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**REVIEW** 

# Forest musk deer (*Moschus berezovskii*) in China: research and protection

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**Abstract.** The forest musk deer (*Moschus berezovskii*) is an endangered artiodactyl species. Males have a musk gland that secretes musk. Musk is the raw material for many medicines and has high economic value. In recent years, because of the impacts of illegal trade and habitat fragmentation, the wild forest musk deer has nearly been driven to extinction, and it has been listed as a key protected animal by many countries. Since 2002, the Chinese government has listed wild forest musk deer populations as first-class nationally protected animals and has conducted many artificial breeding studies. In this article, we review and summarise the biological characteristics and protective measures of wild forest musk deer, the musk synthesis mechanisms and the factors influencing musk yield. We also discuss the problems facing forest musk deer conservation and the development of musk-related medicines.

Key words: behaviour, conservation, endangered species, musk synthesis mechanism

# Introduction

Musk deer (Moschus, Moschidae) are small hornless ungulates belonging to the infraorder Pecora. So far, a total of seven species have been identified, namely forest musk deer (M. berezovskii), Anhui musk deer (M. anhuiensis), Alpine musk deer (M. chrysogaster), black musk deer (M. fuscus), Himalayan musk deer (M. leucogaster), Kashmir musk deer (M. cupreus) and Siberian musk deer (M. moschiferus) (Yang et al. 2003, Yi et al. 2020). This animal has shy, timid and solitary characteristics and mainly lives in mountainous regions from Siberia to the Himalayas. Unlike the female, the male has well-developed upper canine teeth and a musk-producing organ on the abdomen (Britannica 2020). After reaching sexual maturity,

they produce musk to attract females and mark their territory. In recent decades, the population of wild musk deer declined sharply due to the continuous degradation of habitat and a steep increase in illegal hunting activity. Therefore, all species of musk deer have been marked as endangered or vulnerable by the International Union for Conservation of Nature (Nyambayar et al. 2015, Timmins & Duckworth 2015a, b, Wang & Harris 2015a, b, c, Harris 2016).

Musk has great economic value. In addition to being used to make high-end cosmetics and perfumes, musk also has many pharmacological effects, such as exciting and inhibiting the central nervous system, treating anti-myocardial ischaemia, stimulating the cardiovascular system, enhancing immunity, exciting

the uterus and enhancing uterine contractions, exciting the respiratory system, and providing anti-tumour, anti-ulcer, anti-dementia, and anti-bacterial effects, among others (Cao & Zhou 2007, Feng & Liu 2015, Liu et al. 2022). In Chinese clinical practice, musk is used chiefly to treat vascular headaches, stroke, coronary heart disease, angina, ankylosing spondylitis, bedsores, ulcers, and facial nerve paralysis.

The forest musk deer produces (Fig. 1) the largest amount of musk per individual male, and its wild populations are threatened by illegal trade. Since 2002, this species has been listed in Category I of State Key Protected Wild Animals of China. In recent years, research on wild forest musk deer mainly focused on habitat characteristics, living habits and survival status. To protect the wild forest musk deer and increase the musk yield, captive forest musk deer have been bred since the 1960s (Sheng 1992), and the number of breeding forest musk deer in China reached 25,000 at the end of 2019. Although the number of breeding forest musk deer is gradually increasing, the demand for musk exceeds 1,500 kg per year. Therefore, research on captive forest musk deer





Fig. 1. The forest musk deer (a - male, b - female). The male has particularly well-developed upper canine teeth and musk glands. The female has neither canine teeth nor musk glands, and its muzzle is narrower than the male's.

mainly focuses on promoting growth, developing deer culture, and increasing musk yield.

This review will summarise the biological characteristics and protective measures applied to wild forest musk deer. The relationships to the musk gland secretion will be discussed in relation to the physiological and biochemical aspects, behavioural characteristics, population genetic diversity, genome and transcription group. We also discuss the problems faced in forest musk deer conservation and the development of musk-related medicines.

# Methods

Analysed articles were obtained from Google Scholar, PubMed, CrossRef and China National Knowledge Infrastructure using scientific and English and Chinese common names of forest musk deer (Moschus berezovskii/musk deer/musk). Findings were refined based on the title and abstract to identify publications focusing on forest musk deer' biological characteristics and conservation status. These publications' reference lists and citations were also checked to identify further relevant papers. This process was ongoing, with the primary searches occurring from January to July 2021. We collected and analysed 197 papers and books in English and Chinese. Additional searches were conducted from institutional libraries during the writing of this article to locate information on specific topics not adequately captured using those original search terms. Two authors (H. Feng, F. Cao) selected and reviewed the articles.

# The research and protection of wild forest musk deer

The habitats distribution in China

The forest musk deer is a typical forest-dwelling animal. It has a sensitive and timid temperament and is mainly solitary. In keeping with many other deer species, forest musk deer use relatively fixed routes and places for foraging, drinking, and resting.

In general, the main habitats of this animal are mixed coniferous and broad-leaved forests with 50% to 75% crown density (Yan & Jin 1990, Guo et al. 2001). In China, due to human disturbance, vegetation and other factors, the habitats of forest musk deer differ among regions (Jiang et al. 2020). For example, in Baiyu County of Sichuan Province, they inhabit secondary shrub and mixed coniferous broad-leaved forests (Yang et al. 1989). In the Liangshan Dafengding Nature Reserve and Tangjiahe Nature Reserve of Sichuan Province, the density of bamboo under the

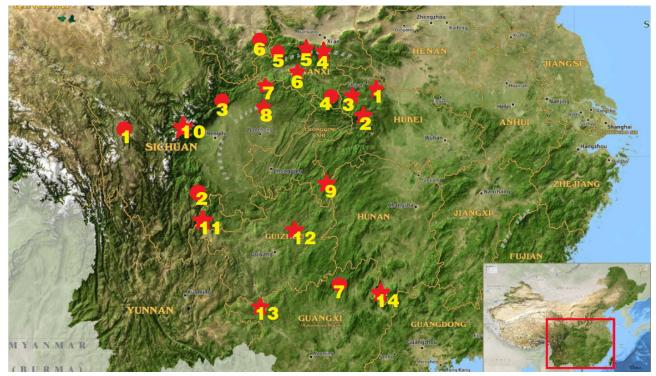


Fig. 2. The habitats and nature reserves of forest musk deer in China. The red circles are the different habitats of forest musk deer. 1) Baiyu County, 2) Liangshan Dafengding Nature Reserve, 3) Tangjiahe Nature Reserve, 4) Zhenping County, 5) Fengxian County, 6) Longshan forest area. The red stars are national nature reserves with forest musk deer as the major species. 1) Nanhe Nature Reserve, 2) Badong Nature Reserve, 3) Duheyuan Nature Reserve, 4) Pingheliang Nature Reserve, 5) Laoxiancheng Nature Reserve, 6) Changqing Nature Reserve, 7) Shuimogou Nature Reserve, 8) Jiulongshan Nature Reserve, 9) Dabanying Nature Reserve, 10) Miyaluo Nature Reserve, 11) Baicaopo Nature Reserve, 12) Baimianshui Nature Reserve, 13) Longtan Nature Reserve, 14) Dupangling Nature Reserve.

mixed coniferous and broad-leaved forest is low, which could be more conducive to the activities of forest musk deer. Therefore, they inhabit evergreen broad-leaved forests and evergreen and deciduous broad-leaved mixed forests (Wang & Sheng 1988, Yang et al. 2011). In Zhenping County of Shaanxi Province, forest musk deer prefer to inhabit nearby

areas with shrubs and coniferous and broad-leaved mixed forests in the middle and high mountains at an altitude of 800-2,800 m. But in Fengxian County of Shaanxi Province, due to the influence of towns and highways, they prefer to inhabit the border area between counties at an altitude of 1,700-2,100 m (Hu et al. 2006). In the Longshan forest area of Gansu

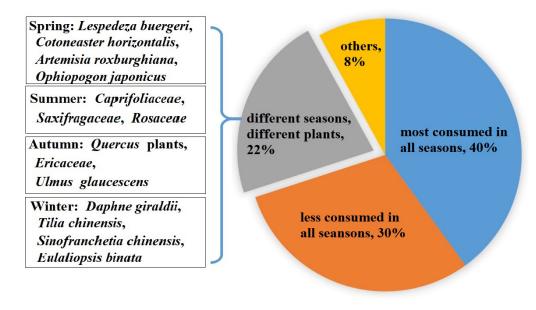


Fig. 3. Pie chart of forest musk deer diet.

Province, forest musk deer are mainly distributed in mountain broad-leaved forests and mountain shrub grasslands (Chen 1987). In Guangxi Province, the local climate is damp-heat, with rich bryophytes and vines, which is conducive to the feeding of forest musk deer. Therefore, they prefer to inhabit sparse, rocky, mountain evergreen broad-leaved forests and areas with vines and shrubs (Xiang 1974; Fig. 2).

### The feeding habits of forest musk deer

Forest musk deer are herbivorous with a wide range of feeding habits and feed on more than 100 plants throughout the year. *Morus alba, Rosa willmottiae, and Perilla frutescens* comprised the main foods of forest musk deer (Hu et al. 2007), accounting for 48% of the annual ingestion. They also like to ingest *Buxus harlandii, Liparis kumokiri, Chionanthus retusus, and Acer mono,* accounting for 30% of the annual ingestion. In different seasons, the forest musk deer use various types of feed. They eat tender shoots in spring, buds and flowers in summer, seeds of plants in autumn, and leaves and twigs of semi-evergreen plants in winter (Jiang et al. 2008, Zhang et al. 2008). The types and specific gravity of forest musk deer ingestion are shown in Fig. 3.

Behaviour and reproductive biology of forest musk deer The forest musk deer exhibits seasonal reproduction. Oestrus begins in mid-October and ends in early

January of the following year. During the oestrus period, males perform distinct rutting calls, follow females, and subsequently display courtship behaviours (combing the hairs, licking the face, and smelling the pudendum of females) that females accept. Copulations are identified as mounts followed by an ejaculatory thrust, and subsequently, the female displays quiet standing or resting behaviour and is no longer actively close to the male (Qi et al. 2011). The gestation period of female forest musk deer is 178-198 days, and the litter size is 1-3 (Sun et al. 2017). The litter size is one at the first pregnancy and two (occasionally three) for the following pregnancy. Fawns are usually born between May and June. Although the weight of male fawns at birth is slightly larger than that of female fawns, the mortality rate is higher than that of female fawns. Therefore, in adulthood, the number of females is significantly higher than that of males (Wang et al. 2016). Maternal and offspring forest musk deer display a typical "hider" relationship. In the first two months after birth, fawns often hide under bushes, mostly lying and resting. The mothers contact the fawns initially via vocalisation. At two months, the fawns are weaned and follow the mother. The individual growth of fawns is very rapid, and they can leave the mother to live independently at the age of six months. The anatomical sexual maturity of forest musk deer is reached at 15 to 18 months. However,

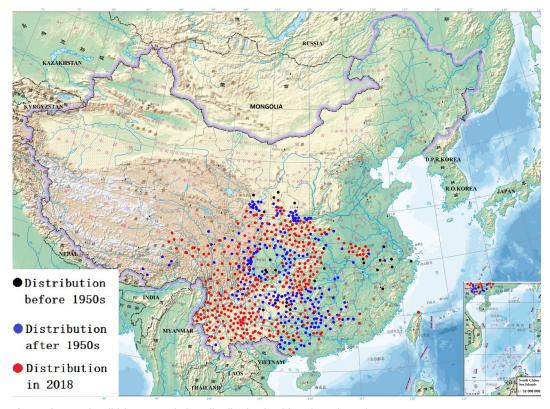


Fig. 4. Changes in wild forest musk deer distribution in China since the 20th century.

captive male forest musk deer must reach maturity at 30 to 36 months (Du & Sheng 1998, Han et al. 2003, Wei 2014). These researches provided the basis for captive forest musk deer feeding management and nutrition studies.

Survival status of and protection of wild forest musk deer Before the 20th century, wild forest musk deer were mainly distributed in central Ningxia Province, the eastern part of the Qinling Mountains, south of the Huai River Valley, China. Since the beginning of the 20<sup>th</sup> century, wild forest musk deer distribution has changed from clumped to point distribution. Wild forest musk deer have been concentrated in three areas, namely, western and northwestern China, the Sichuan Basin and southern Chongqing, at low elevations in Guizhou and central Guangxi Province. The distribution area is approximately 800,000 square kilometres, which has decreased by approximately two-thirds. The quantity of wild forest musk deer has also decreased dramatically. In the 1950s, the quantity of wild forest musk deer was approximately 2 to 3 million; in the 1960s, it fell to 1.25 to 1.5 million; in the 1970s, it was less than 1 million; and in the 1980s, it was estimated to be less than 600,000. Currently, the number of wild forest musk deer is approximately 50,000 in China and 200 in Vietnam (Wen 2018; Fig. 4). China's economic development has had substantial impacts on the quantity and distribution of wild forest musk deer, which experienced a stable period, slow change period, rapid reduction period and recovery period.

#### Stable period

Only one factor affected the wild forest musk deer, so it had little effect on the distribution and quantity of forest musk deer.

### Slow change period

From the 1950s to the 1970s, which was China's national economic recovery period and included the "Great Leap Forward", several inter-provincial highways and railways were built, such as the Baocheng Railway, Cheng-Kun Railway, Gui-Kunming Railway, no. 318 National Highway, no. 108 National Highway, and no. 320 National Highway. In addition, the wild forest musk deer's home range was affected by herbal medicine collection, deforestation, and farmland reclamation. As a result, the population of wild forest musk deer slowly decreased during this period.

### Rapid reduction period

From the 1980s to 2002, the Chinese government implemented reformation and opening, leading to

vigorous economic development. During this period, the distribution of wild forest musk deer sharply decreased, and there were many influencing factors. First, the international price of musk soared to nearly 100 times higher than that in the 1970s. To obtain more musk, large-scale hunting and poaching occurred, which caused the size of the wild forest musk deer population to plummet. Second, deforestation and crop planting also seriously affected the living environment of wild forest musk deer. In addition, factors such as the development of grazing, mining, and tourism led to wild forest musk deer habitat fragmentation. All of these factors placed wild forest musk deer under tremendous pressure.

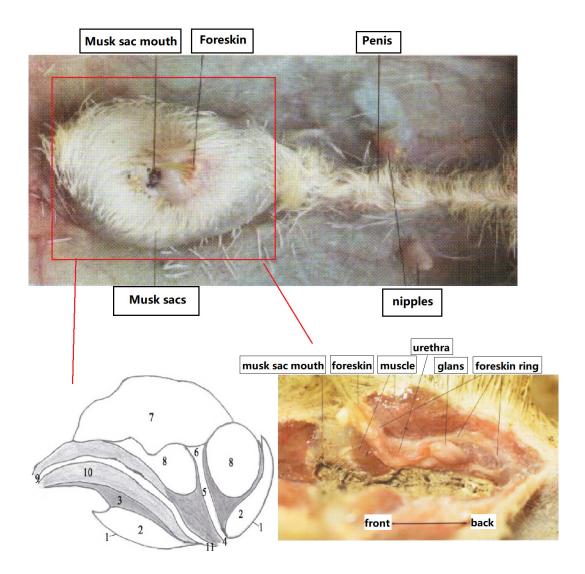
#### Recovery period

Since 2003, with the approval of the State Council, the forest musk deer has been listed as a first-class national protected animal. In addition, the Chinese legislation government implemented poaching, such as the "Wild Animal Protection Law", "Criminal Law", "Regulations of Terrestrial Wild Animal Protections", "Regulations on the Import and Export of Endangered Wild Animals and Plants", and "Regulations on the Protection and Administration of Wild Medicinal Material Resources". At the same time, a number of national and provincial nature reserves have been established. Currently, there are 14 national nature reserves with forest musk deer as the major protected species. To restore the number of wild populations, the reintroduction of captive-bred forest musk deer into the wild was also carried out. On 29 June 2017, eight female and five male captive forest musk deer were released to the wild in Ningshan County, Shaanxi Province. One year later, the activity of six forest musk deer was monitored, and one of the female forest musk deer successfully gave birth to a fawn in the wild (Liu et al. 2020).

Because of the fragmentation and isolation of the wild forest musk deer distribution and the vulnerability of small isolated populations, although protection has been carried out for many years, wild forest musk deer are still endangered. Consequently, indepth research leading to effective and sustained conservation measures is required.

# The research of captive musk deer

China has the largest number of captive forest musk deer, and more than 70% of musk and musk-related products in the world come from China (Sheng 1996). At the end of 2020, the number of breeding forest musk deer in China reached 25,000, 18,000 of



**Fig. 5.** Anatomic diagram of musk gland. 1) skin, 2) sweat glands and sebaceous glands, 3) rhabdoid muscle layer, 4) musk sac mouth, 5) musk excretion tube, 6) musk sac neck, 7) musk sacs, 8) musk secretory glands, 9) penis, 10) foreskin ring, 11) foreskin.

which were in Shaanxi Province, and the total musk production reached 250 kg per year. Although the number of breeding forest musk deer is gradually increasing, the demand for musk is greater than 1,500 kg per year. Due to the enormous gap between musk production and demand, the price of musk has soared from 60 yuan per gram in 2005 to 800 yuan per gram in 2020. To increase the natural musk yield, many studies have been carried out in recent years on the mechanism of musk secretion of forest musk deer.

# Musk synthesis mechanism

The male's musk gland is located between its abdomen and its genitals, covered by the abdominal skin (He et al. 2014). It is composed of a musk secretory gland and a musk sac. The musk secretory gland is the organ that secretes the initial liquid. The musk sac is

the organ that collects and stores the initial liquid and mature musk. The opening of the musk sac is above the foreskin (Wang 2016, Liu et al. 2022; Fig. 5). The epithelial cells of the musk secretory gland consist of both light and dark cells (Bi et al. 1993). From May to June each year, by holocrine and apocrine secretion, the epithelial cells secrete a large amount of cream-coloured initial liquid that contains large amounts of protein, carbohydrates and water. The initial liquid is transported to the musk sac neck; through the musk excretion tube. From July to September, the sebaceous glands continuously secrete sebum. Under the combined action of sebum and microbiota, the initial liquid is transformed into coffee-coloured powdery mature musk in the musk sac.

With the development of genome and transcriptome technology, 21 genes closely linked to musk secretion

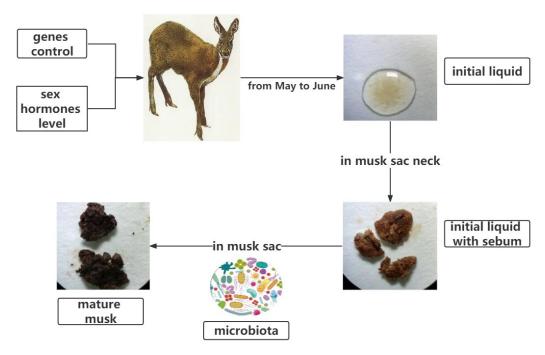


Fig. 6. The musk yield process.

of forest musk deer were found based on the genome of forest musk deer published in 2018 (Fan et al. 2018a, Bu et al. 2019). The genes are akr1d1, mvd, fnta, dhdds, scnn1a, fxyd4, sts, ugt1a3, hsd17b7, cyp1b1, srd5a1, dhcr7, dhcr24, nsdhl, cyp3a5, fdft1, fdps, hmgcl, cyp11a1, cyp17a1 and hsd3b (Xu et al. 2017, Zhou et al. 2019, Yang et al. 2021).

Musk secretion has a close relationship with sex hormone levels. The testosterone, oestradiol and progesterone levels of male forest musk deer of various ages exhibit an obvious pattern in the musk-secreting period, reaching their peak during vigorous musk secretion and then gradually returning to normal baseline levels (Bai et al. 2013, Zhang et al. 2015). The contents of four examined musk components (muscone, cyclopentadecanone, cholesterol, and cholestenol) from June to August were significantly highly negatively correlated with faecal testosterone and estradiol levels (P < 0.01). In contrast, the correlation coefficients were low or not significant from August to April of the following year (Fan et al. 2018b). Forest musk deer folliclestimulating hormone beta (FSHβ) and luteinising hormone beta (LHβ) genes are also associated with aroma secretion (Zou et al. 2004, Wang et al. 2012). In addition, exogenous male sex hormones can induce secretion activity in male forest musk deer and promote musk secretion (Yin & Dai 1990). They also can cause male forest musk deer with the testis and epididymis removed, male forest musk deer with secretion disabilities, and male forest musk deer in the non-secreting period to re-secrete musk. All these showed that increases in sex hormones could promote musk secretion.

The microbiota in the musk sac plays a vital role in the maturation process of the musk (Li et al. 2016). Microbial richness gradually declined during musk maturation (from July to September). The microbiota composition varied significantly between the initial liquid and final solid musk samples. The dominant bacteria were firmicutes, proteobacteria, actinobacteria, and bacteroidetes (Li et al. 2018). Fig. 6 is the musk yield process.

The effecting factors of musk yield

Firstly, inhabited environment. The inhabited environment is an essential ethological factor for



Fig. 7. Breeding pen with bare habitat.



Fig. 8. Breeding pen with grassland.

the captive forest musk deer. Captive forest musk deer are prone to stereotypical behaviours (Xue et al. 2008) (including feeding on non-food materials, stereotypic licking, galloping, to-from-walking, platform-standing, wall-jumping and stereotypic gazing), which directly affect reproduction, survival and musk yield (Qi et al. 2013, Han et al. 2018a, b). Captive forest musk deer kept in bare land (Fig. 7) spend most of their time performing stereotypical behaviours but kept in grassland (Fig. 8) exhibit high behavioural diversity (Zou et al. 2005, Feng et al. 2016). Under the same diet management and surrounding environment, the captive forest musk deer kept in grassland produce more musk than those kept in bare conditions (Cai et al. 2017). In addition, there was a significant positive correlation between the sociality dimension and musk secretion; i.e. higher sociality (individuals showing more intimate contact and co-ingestion) was associated with more musk secretion (Qiao et al. 2018). A too-low captive density will reduce the sociality of the forest musk deer and lead to the propensity for stereotypical behaviours. A too-high captive density will restrict physical development and daily activities. Medium-density (4-6 musk deer per 10 square meters) groups produce more musk than low- and high-density groups (Bai et al. 2019, Han et al. 2019).

Secondly, physical condition. Male forest musk deer can secrete musk from one to 18 years of age, but the period of abundant musk secretion is two to 10 years of age. Sick or injured male forest musk deer secrete less or no musk at this time but gradually secrete a normal amount of musk as the body recovers within 1-2 years.

Thirdly, genetic diversity. Musk yield was positively correlated with genetic diversity (Zhao et al. 2011). The captive musk deer populations with low musk production are characterised by small founder populations and genetic drift caused by non-random mating between individuals (Huang et al. 2013, Fan et al. 2019). This effect implies that the level of genetic diversity in captive populations is decreasing, leading to reduced individual adaptability, population diseases, and high mortality rates of young musk deer. To maintain genetic diversity, adaptability, and musk yield in captive forest musk deer populations, we recommend the following: 1) supplement captive breeding populations with individuals from other captive breeding populations; 2) take measures to ensure that individuals with a low frequency of alleles or rare or private alleles participate in breeding; and 3) establish a clear and comprehensive pedigree for captive populations. Table 1 shows the factors influencing musk yield.

# **Discussion**

## **Problems**

Although significant progress has been made in forest musk deer research at the macroscopic (morphological) to microscopic (molecular) levels, this research is limited compared with that on other species because of multiple factors.

The quantity of wild forest musk deer has decreased Most behavioural and population genetic studies have focused on captive forest musk deer. Due to habitat fragmentation and poaching, the number

Table 1. The influencing factors of musk yield.

Influencing factors	High yield	Low yield
Age	2 to 10 years of age	1 year of age, 11 to 18 years of age
Sex hormone level	High sex hormones level	Low sex hormone level
Breeding pen	With grassland	With bare habitat
Captive density	4-6 musk deer per 10 square meters	1-2 musk deer per 10 square meters, 8-10 musk deer per 10 square meters
Genetic diversity	High genetic diversity	Low genetic diversity

of wild forest musk deer is decreasing. The nature reserves that wild forest musk deer inhabit are mostly in remote mountainous areas with extremely inconvenient transportation and limited economic development. The efforts of people living in nature reserves to protect wild animals are limited. Therefore, wild forest musk deer poaching occurs frequently, which seriously affects wild forest musk deer populations.

### Sample collection difficulty

Because the forest musk deer is a nationally protected animal, it is necessary to apply for permits from animal protection departments for invasive sampling research. Therefore, only the hair, blood, faeces or tissues from dead forest musk deer can be collected, which complicates in-depth studies of forest musk deer molecular biology. At the same time, due to limited forest musk deer resources, the current price of a pair of forest musk deer is 50,000 to 80,000 RMB yuan, which dramatically increases the cost of forest musk deer research. This phenomenon not only seriously hinders research on forest musk deer but also hinders the development of forest musk deer breeding programmes.

Forest musk deer have not been domesticated, which limits research on musk secretion mechanisms

The wild nature of forest musk deer is the greatest obstacle to research on its musk secretion mechanisms. Although studies on breeding forest musk deer have been carried out for many years, the domestication of forest musk deer progressed slowly for various reasons, and newly born forest musk deer are still wild. Furthermore, because the forest musk deer is highly susceptible to stress, the stimulation caused by sample collection during the secretion period may prevent musk secretion in that year. This effect often complicates sample collection, affects the reliability and stability of experimental data, and affects the health of forest musk deer.

# **Prospects**

Ex situ conservation of wild forest musk deer and reintroduction of captive-bred forest musk deer into the wild

Ex situ conservation refers to an animal protection strategy in which wild animals are taken from the natural environment, raised in a different place and then released back into the natural environment. It is an important method for the conservation of rare wild animals. For example, breeding forest musk

deer can relieve the pressure of poaching wild musk deer to obtain musk and provide significant economic benefits. Furthermore, according to differences in genetic structure between captive and wild forest musk deer populations (Jiang et al. 2022), scientific conservation and rational utilisation are carried out to provide valuable experience and information for the protection and restoration of forest musk deer.

Strengthening the domestication of forest musk deer to support musk secretion mechanism research

The wild nature of the forest musk deer is the greatest obstacle to forest musk deer musk secretion mechanism research. Therefore, only by vigorously strengthening forest musk deer domestication research can a solid foundation be laid for breeding, genetic studies, and musk secretion mechanism research. In contrast to the long domestication process of other domestic animals, the forest musk deer have been domesticated through breeding since only 1958. Although the domestication of the forest musk deer is progressing slowly, given the interest of pharmaceutical companies in the musk industry and relevant national policy instituted in recent years, the domestication of forest musk deer will continue to progress.

# Application of new technologies

Research on forest musk deer molecular genetics has made great progress with the continuous development of molecular biotechnology. However, compared with the abundant background genetic diversity in the forest musk deer, molecular biology techniques need to be improved, and relevant research achievements cannot be implemented entirely. Therefore, it is necessary to perform further studies on forest musk deer genetic structure, combine traditional research methods with molecular biotechnology, and conduct scientific conservation and rational utilisation. In addition, with the rapid development of life science technology and the deepening application of proteomics, metabolomics and transcriptomics, forest musk deer research will begin to incorporate comprehensive analysis. Then, the gene regulatory mechanisms of musk secretion and disease resistance will be comprehensively and systematically revealed and clarified, and the production of forest musk deer and their musk will increase. Such research will also lay a foundation for realising forest musk deer marker-assisted selection, accelerating high-quality variety selection, and promoting forest musk deer breeding.

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### **Author Contributions**

H. Feng, F. Cao and Z. Su conceptualised the paper. All authors participated in writing the manuscript and prepared figures. H. Feng and Z. Su supervised the writing and editing of the manuscript. All authors have read and approved the final version of the manuscript.

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