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Genetic quality of the Miyaluo captive forest musk deer (*Moschus berezovskii*) population as assessed by microsatellite loci

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ABSTRACT

The Miyaluo captive forest musk deer population (Sichuan Province, China) is one of the largest captive breeding populations in the world. In order to evaluate the genetic quality and provide available genetic management strategy, seven polymorphism microsatellite loci were applied to assess the genetic variation of the Miyaluo forest musk deer. The results indicated that a total of 168 alleles were detected from these seven microsatellite loci in 361 individuals, and the number of the alleles per locus ranged from 12 to 41 with a mean of 24. The average observed heterozygosity, expected heterozygosity, and PIC were 0.782, 0.854, and 0.837, respectively. Considering the results of the loci Hardy–Weinberg equilibrium test, the comparison of the common allele frequency as well as the private allele between the adults and juveniles, we concluded that the heterozygosity and the genetic diversity of the Miyaluo captive breeding population are increasing due to the input of new individuals from other populations. However, the frequency of some alleles declined sharply, and some were even lost indicating that there is a risk for diversity loss. Thus, we proposed an improved management and breeding strategy for the captive breeding population of the forest musk deer.

1. Introduction

Musk deer (*Moschus* spp.), a rare species only distributed in the forest and mountainous regions of Asia, are currently listed under Appendix 1 in CITES and were all listed under Category I of the State Key Protected Wildlife List of China. Musk Deer are famous for the musk secreted by the musk glands of males, which is highly valued, having a market value higher than gold (Green, 1986). The forest musk deer (*Moschus berezovskii*) is one of the five recognized musk deer species in the world, which are mainly distributed in the southwestern part of China, from Ningxia province in the north to Guangxi province in the south, and from Anhui province in the east to Xizang province (Tibet) in the west (Yang and Feng, 1998; Sheng, 1998). Based on the quantity of musk purchased, Sheng (1998) estimated that there were more than one million forest musk deer in the 1960s,

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while only 600,000 were found in the late 1970s. In the 1980s, with the price of musk soaring, the capture of musk deer became more intensive, leading to over-exploitation. As a consequence, the population of forest musk deer declined dramatically to 300,000 to 200,000 or even as low as 100,000 (Sheng, 1998).

In order to save musk deer from extinction and protect traditional Chinese medicines, the government has encouraged musk-using enterprises to participate in captive breeding programs since the early 1950s (Peng et al., 2008), and the musk deer farming population reached a peak of 3000 individuals in the early 1980s (Zhou et al., 2000). Among all the musk deer species, the captive population of the forest musk deer is the largest (Meng et al., 2006; Wu and Wang, 2006; Sheng and Liu, 2007). The Miyaluo farming population of the forest musk deer in Sichuan province (China), which started in 1958 with less than 50 individuals, was one of the earliest established captive breeding populations. The size of this population has grown rapidly to approximately 400 in 2010. However, this population is still suffering from inbreeding and genetic decline due to nonrandom mating and limited gene flow from different captive and wild populations. Genetic variation is an important component for both short- and long-term persistence of populations (England et al., 2003). The aim of conservation is not only the survival of some populations, but also the long-term conservation of genetic diversity. Genetic diversity is essential to ensure the conservation of the evolutionary potential, which allows the population to adapt to changing environments (Frankham et al., 2002; Guan et al., 2009). Maintaining genetic diversity is a key factor for keeping a population stable and healthy; therefore, it is important to assess and monitor the level of genetic variation in captive deer populations such as that at Mialuo.

In the last decade, various genetic markers, such as 16S rRNA, cytochrome b, mtDNA control region, have been used to help solve problems of genetic diversity in musk deer (Guha et al., 2007; Gilbert et al., 2006; Peng et al., 2008). Compared to other genetic markers, microsatellite makers have been proven to be valuable tools for the characterization and evaluation of genetic diversity because of their advantages of high polymorphism, relatively small size and rapid detection protocols (Ebrahimi et al., 2012; Guo et al., 2010; Gibson et al., 2005). The number of alleles in a microsatellite locus is significant for evaluation of genetic diversity. “Private alleles” is a term originally used in population biology to describe alleles that are unique to a particular endemic species; however, Slatkin termed a “private allele” if it is found in only one of several populations (Slatkin, 1985; Slatkin and Takahata, 1985). In this study, we extend the identification of “private alleles” to different breeding age groups (i.e., adults and juveniles) so that the process of transfer or loss of these unique alleles can be better understood. The presence and number of private alleles in populations identifies an important aspect of genetic diversity which calls for special management procedures to prevent their loss in future populations (Kalinowski, 2004).

Some microsatellite loci have been successfully isolated from the forest musk deer genome (Zou et al., 2005; Xia et al., 2006; Zhang et al., 2007; Zhao et al., 2007). Guan et al. (2009) have analyzed the genetic diversity of 41 captive forest musk deer individuals in the Miyaluo population with eleven microsatellite markers. However, their study did not say whether the present breeding strategy would bring a loss of genetic diversity or not. In the present study, 361 samples from all of the forest musk deer in the Miyaluo farm were collected to evaluate the level of genetic health using seven microsatellite loci. A Hardy–Weinberg equilibrium test was conducted. The common allele frequency as well as private allele numbers was compared between the adult and juvenile forest musk deer. The combined results of this study provide reliable data that can be used to develop a healthy genetic breeding strategy.

2. Materials and methods

2.1. Sample collection and DNA extraction

Hair samples were collected from 361 forest musk deer at the Miyaluo captive breeding farm in Sichuan Province, China. This includes 243 samples collected from all the adult deer present in Miyaluo in September 2008. According to breeding records for the Miyaluo population, 35 adults from the Maerkang forest musk deer farm were introduced into the Miyaluo population after Septermber 2008, so we were unable to obtain samples from these Maerkang deer. In 2010, 118 samples collected from all the juvenile forest musk deer born in 2008 and 2009, which included 6 offspring from 6 females of the introduced Maerkang deer, which were the only Maerkang deer to foster any offspring by 2010. It takes two years for the forest musk deer to reach sexual maturity; therefore, we divided the Miyaluo population into two groups: juveniles, which had not yet reached sexual maturity, and adults, who were sexually mature and capable of participating in breeding. Total genomic DNA was isolated according to the method described by Zou et al. (2005).

2.2. Microsatellite loci amplification

Seven microsatellite markers (mber23f, mber39b, mber81b, mber33zd, mber34zd, mber14h, mber112a) were used in this study, which were chosen from Zou et al. (2005), Xia et al. (2006), Zhang et al. (2007), and Zhao et al. (2007) and based on the level of polymorphism and the stability required to amplify in PCR reactions. Each forward primer was labeled with fluorescent dye, FAM, TET, or HEX (Shanghai Sangon Biological Engineer Technology & Services Co. Ltd, Shanghai, China). The sequence of primers, microsatellite loci repeat motif, annealing temperature, and GenBank accession number are shown in Table 1. The PCR reactions were conducted in a Thermal Cycler S1000 (Bio-Rad). PCR was carried out in a 25 μL reaction mixture, composed of 25 ng of genomic DNA, 1.0–2.0 μm $MgCl_2$ (TaKaRa, Japan), 200 μM of each dNTP, 1.0 μm of each primer, 1 × PCR buffer (TaKaRa, Japan), and 1.0 U of Taq DNA polymerase (TaKaRa, Japan). The reactions were performed using the following PCR procedure: an initial denaturation at 95 °C for 5 min and 35 cycles of denaturation at 94 °C for 30 s, a primer-