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The mating system study of black muntjac (*Muntiacus crinifrons*) based on fecal DNA

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ABSTRACT

In protecting endangered wild animals, to understand the mating system of the given animal clearly in its natural living area is an important research. The black muntjac (*Muntiacus crinifrons*), endemic to China, is an important protected endangered species. The Jiulongshan National Nature Reserve is one of the main distribution areas of black muntjac. However, the mating system of black muntjac has not been known yet. In recent years, fecal DNA has been widely used to study the parents–child relationship of wild animals. In October 2012 and May 2013, by following the fresh foot chains of black muntjac, we collected 148 fecal samples of black muntjac in the Jiulongshan National Nature Reserve. Ninety fecal DNAs were successfully extracted. In addition, Cyt *b* gene was used to identify species of some unrecognized feces. Eight microsatellite loci with high polymorphic characters were applied to identify the genotypes of these fecal samples. Seventy-one alleles were detected in 8 microsatellite loci. The expected heterozygosity (*He*) ranged from 0.607 to 0.826, with an average of 0.712, and the polymorphism information content (*PIC*) ranged from 0.523 to 0.803. SRY gene located Y chromosome was used to identify sexuality of the owners of these feces. Later, the paternity of these individuals was tested by Cervus 3.0. The combined exclusion probability of parent pair is 99.99%. Finally, in order to improve the accuracy of the results, we used Kingroup v2 to calculate the relationship coefficient between individuals. The results showed that 90 feces of DNA belong to 70 individual black muntjacs, which consist of 36 males and 34 females. Parent pair relationship existed in a total of 48 individuals, including 15 parentage groups, 7 father–child relationships, and 5 mother–child relationships. However, parents or offspring of 22 individuals could not been identified. By analyzing the mating relationship between males and females, we found that one male black muntjac mated with multiple females and produced offsprings, whereas female black muntjacs only mated with one male. Therefore, the mating pattern of black muntjacs was polygyny in the Jiulongshan National Nature Reserve. The core area of the Jiulongshan National Nature Reserve, which is the main living area of black muntjacs, contains abundant plants to satisfy the food requirement of black muntjacs. This may be the reason of black muntjacs to choose polygyny as their mating system in the Jiulongshan National Nature Reserve.

1. Introduction

The black muntjac was a typical subtropical mountain forest animals, and only distributed in four provinces, that is, Anhui, Zhejiang, Jiangxi, and Fujian. It was categorized as a Grade I of National Key Protected Animal by the Ministry of Agriculture of China [1] and classified as Vulnerable (VU) by IUCN.

In recent years, with the noninvasive sampling technology [2,3], especially fecal DNA analysis techniques are widely used in molecular ecology research. A battery of studies on paternity, kinship and mating system for the wildlife have been reported using the non-invasive sampling technology, such as chimpanzees (*Pan troglodytes*) [4], brown bear (*Ursus arctos*) [5], baboon (*Papio hamadryas*) [6], white-headed langurs (*Trachypithecus leucocephalus*) [7], red deer (*Cervus elaphus xanthopygus*) [8], Siberian tiger (*Panthera tigris sibilia*) [9,10], giant panda (*Ailuropoda melanoleuca*) [11], and black rhinoceros (*Diceros bicornis*) [12].

Based on the noninvasive sampling technology, previous studies about black muntjac mainly focused on DNA extraction and PCR amplification [13,14], individual and sexual identification [15], genetic diversity of the population [16–18] and so on. No information was reported on the mating system. Mating system is one of the important content in animal behavioral ecology research. In this study, we conducted the research in individual and sexual identification for the collected feces samplesm and analyzed the parent–child relationship between individuals and the mating relationship between males and females to explore the mating system of the black muntjac and carry out the better protective strategy.

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2. Study area

The Jiulongshan National Nature Reserve which is the junction of Zhejiang, Fujian and Jiangxi provinces with an area of 55.25 km^2, is located in the southwest of Suichang county (118°49′—118°55′ E, 28°19′—28°24′ N), Zhejiang province of China. It belongs to the main branch of the Xianxialing mountains of Wuyishan, which is rich in water resources as well as in vegetation. The Jiulongshan National Nature Reserve belonged to the subtropical humid monsoon climate which has obvious seasonal variation, showing obvious vertical zonation. The zonal vegetation type is evergreen broadleaf forest, and glauca (*Cyclobalanopsis glauca*) is the dominant species in the community. The fauna is Oriental realm and sub-realm of the eastern hills and plains of central China.

3. Materials and methods

3.1. Sample collection

According to the observation of black muntjac in captivity, the estrous period mainly in autumn and winter from October to January, is concentrated in the April–July period of calving [19]. So, we investigated the animals in October 2012 and May 2013, by following the fresh foot chains of black muntjac, and collected 148 fecal samples of black muntjac in the Jiulongshan National Nature Reserve (Fig. 1). All the fecal samples were put into cover-sealed plastic bottles containing pure ethanol. During the sampling process, all containers and tools were sterilized in order to prevent man-made pollution. Meanwhile, collection time, locality, altitude, latitude, longitude, collector's name and other information were recorded. Samples were transported to the laboratory and kept at −20 °C.

3.2. Experimental treatment

3.2.1. DNA extraction

DNA was extracted from fecal samples using the OMEGA-Stool DNA Kit according to the manufacturer's instructions. DNA extracted was detected by agarose gel electrophoresis and stored at −20 °C.

3.2.2. Polymerase chain reaction (PCR)

We used 8 microsatellite loci (BM203, BOVIRBP, BM1225, BM1706, RT1, BM888, CSSM41, BMC1009) with high polymorphic characters, to identify the genotypes of these fecal samples (Table 1). Demonstration of these SSR primers could be found in the studies of Wang [18] and Wu [20]. All microsatellite loci unilateral primer 5′ end fluorescently labeled (FAM, HEX, TAMARA) (Table 1). The PCR amplification mixture contained: 5 μl 10 × PCR buffer, 4 μl of 2.5 mmol/l dNTPs, 2.5–3 μl of 25 mM $MgCl_2$, 2.5 μl of 2 mg/ml Bovine serum albumin (BSA), 0.4 μl of 5 U/ml Taq DNA polymerase, 0.8 μl genomic DNA, 1.0 μl of each primer, and sterile water added to a final volume of 50 μl. The following amplification conditions were used: 95 °C for 5 min; 40–45 cycles consisting of 94 °C for 40 s, 40 s at the optimized annealing temperature

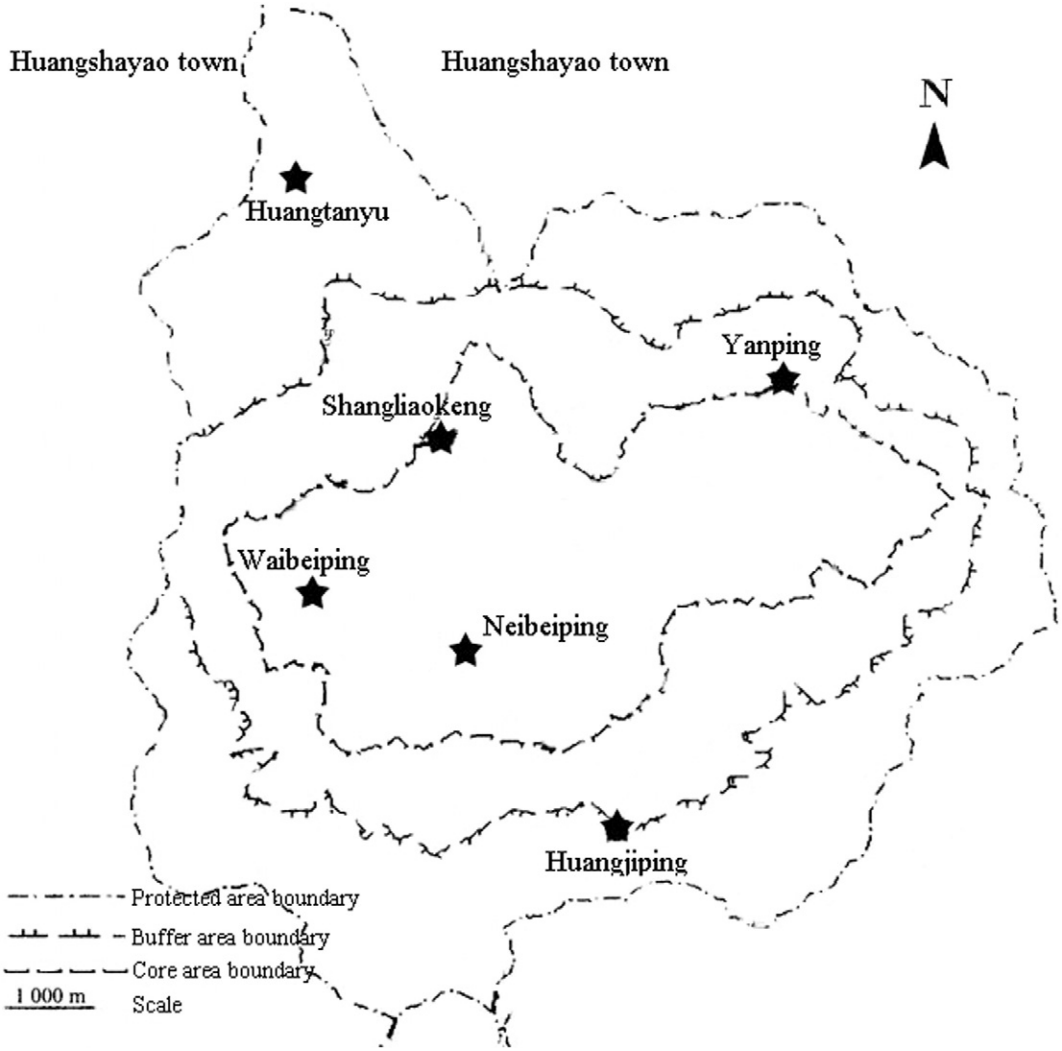

Fig. 1. Profile of studying area in Jiulongshan National Nature Reserve (the pentagram as the sampling area).