

## Age-related musk secretion and body weight in captive forest musk deer (*Moschus berezovskii* Flerov, 1928)

Jing WANG<sup>1,2</sup>, Robert B. WELADJI<sup>3</sup>, Xiuxiang MENG<sup>2,4\*</sup>

1. School of Customs and Public Administration, Shanghai Customs College, Shanghai 201204, China

2. School of Environment and Natural Resources, Renmin University of China, Beijing 100872, China

3. Department of Biology, Concordia University, 7141 Sherbrooke Street West, Montreal, Quebec, H4B 1R6, Canada

4. College of Science, Tibet University, Lasa 850000, Tibet Autonomous Region, China

\* Corresponding author, X. Meng, E-mail: meng2014@ruc.edu.cn

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**Abstract.** Musk is a precious wildlife resource, secreted by adult male musk deer and having role in rutting and reproduction. As predicted by senescence hypothesis, this sexual trait should change with age. By investigating a captive forest musk deer (*Moschus berezovskii* Flerov, 1928) population located in the western plateau of China from 2006 to 2018, we assessed the relationship between animal age, body weight and musk secretion. Aging pattern was found both in body weight and musk secretion. Body weight of males decreased from 10 years old, while musk secretion declined from 4 years of age. The relationship between body weight and musk secretion varied at different age classes. Musk secretion represented positive correlation with body weight for individuals aged 1-4 years, while it was not obvious for older males. Our results provide an understanding of endangered musk deer from the perspective of age-related phenotypic and endocrine change, which can serve as a basis for successful musk deer farming, sustainable musk production and improving *ex situ* conservation.

**Key words:** *Moschus berezovskii*, musk secretion, senescence, body weight, captive breeding.

### Introduction

Senescence, a gradual decrease in fitness traits with advancing age (Ericsson et al. 2001, Kroeger et al. 2018) commonly observed in mammals (Liu et al. 2012, Marangoni et al. 2016), is expressed by a progressive decline in reproduction value and performance or survival rate while aging (Loison et al. 1999, Weladji et al., 2010). Senescence varies among genders, species or populations and the underlying mechanism is controversial (Kirkwood & Rose 1991, Bouwhuis et al. 2011). Age at onset of senescence in Norwegian female red deer (*Cervus elaphus* Linnaeus, 1758) was after the age of 20 and 12 for males (Mysterud et al. 2001), which differ from fallow deer (*Dama dama* Linnaeus, 1758), reaching senescence at about 9 years old (McElligott et al. 2002).

Most senescence studies in animals have been tested from the perspective of survival and reproduction traits. Other physiological or morphological traits, including body condition and antler size of ungulates, that are also key factors of individual's fitness and substantially affect the survival and reproduction, have received little attention (Kroeger et al. 2018). Body mass in reindeer (*Rangifer tarandus* Linnaeus, 1758), soay sheep (*Ovis aries* Linnaeus, 1758), red deer and small body primates exhibit various patterns at senescence (Weladji et al. 2010, Nussey et al. 2012, Mysterud et al. 2001, Hämäläinen et al. 2014), and the senescence in body size which was the determinant of reproduction in females and competitive ability in males, reflecting a fitness change and population dynamics (Bérubé et al. 1999).

Forest musk deer (*Moschus berezovskii* Flerov, 1929) is a small solitary forest ruminant. Musk deer males possess a musk gland specialized for the musk production. It is situated beneath the skin of the abdomen between reproductive organ and umbilicus (Wang & Harris 2015). Musk has been widely used in Asian traditional medicine as valuable medicinal component and perfumery industry as

fixative substances, and is even more expensive than gold (Shrestha 1998, Yang et al. 2003). Musk has a strong musk odor owing to the muscone, and it can be involved in chemical communication concerning maturity and reproduction (Sokolov et al. 1986). Musk is secreted before rutting season, namely from May to July every year and usually last for 3 to 7 days (Zhang 1983). During musk secretion, musk deer males are less active and get easily excited, and they reduce and even stop feeding (Meng et al. 2006). Then, after almost two months' maturity in the secretion pods, musk changes from white paste without scent into significant scented red-brown substance. Associated with males' hormone change, the high energy-consumption secretion has been reported to be affected by age, body condition, farming system, nutritional level and even feeding method (Cheng et al. 2002, Guo et al. 2018), yet the senescence hypothesis has not been tested using those traits. In addition, the way age and other intrinsic factors specifically influence the musk secretion has not been explored.

Here, we use a long-term individual-based data from a known-age male population of captive forest musk deer located at western Sichuan plateau, China, to test the senescence hypothesis on males' musk secretion and body weight, and to assess the potential relationship between musk secretion and body weight varies with age. Firstly, we assumed that body weight and musk production of musk deer decreased at a certain age due to senescence. Individual performance typically peaks at the prime age (Loison et al. 1999, Mysterud et al. 2005). We, therefore, expected that in prime-age body weight and musk production should be higher than those in younger or older males. Secondly, we hypothesized for males that capable of musk secretion, the body weight can be an indicator of health condition and has certain effects on the secretion. Being a secondary sexual trait, musk is costly to produce and maintain, especially for the weak animals (Yang et al. 2003), suggesting that males with better body condition should produce more musk.

Hence, as body weight changes with age, patterns of musk secretion may differ depending on the body weight at different age stage. Thus, we also hypothesized, that the relationship between body weight and male secretion will vary with age classes. Our results can not only deepen the senescence understanding of ungulates, also provide a basis of musk resources management.

## Material and Methods

### Study area

The data are from the Maerkang Musk Deer Breeding Center (MMDBC) in the western plateau of Sichuan Province, China (31°53'N, 102°07'E), situated around 2600 m above the sea level with an annual precipitation around 753 mm and with an average temperature of 8-9°C, and characterized by dry winters and humid summers. In the center, 1-3 males were kept in a mud-grounded enclosure which consisted of a 10×10 m<sup>2</sup> outdoor square and seven lined brick cells, separated by iron-mesh fence, with 2 meters-high ceiling above the floor. All animals were marked with ear tags once birth to realize individual identification with specific age and fed twice a day (around 5 minutes at dawn and dusk) by deer-keepers. The forage was mostly fresh (in summer/spring) or dried leaves (in winter/fall) collected from the nearby natural habitats of wild musk deer.

### Data collection

Musk can be extracted from live males without harming their

growth and following breeding, which is conducted every year and sustainable (Shrestha 1998). In MMDBC, at the beginning of October, male musk deer was physically restrained to be weighted. Meanwhile, a sterile scoop with smooth edge and small groove at the end was gently inserted into musk pod, then by rotating the scoop, the musk was extracted without hurts. For musk's fully collection, the pod was repeatedly pinched and pressed to make sure no substance left. Individual's musk was weighed in fresh once extracted outside, and the process was completed in 10 minutes by the same experienced musk collector to minimize the musk loss and musk deer's stress response. In general, individual's body weight and musk production of about 1259 ungonadectomized male forest musk deer was repeatedly measured in October, namely 2842 cases were recorded from 2006 to 2018. The body weight and musk secretion of males in different age were listed in Table 1.

### Statistical analysis

Data in 2008 and 2011 were excluded for analysis due to male musk deer were not weighted. Because of individual males were repeatedly measured annually, male identity was taken into consideration as a random factor to avoid the pseudo-replication in all Generalized Linear Mixed Model (GLMM) models (Weladji et al. 2010, Nussey et al. 2012) by adopting the lme4 R package (R development Core Team 2007, Bates et al. 2015). The enclosures and year were also taken as fixed effects in models to test whether these predictors affected the response variable. As the impacts of age was predicted to be curved (Ericsson et al. 2001, Myrsterud et al. 2001), polynomial of age was considered in the age-related models. The model selection was performed using Chi-square tests, and we selected the influential variable at  $P < 0.05$ .

Table 1. Individual's musk secretion (Mean±S.D.) at different age of captive forest musk deer in Maerkang musk deer farm from 2006-2018.

Age	Average musk secretion (g)	Average body weight (kg)	N	Age	Average musk secretion (g)	Average body weight (kg)	N
1	8.56±0.16	7.09±0.95	603	11	8.54±0.62	8.08±0.78	79
2	11.92±0.23	7.86±0.87	490	12	8.03±0.82	8.13±0.71	56
3	11.71±0.31	7.95±0.80	366	13	10.06±1.11	8.33±0.69	18
4	10.51±0.36	8.02±0.76	307	14	7.91±1.36	8.56±0.82	12
5	10.92±0.39	8.01±0.75	266	15	7.85±2.50	8.47±0.79	14
6	9.94±0.45	8.09±0.70	194	16	8.47±2.00	8.26±0.75	8
7	9.71±0.52	8.16±0.85	143	17	9.77±2.17	8.12±0.32	6
8	8.35±0.58	8.20±0.76	119	18	8.51±3.41	7.62±0.58	5
9	9.47±0.59	8.20±0.71	99	19	-	-	0
10	9.92±0.58	8.16±0.82	55	20	7.75	7.65±0.35	2

## Results

### Musk secretion

In MMDBC from 2006 to 2018, the annual musk production was 2658.76±389.29 g ( $n = 11$ ), and the average musk production per male was 10.29±6.15 g ( $n = 2842$ ). Musk secretion had significant interannual and enclosure variances ( $\chi^2 = 35.14$ ,  $df = 1$ ,  $P < 0.001$ ;  $\chi^2 = 61.27$ ,  $df = 11$ ,  $P < 0.001$  respectively), and age significantly influenced musk secretion in a quadratic approach ( $\beta_{age} = 0.276 \pm 0.112$ ,  $P = 0.014$ ;  $\beta_{age^2} = -0.026 \pm 0.008$ ,  $P = 0.001$ ). Fitted line indicated that musk secretion slightly increased until about the age of 4 years then progressively decreased afterwards (Fig. 1).

### Body weight

1259 males, aged between 1 and 20 years were repeatedly weighed from 2006 to 2018, and the average body weight was (7.82 ± 0.92) kg. Body weight of males differed significantly among years ( $\chi^2 = 194.72$ ,  $df = 10$ ,  $P < 0.001$ ) and

enclosures ( $\chi^2 = 32.28$ ,  $df = 11$ ,  $P = 0.0007$ ). Age significantly affected body weight in a quadratic approach as well ( $\beta_{age} = 0.268 \pm 0.016$ ,  $P < 0.001$ ;  $\beta_{age^2} = -0.013 \pm 0.001$ ,  $P < 0.001$ ), the fitted model indicated that body weight of male forest musk deer peaked at 10 years and gradually declined afterwards (Fig. 2).

### Relationships between musk secretion and body weight at different ages

Taking into account the effect of age on musk secretion and body weight, it appears that the pattern varied with age class. The fitted line among individuals of 1-4 years old indicated the musk secretion significantly increased with body weight ( $R^2=0.065$ ,  $df=1764$ ,  $P < 0.001$ ), while fitted line among 5-9 years old indicated that, though the musk secretion had a slightly increase with body weight, the tendency is not significant ( $df=819$ ,  $P = 0.28$ ), and the similar trend also found in the individuals that older than 10 years old ( $df=253$ ,  $P = 0.73$ ). Overall, musk secretion have increased

with body weight until about the age of 4 (Fig. 3a), and plateau thereafter (Fig. 3b, 3c).

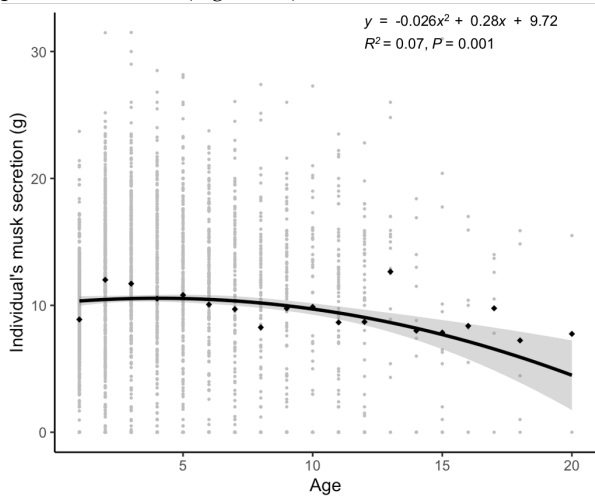


Figure 1. Musk secretion of forest musk deer in MMDBC in relation to age. Grey dots indicate the observed individual's musk secretion at a certain age. Dark dots indicate the average musk secretion at a specific age. Fitted line with equation based on the observed data with the grey 95% confidence interval was provided.

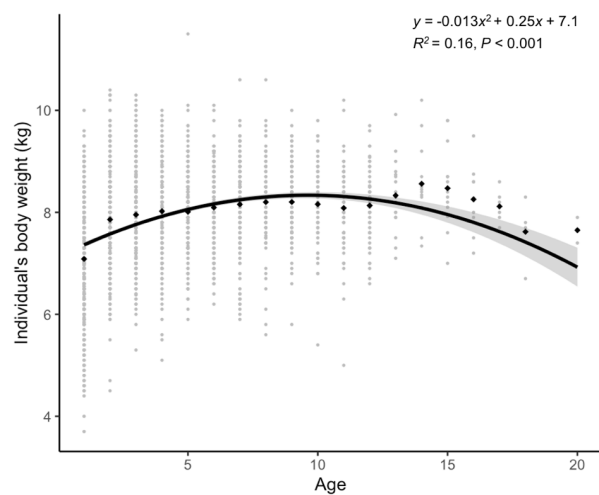


Figure 2. Body weight of forest musk deer in MMDBC in relation to age. Grey dots indicate the observed individual's body weight at a certain age. Dark dots indicate the average body weight at a specific age. Fitted line based on the observed data with the grey 95% confidence interval was provided.

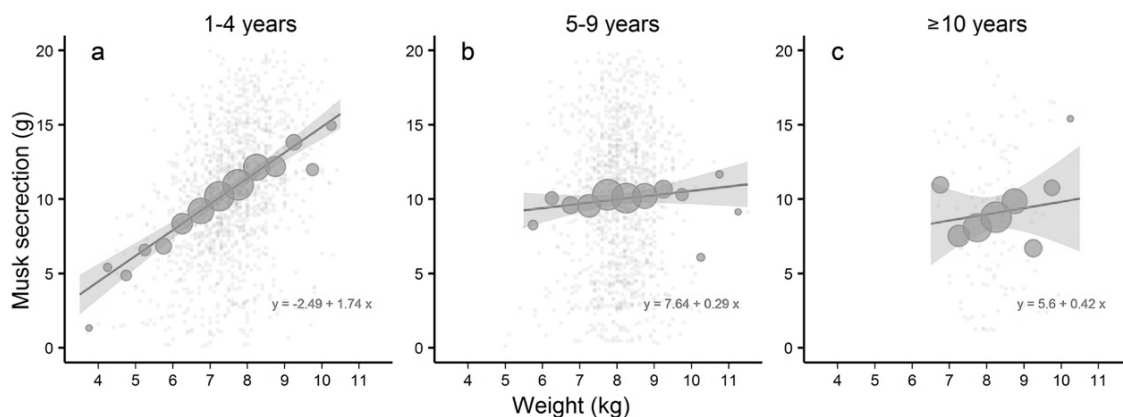


Figure 3. Musk secretion of forest musk deer in MMDBC in relation to body weight among the age of (a) 1–4 years' individuals (b) 5–9 years' individuals and (c) older than 10 years' individuals. Grey dots indicate the observed individual's musk secretion at certain body weight. Blue circles indicate the average musk secretion at a 0.5 kg body weight interval. Fitted line with 95% confidence interval was provided.

## Discussion

### Age-related body weight and musk secretion

By recording the body weight of male forest musk deer during the musk secretion period, we found that body weight of male forest musk deer increased obviously until the age of 10, then represented a downward tendency afterwards. In accordance with our results, most ungulates reach the maximum body condition at the prime age: Weladji et al. (2010) reported that the body mass of reindeer peaked at the age of 10–11, Festa-Bianchet & Côté (2008) reported that the peak of 6 years for mountain goat (*Oreamnos americanus* de Blainville, 1816), Gaillard et al. (1992) reported that the peak of 8–10 years for bighorn sheep (*Ovis canadensis* Shaw, 1804) and they also had the later decrease in body condition at older ages.

Musk secretion, a key secondary sexual trait for the

endangered male musk deer, is involved in chemical communication, hormone level and reproductive success (Sokolov et al. 1986). Our results showed that, the average musk secretion of male forest musk deer was 10.29 g per head in MMDBC. Due to the species-specific difference and population size variances, this is different from other studies: for the captive alpine musk deer (*Moschus chrysogaster* Hodgson, 1839) it was 7.90 g per head (Li et al. 2012), Himalayan musk deer (*Moschus leucogaster* Hodgson, 1839) – 25g per head (Khan et al. 2016), Siberian musk deer (*Moschus moschiferus* Linnaeus, 1758) – 11.24 g per head (Bi et al. 1985). Dai and Yin (1991) suggested a 14.66–16.13 g per head secretion for the forest musk deer in small-groups. Our results also showed that the prime-age of musk secretion ranged from 2 to 10 years, which includes the 2–5 years prime period suggested by Homes (1999). Differences may be related to the study methods and spatial-temporal

differences.

Though the regression model between age and body weight or musk secretion had a relatively small  $R^2$ , which may be affected by the interaction between body weight and musk secretion, enclosure, and year variances as well, age-related decline in both musk production and body weight represented various onsets, indicating that body weight and musk secretion varied with age in a quadratic manner. The age-related patterns supported by our finding of a musk secretion decline at 4 years and a body weight decline at 10 years, thus our first hypothesis was proved.

Heavier body weight and larger musk production were found in prime-age males compared to young and older ones. This allowed prime-age males to maintain a high rank and maximize their current and future fitness by being ready for the following rutting season (Green 1987, Meng et al. 2011). Studies on trait asynchrony in aging rates within populations showed that all survival-related traits should senesce at the same rate, since the assumption that the intensity of selection for a trait increases as its mean value across the population decreases (Williams 1999). However, our results showed that the onset of senescence of body weight and musk secretion for male forest musk deer varied, and this related to relatively weak correlation between body weight and musk secretion, as well as the various thresholds of age-specific change pattern. In accordance with our results, 20 phenotypic traits including vital rates and body mass of Soay sheep showed a striking asynchrony of senescence within the population (Hayward et al. 2015).

#### Relationship between body weight and musk secretion at age classes

For males capable of musk secretion, a positive correlation was found between body weight and musk secretion for age of 1–4 years' individuals, and the correlation coefficient suggested that heavier body weight contribute to more musk secretion, whereas this trend was not significant in senile males. Thus, our second hypothesis that potential relationship between musk secretion and body weight was supported. Musk secretion is costly for males, and those with poor body condition must shorten the secretion duration and reduce the musk quantity to secure survival and restoration for the following rutting season (Shrestha 1998, Meng et al. 2006, He et al. 2014). Prikhod'ko (2003, 2008) reported that, for Siberian musk deer (*Moschus moschiferus* Linnaeus, 1758), gonadectomized males cannot secrete musk, though had high body mass indicators, which is not mutually exclusive with our results. Male forest musk deer involved in our study were all capable of musk secretion with well-functioning preputial gland. Under this circumstance, a significant correlation between musk secretion and body weight was found in males of 1–4 years old.

Reliable signals must be costly and cannot be produced by an individual with low body condition (Kotiaho 2000). Musk is one pheromone secreted only by the adult male musk deer and involved in sexual chemical communication during rutting and mating. As a costly physiological activity, the secretion is related positively to a health condition, requiring substantial investments of resources (Sheng & Liu 2007). Males with a better body condition can afford the

costly energy consumption to obtain better olfactory sexual communication by producing more musk. Young males with heavier body weight can secrete more musk to obtain a higher sexual attraction, which benefits the mating success compared with senile males with higher rank and more reproduction experiences. Besides, young males with heavier body weight are physically mature and able to compete effectively for access to estrous females with other older large-bodied and more experienced males and are likely reflecting the most successful strategy for maximizing individual fitness.

Body weight and musk secretion are notable and suitable indexes to estimating senescence for male forest musk deer, and showing different onsets of senescence, which is affected by the relationship between body weight and musk secretion. Young males secreted more musk with heavier body weight for better sexual attraction to maximize individual fitness. Our results enhance the understanding of forest musk deer biology and have important implications for the sustainable management of musk resource in captive populations.

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#### **Ethics approval**

Work with animals was in accordance with the guidelines established by the Academic Committee of School of Environment and Natural Resources, Renmin University of China.

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