Postprint: Parentage Analysis and Mating System of Reeves' s Muntjac Based on Fecal DNA

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Abstract

From April 2014 to January 2015, a total of 634 fecal samples and 2 muscle samples were collected within the Gutianshan National Nature Reserve. Through rigorous screening, 390 samples suitable for PCR amplification were ultimately obtained. Genotyping using 8 highly polymorphic microsatellite loci identified 177 Reeves' muntjac individuals. SRY gene sex identification revealed 84 males and 93 females among the study samples. For the 8 microsatellite loci analyzed in the 177 samples, the mean number of alleles (A) was 11, mean observed heterozygosity (Ho) ranged from 0.960–1.000 with an average of 0.9685, mean expected heterozygosity (He) ranged from 0.799–0.887 with an average of 0.8429, and polymorphic information content (PIC) ranged from 0.766–0.872 with a mean of 0.8214. The high heterozygosity levels indicate a population with rich genetic diversity. Parentage analysis conducted using Cervus 3.0 achieved a 100% assignment rate for the 8 microsatellite loci at both 95% and 80% confidence levels. A total of 24 father-mother-offspring pairs, 23 mother-offspring pairs, and 19 father-offspring pairs were identified, involving 104 individuals. Kinship analysis of the Reeves' muntjac mating system revealed that it belongs to polygyny, but not as a currently recognized subtype; rather, it may represent a form of polygyny termed the "checking strategy."

Full Text

Parentage Verification and Mating System of Chinese Muntjac (*Muntiacus reevesi*) Based on Fecal DNA

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Abstract

Microsatellite technology was utilized in the present study to investigate paternity testing, mating system, genetic diversity, and population quantity of Chinese muntjac (*Muntiacus reevesi*) to increase knowledge of relationships between members of the species, reproductive strategies, and evolutionary potential. Therefore, the present study aims to facilitate in-depth research and protection of the species. We collected 634 fecal samples and 2 muscle samples from Chinese muntjac in Gutianshan National Nature Reserve in January 2013 and April 2014. After repeated experiments, 8 microsatellite loci with stable amplification and high polymorphism information were selected, and 442 samples were utilized for polymerase chain reaction (PCR) amplification. Based on preliminary identification of fecal morphology, a verification test was conducted with the mitochondrial cytochrome *b* gene (*Cyt b*), which served as a supplement to species identification. Individual identification for Chinese muntjac was performed by contrasting the fingerprints synthesized by the eight microsatellite marker genotypes. The *SRY* gene was amplified three times to reduce any false negative influence on the fecal samples, where the target band was identified as male when it appeared more than two times. The *SRY* gene and 8 microsatellite loci were utilized for sexual and individual identification, respectively, and 177 individuals, including 94 females and 83 males, were identified. The results showed that the total number of microsatellite alleles was 88, and the mean number of alleles was 11, ranging from 8 to 16. The mean observed and expected heterozygosities were 0.9685 (0.960–1.000) and 0.8429 (0.799–0.887), respectively. The polymorphism information content ranged from 0.766 to 0.872, with an average of 0.821. Based on the above data, the Chinese muntjac population in Gutianshan National Nature Reserve has a high level of genetic diversity. The identification rate of 8 microsatellite loci from Cervus 3.0 was 100% when the confidence level was 95% and 80%. Among 24 parentage groups, 19 father-child relationships and 23 mother-child relationships were obtained among 104 individuals. The result of the relationship analysis showed that the mating system of Chinese muntjac belongs to polygyny. However, it does not belong to any known subtypes, and this polygyny might be called a "check strategy." During the female reproductive period, once the female individual is pregnant she will not mate with any other males. However, the relationship is not fixed and the female might still mate with another male in the next breeding season. Therefore, in the present study, the mating phenomenon of a female and several males appeared in different breeding periods.

Keywords: Chinese muntjac (*Muntiacus reevesi*); parentage identification; genetic diversity; mating system

Introduction

Muntiacus reevesi is a small deer species (Artiodactyla, Cervidae) endemic to China, widely distributed in central and southern regions of the country, and holds important economic value. In 2008, the International Union for Conservation of Nature (IUCN) Red List of Threatened Species classified it as Least Concern (LC). As deer species that live solitary lives are not only active but also inhabit dense forests, direct observation in the wild is difficult. Consequently, research on the social structure and mating systems of cervids is limited, with studies conducted either through direct observation in captivity or via radio telemetry technology. Current research on Chinese muntjac primarily focuses on macro-ecology, cytogenetics, and evolutionary biology. With the development of non-invasive sampling for wildlife, fecal samples have become the most potentially valuable research material due to their ease of collection. Combined with microsatellite markers, this approach has been widely applied in individual identification, parentage testing, genetic diversity assessment, and home range analysis in cervids. While many studies have used microsatellite markers to investigate mating systems in species such as polar bears (*Ursus maritimus*), broad-snouted caiman (*Caiman latirostris*), and fan-tailed gerygone (*Gerygone flavolateralis*), reports using feces to study mating systems are rare, with only black rhinoceros (*Diceros bicornis*) and black muntjac (*Muntiacus crinifrons*) being documented. This makes molecular biological approaches a new perspective for studying the mating system of Chinese muntjac. Understanding relationships among population members, species' evolutionary potential, and resistance to adverse environments provides a novel research perspective for analyzing the genetic diversity of Chinese muntjac populations. This paper, based on fecal samples of Chinese muntjac, conducts individual identification and sex determination through microsatellite markers, performs parentage testing, and explores the mating system of Chinese muntjac and the role of mating systems in maintaining population genetic diversity, which is significant for developing effective conservation and management strategies and provides a reference for mating system studies in other species.

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1. Sample Collection

The sampling site for this study was Gutianshan National Nature Reserve, located in northwest Sujiang Town, Kaihua County, Quzhou City, Zhejiang Province, bordering Wuyuan, Jiangxi. The reserve has a typical mid-subtropical monsoon climate with distinct seasonal changes, an average annual precipitation of 1963.7 mm, average temperature of 15.3°C, and a frost-free period of approximately 150 days. The reserve, with its main peak Qingjian at 1258 m elevation (118°03 49.7 –118°11 12.2 E, 29°10 19.4 –29°17 41.4 N), features typical mid-subtropical evergreen broad-leaved forest and abundant Chinese muntjac resources, making it representative for research.

To maximize collection of fresh Chinese muntjac feces within the reserve, sampling transects were established following the methodology of Zheng Xiang et al. [16]. Ten repeated samplings were conducted with a unilateral width of 10 m, spacing greater than 6 km between transects to avoid obtaining duplicate fecal samples from the same individual. While adult black muntjac and Chinese muntjac feces are easily distinguishable, feces from juvenile individuals of both species are easily confused due to immature intestinal development, making misidentification likely. For those juvenile samples that could not be definitively identified, sampling was performed using disposable sterile gloves, and fecal samples were placed in sterile bags containing anhydrous ethanol, labeled with collection time, altitude, longitude and latitude, and transported back to the laboratory. A total of fecal samples were obtained from multiple samplings, along with 2 muscle samples obtained locally.

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2. DNA Extraction and Species Verification

Fecal and muscle tissues were processed using the OMEGA-Stool DNA Kit and phenol-chloroform extraction method [18], respectively. Samples were stored at -20°C for subsequent analysis. Based on preliminary identification through fecal morphology, the mitochondrial cytochrome *b* gene (*Cyt b*) was amplified for verification. This method serves as a molecular marker for species identification with advantages of simplicity and accuracy and has been widely applied in mammals [19-21]. *Cyt b* was used as a supplement to species identification.

3. Microsatellite Primer Selection and PCR Amplification

Primers and reaction systems referenced studies by Wang et al. [22-23]. After multiple experiments, 8 microsatellite loci with stable amplification and high polymorphism information content were selected for Chinese muntjac microsatellite analysis. Primers were synthesized by Shanghai Sangon Biotech. When amplifying the sex-determining gene, to reduce the influence of false negatives, the *SRY* gene was amplified three times, with samples showing bands more than twice identified as male. Primer sequences and amplification systems referenced Lu et al. [24]. The eight microsatellite primer sequences are shown in .

** Eight Microsatellite Primer Sequences (5 -3)**

Locus	Primer Sequence (5 -3)	Repeat Types	Allele Size	Annealing Temperature	Fluorophores
Mre61	F: AAGGGGGACGTTG-GTTTGAC R: GATGCCTGT-GTGGACA-GAGTTTGA	(AC)18	5	6FAM	
Mreg25	F: GT-TACAGCTC-CGTTTTAC-CACTCA R: AAAGCCTG-CAAAAATA-GAACAAAG	(AG)24	5	6FAM	
Mreg283	F: GGTAACCT-GCAAGTCCT-GTTC R: GTAGTTGGA-GAACGCCAA-GAC	(AG)13	5	6FAM	
Mreg22	F: CAAG-CAATAAGTG-GCCTCT-GAAG R: GGAG-CAACTTGCT-GCCTTTGC	(AG)17	5	HEX	
Mreg252	F: GCAACT-GCCA-GACTTTGCTG R: ACTTGAG-GCAGGCACG-GTC	(AG)34	5	HEX	
Mreg260	F: AGGGCG-GTAAATG-GAAAACA-GAA R: TCCCCATGA-CAACGAA-GAGC	(AG)19(GT)15	5	HEX	

Locus	Primer Sequence (5 -3)	Repeat Types	Allele Size	Annealing Temperature	Fluorophores
Mreg196	F: AGGAA-GAACTTGCTG-GTAAAAATG R: TCTTGTCTTC-TATCTG-GAGTCTGC	(AG)31	5 TAMRA		
Mre39	F: AATTGGGA-GACTGGGACT-GAGA R: TGAATGAAT-GAAGCT-GCTTGTAA	(AC)17	5 TAMRA		

4. Data Analysis

Microsatellite locus amplification products were genotyped using an ABI 3700 DNA sequencer. Genotyping results were analyzed using GeneMarker for individual identification of Chinese muntjac by contrasting fingerprint patterns synthesized from the 8 microsatellite marker genotypes [25]. POPGENE 3.2 [28] was used to calculate allele numbers, mean observed heterozygosity, mean expected heterozygosity, and polymorphism information content (PIC). Cervus 3.0 [19,27] was used for parentage identification. Since both paternal and maternal parents were unknown in the experimental samples, all identified males were used as candidate fathers and all females as candidate mothers, with sampling probabilities set at . Kinship coefficients between individuals were calculated using Kingroup v2 [19,27] as a supplement to parentage identification.

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1. Sex Identification and Individual Identification

Among the Chinese muntjac fecal samples, samples could be repeatedly and stably extracted, and could amplify products detectable by agarose gel electrophoresis. Partial results are shown in [Figure 1: see original paper]. Further amplification showed that the obtained quantities could meet subsequent experimental requirements. To further confirm that collected samples were Chinese muntjac, mitochondrial cytochrome *b* gene was amplified from equal volumes of fecal samples and the two muscle samples. Sequencing of the products showed that all samples had extremely high similarity to Chinese muntjac *Cyt b* gene sequences in the database. Samples were amplified for microsatellite loci, and those with insufficient loci were discarded. Agarose gel electrophoresis of the *SRY* gene amplification showed a target fragment size of 220 bp [Figure 1: see

original paper], with relatively small brightness differences. Except for sequencing failures, samples were used for analysis.

[Figure 1: see original paper] PCR Amplification Results of *Sry* Gene in *Muntiacus reevesi*
1-22: Different fecal sample DNA; 23: Female muntjac muscle DNA; 24: Male muntjac muscle DNA

A total of 177 Chinese muntjac individuals were identified, including 94 females and 83 males.

2. Genetic Diversity Analysis of Microsatellite Loci

Genetic information parameters for the 8 microsatellite loci in 177 individuals showed a mean allele number of 11.00, mean observed heterozygosity of 0.9685 (0.960–1.000), mean expected heterozygosity of 0.8429 (0.799–0.887), and polymorphism information content (PIC) ranging from 0.766 to 0.872, with an average of 0.8214, indicating highly polymorphic loci (PIC > 0.5). Details are shown in .

** Genetic Parameter Analysis of 8 Loci in 177 Chinese Muntjac Samples**

Locus	Allele Number	Sample Size	Observed Heterozygosity	Expected Heterozygosity	PIC	E-1P	E-2P	E-PP	Fis (Null)	HWE
Mreg2	16.000	0.887	0.873	0.620	0.766	0.915	-0.1343	0.0626		
Mreg1	12.957	0.879	0.867	0.618	0.764	0.921	-0.1256	0.0472		
Mreg5	15.966	0.875	0.859	0.588	0.742	0.898	-0.1345	0.0525		
Mre6	11.943	0.844	0.823	0.523	0.689	0.862	-0.1747	0.0586		
Mreg8	8.969	0.837	0.816	0.512	0.680	0.857	-0.2195	0.0845		
Mreg7	5.977	0.824	0.807	0.503	0.674	0.861	-0.2684	0.1017		
Mre3	9.976	0.797	0.770	0.434	0.613	0.801	-0.2436	0.1196		
Mreg10	9.960	0.799	0.767	0.425	0.603	0.786	-0.2083	0.0962		

Note: E-1P: Exclusion probability of the first parent; E-2P: Exclusion probability of the second parent; E-PP: Exclusion probability of parent pair; Fis: Inbreeding coefficient; HWE: Hardy-Weinberg equilibrium

The cumulative exclusion probability for the 8 loci was greater than 99.99%. All loci showed high heterozygosity levels, and the Chinese muntjac population in Gutianshan represents a genetically diverse population.

3. Parentage Exclusion Rate Analysis of Microsatellite Loci

The ability of microsatellite markers to exclude non-parents in parentage identification depends on the number of microsatellite loci and allele polymorphism. As the number of loci increases, the exclusion probability increases accordingly [29]. The 8 microsatellite loci selected in this study yielded 88 alleles with a mean allele number of 11.00 and PIC ranging from 0.767 to 0.873, all representing highly polymorphic loci. Compared with other cervids, the allele number and PIC of Chinese muntjac (0.799–0.887, mean 0.8429) are higher than those reported for Père David' s deer (*Elaphurus davidianus*) [31], black muntjac (*Muntiacus crinifrons*) [32], white-tailed deer (*Odocoileus virginianus*) [33], red deer (*Cervus elaphus*) [11], and sika deer (*Cervus nippon*) [10], indicating rich genetic diversity.

Genetic diversity refers to genetic variation within a species, including variation among different populations and among individuals within the same population, and represents the evolutionary potential of a species [11]. The high genetic diversity detected in the Gutianshan Chinese muntjac population suggests strong viability and adaptability. The mean expected heterozygosity (He) of 0.8429 is higher than that reported for other cervids, including Père David' s deer (0.374, mean 0.74) [31], black muntjac (0.680–0.836, mean 0.78) [32], white-tailed deer (0.056–0.598, mean 0.723) [33], red deer (0.61–0.81) [11], and sika deer (0.685) [10].

Null alleles are a primary factor affecting the accuracy of parentage identification using microsatellites [10]. In this study, the frequency of null alleles was extremely low, and all samples could stably amplify at least alleles from the 8 microsatellite loci, thus not affecting identification accuracy. When loci were randomly selected from the 8 microsatellite markers, the probability of finding the mother from female muntjacs and the father from individuals exceeded . To improve identification accuracy, all 8 loci were used for parentage analysis. Kinship coefficients derived from microsatellite data typically fluctuate around theoretical values. In this study, the average parent-offspring kinship coefficient was 0.5316, close to the theoretical value of 0.50, and the average kinship coefficient among offspring was 0.3064, also approaching theoretical values, further verifying the accuracy of parentage identification.

Hardy-Weinberg equilibrium (HWE) test results showed that microsatellite loci Mreg260, Mreg283, and Mre61 deviated from equilibrium. For randomly mating populations without external selection, immigration, emigration, or mutation, HWE describes the equilibrium state of genotype frequencies. Deviation from HWE may result from inbreeding, population substructure, or null alleles [37]. The inbreeding coefficient (Fis) is an important parameter for evaluating

whether inbreeding exists in a population. A negative Fis value indicates no inbreeding [38]. The Fis value for the Gutianshan Chinese muntjac population was -0.1886, indicating no inbreeding. Chinese muntjac have large home ranges, and within the Gutianshan area, there are no geographical barriers blocking individual migration and gene flow, so no population substructure exists. The extremely low frequency of null alleles also excludes them as a cause of deviation. However, the large-scale migration of muntjac and habitat fragmentation within the reserve may prevent random mating, causing the observed deviation from HWE.

4. Parentage Identification

Cervus 3.0 software can perform parentage identification using the "Parent pair (Sex known)" module. Results are presented in the Appendices. A total of father-offspring relationships were identified among 104 individuals, with kinship coefficients ranging from 0.5044 to 0.6332 (mean 0.5273). individuals could not be assigned parentage. The relationship structure diagrams for three pedigrees are shown in [Figure 2: see original paper].

[Figure 2: see original paper] Relationship Between Combined Probability of Exclusion and Number of Microsatellite Loci
CE-1P, CE-2P, CE-PP: Cumulative exclusion probability arranged from high to low PIC; CE-1P , CE-2P , CE-PP : Cumulative exclusion probability arranged from low to high PIC

5. Mating System

Pedigree analysis revealed Chinese muntjac mating relationships. Phenomena of one female mating with multiple males and one male mating with multiple females were observed. The kinship coefficients among offspring produced by one female mating with multiple males ranged from 0.2759 to 0.3844 (mean 0.3064), while kinship coefficients among offspring produced by one male mating with multiple females ranged from 0.2849 to 0.3315. These results indicate that the Chinese muntjac mating system remains polygynous but does not belong to any currently known subtypes.

[Figure 3: see original paper] Genetic Pedigree Charts of 1, 2, and 3

** Parentage and Kinship Coefficients in *Muntiacus reevesi*****

Pedigree	Mother	Father	Offspring	(m,o)	(f,o)
A1( )	B51( )	0.5637	C82( )	0.6083	
C73( )	0.5137	0.5637	B71( )	0.5637	0.6083
A48( )	0.5637	A49( )	0.5044	0.5137	
C71( )	A58( )	0.5044	0.5436	C87( )	0.5436
0.5436	D35( )	0.5044	C48( )	0.5436	0.5637
C75( )	0.5637	C83( )	0.5974	0.5044	

Pedigree	Mother	Father	Offspring	(m,o)	(f,o)
A13( )	0.6083	0.6083	A86( )	0.5436	
A54( )	0.5303	0.5137	D42( )	0.5137	0.5436
D26( )	0.6083	A20( )	0.5436	B41( )	0.5137
0.5436	E39( )	0.5436	A84( )	0.5637	
B67( )	0.5137	E26( )	0.5044	0.5137	
E16( )	0.5044	0.5044	B4( )	B8( )	
B5( )	0.5436	0.5637	0.5637	C21( )	0.6083
0.5637	A61( )	0.6083	0.5637	C50( )	0.5637
0.5637	A38( )	(m,o): Kinship coefficient between mother and offspring; (f,o): Kinship coefficient between father and offspring			

** Kinship Coefficients Among Offspring**

Pedigree	Offspring	Relationship Coefficient	Pedigree	Offspring	Relationship Coefficient
A1( )	B51( )	0.3315	B41( )	A84( )	0.2883
B51( )	C73( )	0.3290	A84( )	B67( )	0.3146
A1( )	C73( )	0.3315	B41( )	B67( )	0.3315
D35( )	A49( )	0.3315	B4( )	B8( )	0.2849
A48( )	A49( )	0.2883	B8( )	B5( )	0.3315
A49( )	C71( )	0.3290	B4( )	B5( )	0.2759
A48( )	C71( )	0.3315	A61( )	C50( )	0.2849
A58( )	C87( )	0.3315	A12( )	A35( )	0.2849
C83( )	A54( )	0.2883	A15( )	A74( )	0.3315
A13( )	D42( )	0.3146	D4( )	E17( )	0.2849
C75( )	C83( )	0.3146	B57( )	B60( )	0.3315
C83( )	A13( )	0.3315	B1( )	A22( )	0.2883
C75( )	A13( )	0.2759	D88( )	A27( )	0.2849
D42( )	D26( )	0.3315	B37( )	D33( )	0.2849
A20( )	E39( )	0.3844	B13( )	B18( )	0.2849

Discussion

1. Parentage Identification and Genetic Diversity Analysis When using microsatellite markers for parentage identification, the ability to exclude non-parents depends on the number of microsatellite loci and allele polymorphism. As the number of microsatellite loci increases, the exclusion probability increases accordingly [29]. The 8 microsatellite loci selected in this study yielded 88 alleles with a mean allele number of 11.00 and PIC ranging from 0.767 to 0.873, all representing highly polymorphic loci. Compared with other cervids, the allele number and PIC of Chinese muntjac (0.799–0.887, mean 0.8429) are higher than those reported for Père David' s deer (*Elaphurus davidianus*) [31], black muntjac (*Muntiacus crinifrons*) [32], white-tailed deer (*Odocoileus virginianus*) [33], red deer (*Cervus elaphus*) [11], and sika deer (*Cervus nippon*) [10], indicating rich genetic diversity.

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Null alleles are a primary factor affecting the accuracy of parentage identification using microsatellites [10]. In this study, the frequency of null alleles was extremely low, and all samples could stably amplify at least alleles from the 8 microsatellite loci, thus not affecting identification accuracy. When loci were randomly selected from the 8 microsatellite markers, the probability of finding the mother from female muntjacs and the father from individuals exceeded . To improve identification accuracy, all 8 loci were used for parentage analysis. Kinship coefficients derived from microsatellite data typically fluctuate around theoretical values. In this study, the average parent-offspring kinship coefficient was 0.5316, close to the theoretical value of 0.50, and the average kinship coefficient among offspring was 0.3064, also approaching theoretical values, further verifying the accuracy of parentage identification.

Hardy-Weinberg equilibrium (HWE) test results showed that microsatellite loci Mreg260, Mreg283, and Mre61 deviated from equilibrium. For randomly mating populations without external selection, immigration, emigration, or mutation, HWE describes the equilibrium state of genotype frequencies. Deviation from HWE may result from inbreeding, population substructure, or null alleles [37]. The inbreeding coefficient (Fis) is an important parameter for evaluating whether inbreeding exists in a population. A negative Fis value indicates no

inbreeding [38]. The Fis value for the Gutianshan Chinese muntjac population was -0.1886, indicating no inbreeding. Chinese muntjac have large home ranges, and within the Gutianshan area, there are no geographical barriers blocking individual migration and gene flow, so no population substructure exists. The extremely low frequency of null alleles also excludes them as a cause of deviation. However, the large-scale migration of muntjac and habitat fragmentation within the reserve may prevent random mating, causing the observed deviation from HWE.

2. Mating System of Chinese Muntjac Mating systems represent an evolutionarily stable strategy (ESS). The mating system adopted by animals in reproductive behavior depends on interactions between individuals and adaptation to external environments. The main ecological factors affecting animal mating systems may be the temporal and spatial distribution of food and nesting sites [40]. Most cervids have polygynous mating systems [41], which mainly include three subtypes: (1) Resource-defense polygyny, where males occupy resources essential for reproduction to attract breeding females; (2) Female or harem defense polygyny, where males directly occupy multiple mates, with females typically living in groups; and (3) Male dominance or lek polygyny, where males compete through social hierarchy or courtship displays independent of resource occupation.

Reproduction in muntjac species such as *Muntiacus muntjak* is characterized by lack of seasonality, with year-round breeding and no fixed estrus or mating periods. Chinese muntjac prefer solitary lives with low interaction frequency among individuals [43]. The mating system remains polygynous but does not belong to any currently known subtypes. Wegge [4] studied the mating system of Indian muntjac and concluded it was neither monogamous nor any known subtype of polygyny. Odden [44] described it as an "inspection or roaming strategy" polygyny, where males regularly check their territories for receptive females to mate with multiple partners. Chinese muntjac and Indian muntjac are not only close evolutionary relatives but also share similar behaviors and lifestyles, both being solitary in the wild with non-fixed estrus and mating periods, and males maintain individual territories [4,45]. Therefore, we can infer that Chinese muntjac mating systems should be similar to Indian muntjac.

Chinese muntjac exhibit obvious marking behaviors during estrus, with males marking more frequently when entering estrus [46]. While leaving their own information, males also check female information within their territories to mate with more females. Once a female becomes pregnant during her reproductive period, she will not mate with other males [47]. However, this mating relationship is not fixed, and females may mate with different males in the next breeding season. Therefore, the phenomenon of one female mating with multiple males observed in this study should occur between different female reproductive periods. This mating system allows males with strong searching abilities to mate with multiple females in breeding condition, increasing mating success probabili-

ties for solitary Chinese muntjac during breeding periods. For females, non-fixed mating relationships increase gene exchange between sexes, enhancing offspring heterozygosity, which is an important reason for the high genetic diversity observed in the Gutianshan Chinese muntjac population.

Conservation Recommendations

To better utilize the role of Gutianshan Nature Reserve, regular monitoring of Chinese muntjac population dynamics should be conducted to understand population quantity change patterns. Simultaneously, monitoring of habitat quality within the reserve should be strengthened to estimate the environmental carrying capacity for Chinese muntjac and to formulate and adjust corresponding conservation measures in a timely manner. Research efforts on Chinese muntjac should be enhanced to achieve comprehensive understanding of factors affecting survival and reproduction. Local residents' awareness of wildlife conservation should be strengthened to reduce hunting pressure and allow muntjac populations to expand further. In experimental zones of the nature reserve, establishing Chinese muntjac breeding farms could be considered for artificial breeding and population propagation, opening new pathways for in-situ conservation, ex-situ conservation, and resource utilization.

Appendix 1: Mother-Offspring Identification Results from Cervus 3.0 Software

** The Identified Results of Mother and Offspring from Cervus 3.0**

Offspring ID	Loci Typed	Candidate Mother ID	Loci Typed	Mismatching Loci	Pair LOD Score	Pair Delta Confidence
					11.83	
					11.83	
					10.41	
					10.41	

Standard Δ value = 0.00; +: 0.62 > Standard Δ value

Appendix 2: Father-Offspring Identification Results from Cervus 3.0 Software

** The Identified Results of Father and Offspring from Cervus 3.0**

Offspring ID	Loci Typed	Candidate Father ID	Loci Typed	Mismatching Loci	Pair LOD Score	Pair Delta Confidence
					10.64	

Standard Δ value = 0; +: 0 < Standard Δ value

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Note: Figure translations are in progress. See original paper for figures.