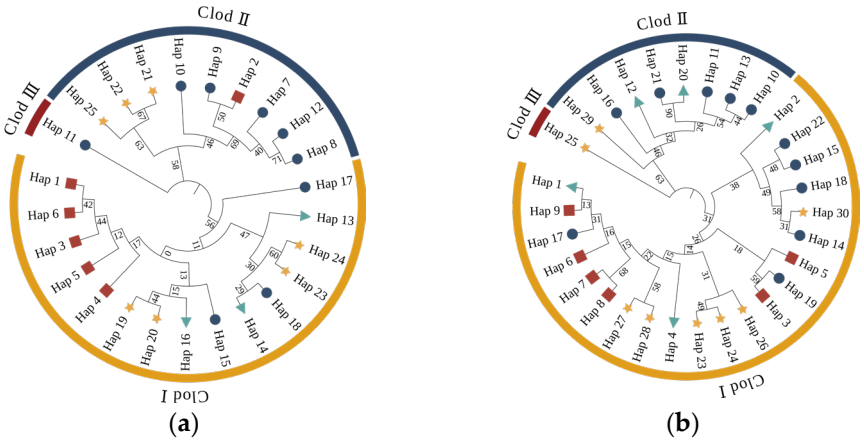

(**a**) (**b**)

Figure 3. Neighbor-Joining (NJ) trees of haplotypes derived from Cyt b gene (a) and D-loop region (b). Note: Rectangles represent haplotypes unique to the JF population; circles indicate haplotypes unique to the SM population; stars denote haplotypes specific to the NEM population; triangles indicate shared haplotypes among populations.

3.3. Genetic Differentiation and Gene Flow

The Mitochondrial Cyt b gene and D-loop region consistently indicated the highest genetic differentiation for the JF population relative to the SM and NEM populations (Cyt b F_{st}: JF-SM=0.27405, JF-NEM=0.25385; D-loop F_{st}: JF-SM=0.23394, JF-NEM=0.26201). This pronounced differentiation suggests significant geographical isolation affecting the JF population. In contrast, genetic differentiation between the SM and NEM populations was notably lower (Cyt b F_{st} =0.09604; D-loop F_{st} =0.06227), implying higher genetic connectivity between these two groups (Tables 4, 5).

Gene flow (N_m) analyses showed notable disparities between the two markers. The D-loop region consistently detected higher gene flow intensities compared to the Cyt b region, particularly between the SM and NEM populations (D-loop N_m =3.76494 vs. Cyt b N_m =2.35307). This discrepancy reflects the D-loop's greater sensitivity to more recent gene exchange events. Intriguingly, the SM population appears to act as a gene flow "hub," exhibiting a noticeably higher unidirectional gene flow towards the JF population (Cyt b N_m =0.66218; D-loop N_m=0.81883) than in the reverse direction. This asymmetrical gene flow pattern may be attributed to the central geographical location of the SM population or its potentially larger effective population size.

Table 4. Genetic Differentiation (F_{st}, upper right) and Gene Flow (N_m, lower left) among tufted deer Populations Based on Cyt b Sequences.

F_{st} / N_m	JF	SM	NEM
JF		0.27405	0.25385
SM	0.66218		0.09604
NEM	0.73482	2.35307	

Table 5. Genetic Differentiation (F_{st}, upper right) and Gene Flow (N_m, lower left) among tufted deer Populations Based on D-loop Sequences.

F_{st} / N_m	JF	SM	NEM
JF		0.23394	0.26201
SM	0.81883		0.06227
NEM	0.70417	3.76494	

3.4. Neutrality Tests and Demographic History

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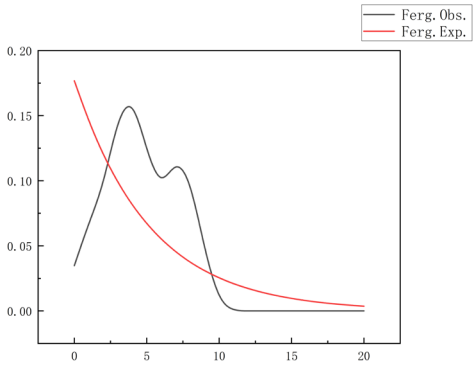
Mitochondrial DNA analyses of three tufted deer populations (JF=13, SM=21, NEM=12) in the Chongqing region revealed distinct demographic histories across different temporal scales (Table 6).

Analyses based on the Cyt b gene consistently showed positive Tajima's D values across all populations (JF=0.24882, p=0.638; SM=0.75692, p=0.780; NEM=0.84804, p=0.841). However, only the NEM population exhibited a significantly negative Fu's FS value (-3.40205, p=0.036). Coupled with a broad, unimodal mismatch distribution (peak at 5-10 nucleotide differences, maximum value 0.15)(Figure 4, a), these findings suggest that all populations may have experienced a slow expansion following late Pleistocene climate fluctuations and have since maintained relatively stable effective population sizes. The genetic signal within the NEM population appears more complex, where the disparity between Tajima's D (positive) and Fu's FS (negative) values might indicate substructure driven by complex topography.

In contrast, the D-loop region revealed more recent demographic dynamics. The SM population exhibited a highly significant negative Fu's FS value (-5.73756, p=0.004), while the JF population, although not statistically significant (p=0.071), also showed indications of expansion (Fu's FS = -2.55315). These characteristics, combined with a unimodal mismatch distribution (peak at 5 differences, maximum value 0.12)(Figure 4, b), strongly support a rapid population expansion during the mid-Holocene (approximately 3,000-5,000 years ago). This expansion was likely influenced by improved climatic conditions and habitat expansion during that period. Concurrently, the positive Tajima's D value for the NEM population (0.89333, p=0.863) suggests either relative genetic stability or the persistence of cryptic substructure. The divergence in results between the two gene regions (Cyt b reflecting ancient history, D-loop reflecting recent events) underscores the critical importance of utilizing multi-scale genetic markers for comprehensive studies of species' demographic histories.

Table 6. Tajima's D and Fu's FS Values for tufted deer Populations Based on Cyt b and D-loop Sequences.

Genetic Marker	Group	Tajima's D	Tajima's D p-value	FS	FS p-value
Cyt b	JF	0.24882	0.63800	-0.31705	0.44100
	SM	0.75692	0.78000	-2.89506	0.08800
	NEM	0.84804	0.84100	-3.40205	0.03600
	Mean	0.61793	0.75300	-2.20472	0.18833
	Standard Deviation	0.32289	0.10416	1.65431	0.22036
D-loop	JF	-1.37337	0.08100	-2.55315	0.07100
	SM	-0.19214	0.48100	-5.73756	0.00400
	NEM	0.89333	0.86300	-2.08194	0.13900
	Mean	-0.22406	0.47500	-3.45755	0.07133
	Standard Deviation	1.13369	0.39103	1.98855	0.06750



(**a**)

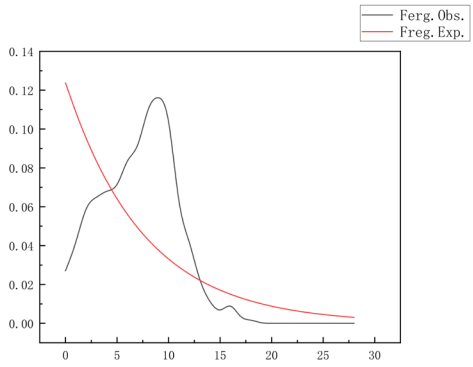


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Figure 4. Observed versus Expected Mismatch Distribution Curves for tufted deer Populations Based on (a) Cyt b and (b) D-loop Sequences.

4. Discussion

Genetic diversity analysis of tufted deer populations in Chongqing revealed significant inter-population differences. The mitochondrial Cyt b gene region exhibited a notable AT bias (58.4%, T=30.7%), consistent with the typical characteristics of mammalian mitochondrial coding genes [36–38]. This bias may reflect evolutionary pressures related to energy metabolism. In contrast, the D-loop region displayed a more balanced nucleotide composition (AT=51.7%, GC=48.3%), indicating less base preference in this non-coding region, potentially influenced by replication or transcriptional regulation. Comparatively, the D-loop region showed markedly higher genetic diversity than Cyt b across several metrics: number of polymorphic sites (S=43 vs. 17), nucleotide diversity (π=0.02127 vs. 0.01424), and haplotype diversity (Hd=0.97295 vs. 0.96522). This aligns with the D-loop's known rapid mutation rate and its higher sensitivity in detecting recent population changes [17,18]. Among the studied populations, the NEM population exhibited higher Cyt b diversity (Hd=0.96970, π=0.01602). This implies that the NEM population either experienced considerable historical expansion or maintained a larger effective population size [39,40]. Conversely, the SM population showed the highest D-loop diversity (Hd=0.97143, π=0.01982), which may indicate stronger gene flow and better habitat connectivity within this region [41]. The significantly lower diversity observed in the JF population (Cyt b Hd=0.87179; D-loop Hd=0.91026) is potentially linked to a historical bottleneck event [42].

Mitochondrial haplotype network analysis (Figure 2) and Neighbor-Joining (NJ) tree analysis (Figure 3) collectively illustrate the complex and diverse genetic structure and evolutionary history of tufted deer populations. Within the Cyt b network, two high-frequency central haplotypes (H13 and H14) were broadly shared by the SM and NEM populations. This strongly indicates historical gene flow between these two populations. Such shared central haplotypes are typically characteristic of recent population expansion or remnants of historically larger populations. In contrast, the D-loop network exhibited higher diversity, comprising 30 haplotypes, and a more intricate multi-centric structure [43]. Notably, the SM population displayed the richest haplotype diversity across both mitochondrial markers, possessing the highest number of unique haplotypes and reflecting the greatest genetic variability within the entire dataset. This not only suggests a potentially stronger adaptive capacity for the SM population but also implies a unique evolutionary history or ecological advantage [44], such as serving as a diffusion center or a glacial refugium [45]. The analysis of the NJ tree for both the Cyt b and D-loop regions demonstrated the presence of multiple branches, with no distinct regional-specific haplotype clusters. This suggests frequent migration and gene flow among regions, leading to genetic admixture across populations. Notably, both Cyt b and D-loop regions contained low-frequency, region-specific haplotypes. This may reflect the influence of geographical isolation or local adaptation during historical evolution in different areas, allowing certain genetic lineages to evolve relatively independently. Overall, haplotype analysis revealed a complex genetic structure in tufted deer populations, demonstrating both extensive gene flow and some degree of regional specificity, indicating potentially intricate migration and dispersal pathways.

Population genetic structure analysis revealed persistently high gene flow between the SM and NEM populations (N_m > 2.35). This suggests that the Yangtze River does not present an absolute barrier to isolation for these two habitats, a finding consistent with Sun et al. (2016), who also noted that the Yangtze River is not an insurmountable barrier for gene exchange in certain species [24]. In contrast, the JF population, sampled from the Jinfo Mountain National Nature Reserve, exhibited significant genetic isolation (F_{st} > 0.23). This strongly indicates substantial geographical isolation surrounding this reserve. The unique and steep topography of Jinfo Mountain likely restricts gene flow between this population and other groups [46]. Notably, the D-loop region detected stronger recent gene flow, approximately 60% higher than that observed in Cyt b. This suggests that while

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Jinfo Mountain National Nature Reserve provides habitat protection, ongoing habitat fragmentation due to surrounding human activities may still exacerbate genetic isolation.

The evolutionary trajectory of tufted deer populations exhibits multi-scalar complexity. Overall, the consistently positive Tajima's D values suggest that populations may have experienced a period of slow or stable expansion [47,48]. Specifically, the unimodal mismatch distribution (peak at 5 differences) and negative Fu's Fs values for the mitochondrial D-loop region, particularly the significantly negative Fu's Fs value for the NEM population (–3.40) and the SM population (–5.73), strongly support a hypothesis of recent population expansion or recovery. This expansion might be attributable to favorable conditions arising from climate change or other environmental factors, which facilitated rapid population growth and an increase in genetic diversity [49–52]. However, at a regional scale, significant genetic differentiation and restricted gene flow were observed among different populations, indicating a degree of geographical or historical isolation. Notably, the SM and NEM populations maintained relatively strong gene flow, while the JF population remained comparatively isolated.

Based on genetic analyses of the three tufted deer populations (SM, JF, NEM) in Chongqing, the following conservation recommendations are proposed: (1) Prioritize protection of the Simian Mountain (SM) population, which exhibits the highest genetic diversity among the regional groups. (2) For the Jinfo Mountain (JF) population, characterized by significant genetic isolation ($F_{st} > 0.23$) and lower haplotype diversity (Hd = 0.87179–0.91026), measures such as establishing ecological corridors, implementing regular genetic monitoring, and pursuing assisted migration should be adopted to promote gene flow. (3) Develop a long-term genetic monitoring and research framework, incorporating nuclear genome analysis to validate mitochondrial DNA findings and track temporal changes. These strategies aim to enhance population connectivity and conserve genetic diversity, ensuring sustainable management of tufted deer in Chongqing.

5. Conclusions

This research comprehensively evaluated the genetic diversity and population structure of tufted deer in the Chongqing area by integrating analyses of the mitochondrial Cyt b gene and D-loop regions. Our findings reveal rich genetic variation and diverse migration patterns within these populations. High genetic diversity is predominantly concentrated in the Simian Mountain area, indicating its crucial role in maintaining the species' genetic resources. Conversely, the observed genetic isolation of the Jinfo population highlights the need for enhanced connectivity conservation. Overall, the presence of both isolation and exchange among different populations poses varying pressures on their long-term survival. Therefore, we recommend implementing targeted regional protection, ecological corridor construction, and continuous monitoring strategies to ensure the genetic diversity and ecological stability of tufted deer.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.: Figure S1: PCR electrophoresis results of tufted deer Cyt b gene and D-loop region.s; Figure S2: Electrophoresis results of gel-extracted PCR products from the tufted deer Cyt b gene and D-loop region; Figure S3: Colony PCR electrophoresis results of the tufted deer Cyt b gene and D-loop region.

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