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Postprint: Genetic Structure of Shennongjia Sichuan Snub-Nosed Monkeys Based on Microsatellite DNA

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Abstract

The Sichuan snub-nosed monkey (Rhinopithecus roxellana) is a rare and endangered species endemic to China, and understanding its population genetic structure and key influencing factors is of great significance for the conservation of this species. This study focused on the Sichuan snub-nosed monkey population in Shennongjia, Hubei, the easternmost distribution in China, and investigated the genetic diversity and genetic structure of this population based on non-invasive DNA technology, molecular biology methods such as microsatellite DNA genetic markers, and landscape genetic parameters, aiming to provide a theoretical basis for research on the Sichuan snub-nosed monkey and the sustainable development of its populations. Using 12 polymorphic microsatellite loci, a total of 62 microsatellite alleles were detected in 455 fecal samples of Sichuan snub-nosed monkeys; 316 distinct individuals were identified; the population' s mean expected heterozygosity, mean observed heterozygosity, and polymorphic information content were 0.626, 0.559, and 0.650, respectively; Nei' s genetic distance among groups ranged from 0.046 to 0.139, and the differentiation coefficient ranged from 0.015 to 0.046. The results indicate that, compared with Sichuan snub-nosed monkey populations in other regions, the Shennongjia population has a relatively low level of genetic diversity, and there is a trend of genetic differentiation within the population; combined with landscape parameter analysis, it was shown that geographic distance is not the main factor affecting genetic distance among Shennongjia Sichuan snub-nosed monkey groups, but shrubs and grasslands in the habitat, as well as human activity disturbance, may be the main factors affecting genetic exchange in the Sichuan snub-nosed monkey.

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Full Text

Preamble

Genetic Structure of the Golden Snub-Nosed Monkey in Shennongjia National Nature Reserve Based on Microsatellite DNA Markers

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Abstract: The golden snub-nosed monkey (*Rhinopithecus roxellana*) is an endemic and extremely endangered species in China. Understanding its population genetic structure and key influencing factors is critical for the conservation of this species. This study investigated the genetic diversity and structure of the golden snub-nosed monkey population in Shennongjia National Nature Reserve—the easternmost isolated population in China—using non-invasive sampling techniques, microsatellite genetic markers, molecular biology methods, and landscape genetic parameters. The findings provide a theoretical foundation for research and sustainable development of golden snub-nosed monkey populations. From 455 fecal samples, 316 individual golden snub-nosed monkeys were identified across 12 polymorphic microsatellite loci. The population' s mean expected heterozygosity, mean observed heterozygosity, and polymorphism information content were 0.626, 0.559, and 0.650, respectively. Nei' s genetic distance ranged from 0.046 to 0.139, and the fixation index (FST) ranged from 0.015 to 0.046. Compared with other regional populations, the Shennongjia golden snub-nosed monkey population exhibited relatively low genetic diversity with a trend of internal genetic differentiation. Landscape parameter analysis indicated that geographic distance was not the primary factor affecting inter-group genetic distance; rather, shrub and grassland habitats and human disturbance were the main factors influencing genetic exchange.

Keywords: Shennongjia Nature Reserve; golden snub-nosed monkey (*Rhinopithecus roxellana*); genetic structure; microsatellite marker; landscape genetics

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Introduction

The golden snub-nosed monkey (*Rhinopithecus roxellana*) is a rare and endangered species endemic to China. Due to habitat degradation and other factors, golden snub-nosed monkey populations have become isolated in three regions: Qinling, Sichuan-Gansu, and Hubei Shennongjia [1-3]. The Shennongjia population represents the easternmost distribution, has the smallest population size, and possesses the lowest genetic diversity among the three geographic populations [4-5, 8-9]. However, studies have shown this population plays an important role in the genetic evolution of the species, making its research and conservation critically important [4-7].

Habitat fragmentation can lead to population subdivision, causing significant negative impacts on small, endangered populations [9]. Small, isolated populations affected by habitat fragmentation experience reduced genetic diversity due to genetic drift and inbreeding [10-14]. Landscape genetics, which integrates conservation genetics with habitat and landscape research methods, quantitatively determines how landscape features affect population genetic structure and diversity [15-19]. Chinese scholars have applied this approach to study giant pandas (*Ailuropoda melanoleuca*) [20-22], crested ibis (*Nipponia nippon*) [23-24], Yunnan snub-nosed monkeys (*Rhinopithecus bieti*) [25-26], and Tibetan antelope (*Pantholops hodgsonii*) [27-28].

The golden snub-nosed monkey population in Shennongjia Nature Reserve is concentrated in Dalongtan, Qianjiaping, and Jinhouling, forming three artificial provisioning groups with approximately 1,200 individuals comprising 8-9 one-male multi-female family units and 2 all-male units [29-30]. This study aims to analyze the genetic diversity of Shennongjia golden snub-nosed monkeys, understand gene flow among different groups, identify key landscape and habitat factors hindering genetic exchange, and provide scientific basis for conservation and management strategies.

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1. Study Materials

The Shennongjia golden snub-nosed monkey population is concentrated in Qianjiaping, Jinhouling, and Dalongtan. Fecal samples were collected from 11 sites across four main distribution areas where monkeys frequently appeared. During collection, fresh surface feces were gathered using sterile disposable plastic gloves, stored in Ziploc bags with silica gel, and preserved at -20°C. Sampling time, location, and habitat descriptions were recorded. A total of 455 fecal samples were collected from the Dalongtan group (DLT-1: 53, DLT-2: 23, DLT-3: 60, DLT-4: 18), Jinhouling group (JHL-1: 19, JHL-2: 19, JHL-3: 9, JHL-4: 5), Qianjiaping group (QJP-1: 10, QJP-2: 47), and the Dalongtan provisioned group (DLT-R).

[Figure 1: see original paper] Distribution of golden snub-nosed monkey fecal sampling locations

2. DNA Extraction and Microsatellite Markers

DNA was extracted from fecal samples using the QIAamp DNA Stool Kit (Qiagen) following the manufacturer' s protocol. Twelve polymorphic microsatellite loci with high polymorphism were selected from published literature [8-9, 31] that could be stably amplified in fecal samples: D1S1656, D1S533, D3S1768, D6S1056, D6S474, D6S493, D7S794, D10S1432, D10S676, D17S1290, D3S1766, and D9S905.

3. Microsatellite Amplification and Genotyping

The PCR amplification system consisted of: 1× PCR buffer (MgCl), 0.2 mmol/L dNTPs, 1 mol/L forward fluorescent-labeled primer and 1 mol/L reverse primer, 2 g BSA, 0.6 U HotMaster Taq polymerase, and 10-20 ng DNA template. Cycling conditions were: 94°C for 5 min; 35 cycles of 94°C for 45 s, 50-60°C for 30 s, 72°C for 45 s; final extension at 72°C for 10 min; and storage at 4°C. Negative controls were included in each amplification, and each sample was amplified three times.

4. Data Analysis

Amplification products were analyzed on an ABI-3730XL genetic analyzer using GeneMapper V4.0 software [32] with manual verification. Micro-Checker V2.2.3 software [33] was used to detect null alleles or allele dropout. Cervus software [34-35] calculated the probability of identical genotypes between unrelated individuals (PID) and full siblings (PIDsib). Individuals were identified when samples differed at 1 locus [32]. Samples with genotypes at <8 loci were excluded. Genetic diversity indices including allele number, observed heterozygosity (Ho), expected heterozygosity (He), and polymorphism information content (PIC) were calculated. Genepop V4.0 [36] tested for Hardy-Weinberg equilibrium (HWE) at each locus. Fstat 2.9.3.2 [37] calculated genetic differentiation coefficients (FST) and inbreeding coefficients (FIS) among groups. Genetic distances between populations were calculated, and gene flow was estimated from FST values [38-39]. ArcGIS 9.3 software [40] calculated geographic distances between sampling sites, and isolation-by-distance (IBD) models were tested to examine relationships between geographic and genetic distances [41].

Results

Individual Identification

No null alleles or allele dropout were detected at any locus. The cumulative PID for unrelated individuals was 2.96×10^{-1} and PIDsib was $9.38\times10^{}$, both below the threshold for reliable individual identification. From 455 fecal samples, 316 individuals were identified: Dalongtan group (153), Jinhouling group (52), Qianjiaping group (57), and Dalongtan provisioned group (18).

Population Genetic Diversity

A total of 62 alleles were detected across 12 loci, with 4-7 alleles per locus (mean = 5.17). Allele distribution was uneven, with 35.48% of alleles having frequencies <0.1, accounting for 21.53%-43.56% of low-frequency alleles in different groups. The Shennongjia population showed mean He = 0.626, Ho = 0.559, and PIC = 0.650. Among the four groups, He ranged 0.578-0.639, Ho ranged 0.515-0.610, and PIC ranged 0.600-0.641, with no significant differences among groups.

Population Genetic Structure

Nei's genetic distances among the four Shennongjia groups ranged from 0.046 to 0.139. The overall FST was 0.042, with pairwise FST values ranging from 0.015 to 0.046, corresponding to gene flow estimates of 2.697-21.010 (mean = 8.020). The greatest genetic differentiation occurred between the Dalongtan provisioned group and Jinhouling group, while the smallest was between Jinhouling and Qianjiaping groups. Inbreeding coefficients ranged from -0.048 to 0.095.

Nei' s genetic distances among 11 golden snub-nosed monkey groups at study sites
FST values among 11 golden snub-nosed monkey groups at study sites

Landscape Genetic Analysis

GIS-based habitat suitability mapping revealed that golden snub-nosed monkeys prefer fir-dominated coniferous forests, birch-dominated deciduous broadleaf forests, pine-dominated mixed forests, and other woodlands, with some shrub areas and a road crossing the habitat [43]. Isolation-by-distance models showed no significant correlation between genetic distance and geographic distance (r = 0.214, P = 0.115) or between gene flow and geographic distance (r = 0.137, P = 0.320).

[Figure 2: see original paper] Layered graph of suitable habitat for golden snub-nosed monkeys in Shennongjia National Nature Reserve
[Figure 3: see original paper] Correlation analysis between genetic distance and geographic distance, and between gene flow level and geographic distance for golden snub-nosed monkeys in Shennongjia National Nature Reserve

Discussion

Genetic diversity is essential for maintaining species' adaptive capacity and evolutionary potential. The Shennongjia golden snub-nosed monkey population is an isolated population at the easternmost edge of the species' distribution with relatively small population size. Understanding its genetic structure and key habitat factors is crucial for conservation management.

This study found that Shennongjia golden snub-nosed monkeys have relatively low genetic diversity (He = 0.626, Ho = 0.559, PIC = 0.650) compared to other regional populations [8-9, 44], consistent with previous research [8-9, 44]. The high proportion of low-frequency alleles (21.53%-43.56%) indicates potential risk of allelic loss through genetic drift, which could further reduce heterozygosity and population viability. Small populations are particularly sensitive to genetic drift, where advantageous alleles may be lost while deleterious alleles are retained, leading to inbreeding depression and reduced adaptive potential [11].

The overall FST of 0.042 suggests moderate genetic differentiation among groups. The presence of gene flow (2.697-21.010) indicates opportunities for group fusion, consistent with the species' multi-level social system where stable social structures coexist with group fission-fusion dynamics [47-48]. Given the relatively low genetic diversity, we recommend: (1) managing the sex ratio and age structure in the provisioned group to maintain adaptive potential, and (2) establishing ecological corridors to enhance gene flow.

Landscape genetic analysis revealed that geographic distance does not significantly explain genetic differentiation. Instead, shrub/grassland habitats and human disturbance appear to be primary barriers to dispersal. The Dalongtan-3 group showed greater genetic distance from other Dalongtan groups (0.083-0.167), possibly due to surrounding shrubland that monkeys rarely traverse [49]. Similarly, the tourism road crossing the reserve may impede dispersal, as monkeys avoid crossing during peak tourist seasons (summer) and only occasionally use both sides in winter-early spring [50]. Road construction, pollution, and noise disturbance likely hinder gene flow.

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