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Review Article

Population Status and Research Progress of Père David's Deer (*Elaphurus davidianus*) in China

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Authors' Contribution
JZ designed the study, provided research funding. HZ collecting the data and wrote the manuscript. HW and PL Both Huajun Wen and Pengfei Li contributed to the data collection and investigation. GT, HC and LZ collected and updated the latest research progress and population situation.

Key words
Père David's deer, *Elaphurus davidianus*, population status, Research progress

ABSTRACT

The Milu has been called Père David's deer (*Elaphurus davidianus*). It is a unique species in China, which has been extinct in China for nearly 200 years. In 1980, the Milu was reintroduced into China and has now become a first-class protected animal in the country. Moreover, research on the Milu has begun. After 30 years of breeding and development, the Milu population has formed stable captive or wild populations in Beijing, Dafeng, Shishou and Dongting Lake. It has become a typical example of the successful reintroduction of a species. Because of the precision of the information on the Milu in biology and population history, the research on Milu has developed rapidly in recent years. In this paper, the authors summarize the principal research work of 30 years on the population development, habitat, disease dynamics, and biological or genetic characteristics of Père David's deer in detail, review the research status and progress and examine the trends in the development and direction of Milu research in China, which should provide a reference for the healthy development and protection of the Milu population.

INTRODUCTION

The Milu is a national first-class protected animal in China and an endangered species in the world. It originated in the middle and lower reaches of the Yangtze River and has a history of nearly 3 million years. Approximately 150 to 200 years ago, the wild Milu population became extinct due to human activity and changes in the natural environment. At the end of the 19th century, more than 30 captive Milu were sent overseas to keep them safe during the war, and the native Chinese Milu population disappeared completely (Fang *et al.*, 2009; Zhang *et al.*, 2011; Bai *et al.*, 2012). In approximately 1900, the English 11th Duke of Bedford, the Lord of Woburn Abbey, raised and bred the world's last 18 Milu on his estate. In the 30 years from 1956 to 1986, the Woburn Abbey Manor and the World Wildlife Fund (WWF) presented 83 Milu to China four times (Yang *et al.*, 2002). In the following 30 years, the Chinese Milu population in Beijing, Dafeng, Shishou, Dongting Lake and other places continued to multiply (Table I); it has reached nearly 7000, an increase in the population of 700 times so far (Fig. 1) (Ding *et al.*, 2006; Yang *et al.*, 2007; Ding *et al.*, 2014).

RESEARCH PROGRESS ON HABITAT OF PÈRE DAVID'S DEER

At present, the habitat selection of the Milu has only been reported in some papers. Its habitat in the summer and autumn often provides abundant water, food, and a high degree of concealment. He *et al*. (2007) studied the habitat selection of the Shishou Milu in Hubei Province. The decisive factors for habitat selection of the Père David's deer are different due to seasonal changes. The requirement for food is the decisive factor for the seasonal selection of the habitat. Water is also a selection criterion in different seasons. The extent to which the deer change their habitat in the summer and their choice of habitat depend more on water. Zou (2012) found that the habitat of the Milu in the spring and autumn was extremely rich in food and water. Food and anthropogenic disturbance are the primary limiting factors in the microhabitat selection of resting grounds for the Milu. Because the fire trace areas in the winter are basically covered by new-born acoustic bundle or washed away by rain water, the influence of fire marks is not considered in the spring and autumn. As

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the Yangtze River enters a high water season in summer, the vast majority of the Milu habitat becomes covered by floods. Therefore, the roads are basically inaccessible, and coupled with the hot weather, the number of people who enter the habitat of Milu is also greatly reduced. Food and temperature are the primary limiting factors in the microhabitat selection of the Milu resting ground. In the winter, when the Yangtze River enters a dry season, the edible plant species of the Milu diet within and out of the reserve, as well as the vegetation height and coverage, are greatly reduced. Compared with the other seasons, the food was relatively scarce during the winter, and the low temperature had a greater impact on the Milu within the microhabitat of the resting land. Zou *et al.* (2013) explained that in the winter the primary habitat of the Milu in Shishou was abundant in food, high in vegetation coverage and offered ready concealment. A study on the annual habitat selection of the male deer in the semi-wild Milu in the Dafeng Milu National Nature Reserve shows that the Milu tend to choose the location of migratory bird communities, which have a higher plant density and plant height and greater aboveground biomass in the autumn and winter seasons. In the spring and summer, the Milu prefer to choose the location of alternate plant communities with lower plant heights and smaller aboveground biomass. The Milu that were released preferred to choose a location close to a water source and a shallow water swamp but at a medium distance from the ocean. Based on the principal component analysis of the eight ecological factors, the factors affecting the habitat selection of Milu include aboveground biomass, plant height, water factor (water distance, distance from the shoal), and the interference factor (distance from human interference) (Wu *et al.*, 2011).

On this basis, Li *et al.* (2016) found that the discriminant function of plant coverage, food abundance, distance from the road, concealment, wind speed, distance from a water source and distance from concealment can distinguish the nocturnal resting land of the Milu in different seasons. Moreover, the nocturnal resting land characteristics of the Milu in different seasons partly overlapped, which may be related to the difference in food, water, temperature and man-made disturbance in different seasons.

Xu *et al.* (2013) found that the behavioral frequency of the natural wild Milu owners and the scattered Milu owners varied in different habitats, indicating that the Milu owners expressed different types of behavior in different habitat types. Simultaneously, man-made interference and the flooding season of the Yangtze River also affected the expression of the behavior of the owners of the natural wild Milu populations to some extent and the strategies of the owners of Milu groups inside and outside the reserve to adapt to different habitats by regulating the behavior of the deer. It would be highly significant to enlarge the population size of the Shishou Milu in Hubei Province.

Table I.- Summary and population distribution of four Milu Nature reserves in China.

	Dafeng Milu Nature Reserve, Jiangsu Province	**Shishou Milu Nature Reserve, Hubei Province**	**Nanhaizi Milu Park, Beijing**	**East Dongting Lake National Nature Reserve, Hunan Province**
Geographic Coordinates	33°5'N, 120°49'E	29°49’N,112°33’E	39°46’N,116°26’E	28°59’~29°38’N,112°43’~113°15’E
Annual Mean Temperature (°C)	14.1	16.5	13.1	17.5
Relative Humidity (%)	80	80	65	80
Average Annual Rainfall (mm)	About 1000	About 1200	About 600	1100~1400
Altitude (m)	1.0~2.0	32.9~38.4	About 31.5	30~35
Floor Space (hm^2)	78000	1567	55	190300
Vegetation Condition	salt meadow, swamp vegetation, aquatic vegetation, deciduous broad-leaf forest and sparse shrubwood, etc.	Swamped meadow, Lake vegetation, swamp vegetation, shoal grassland and dry willow thickets, etc.	aquatic vegetation, thicket, Arbor forests, etc.	hygrophyte, aquatic plant, floating-leaved plant, submerged plants and floating plants
Number of Population	About 1500	About 1100	About 120	About 200

Fig. 1. Distribution map of living Milu in China.

The Milu have a strong reproductive capacity and adaptability to the surrounding environment. The reintroduction of the deer into the Beijing and Dafeng Milu sanctuaries is strong proof that the population in these two sanctuaries has rapidly increased, and the deer are exhibiting strong growth and are in good condition. At the end of 1992, the population of Milu in Beijing increased to more than 200. Since the introduction of the Milu population, the fertility and survival rate of the population have been stable at a high level for a long time (Li *et al.*, 2015). The population density of Milu is increasing daily. To this end, we need to establish more breeding bases to facilitate the rehabilitation and reintroduction of the deer.

Therefore, domestic researchers have conducted a substantial amount of research on the choice of Milu habitat. The habitat of the deer was affected by its own activity. It was found that the activities of the deer had different effects on the physical and chemical properties of soil when the water content, organic matter and pH of several typical habitats used by the Milu were compared. The activities of the Milu aggravate soil salinization without exception but can increase the available nitrogen, phosphorus and potassium contents of the plants. The abundant natural resources in the field and semi-field environments that are readily acclimatized by the deer result in cluster activities that will reduce the content of the soil organic matter. In addition, low-density wild population activity and a high-density population can effectively slow down the process of soil salinization (Zhu *et al.*, 2016). Zhao *et al.* (2010) examined the wetland vegetation in the third core area of the Dafeng Milu National Nature Reserve in Jiangsu Province as an example and found that the dominant species in the community were obvious; the associated species were few; the species composition was monotonous, and all of them were herbaceous plants. The distribution of wetland vegetation from offshore to inshore beaches presents obvious regular changes: the species diversity, ecological dominance and community evenness of *Spartina alterniflora* community → *Suaeda salsa* community → *Zoysia macrostachya* community → *Phragmites communis* community → *Puccinellia tenuiflora* community were lower, and the species richness showed a trend from low to high and then to low.

The results of the PCA and plant physiological

characteristics analysis suggested that the soil salt gradient was the primary factor affecting the distribution of the plant communities. Liu *et al.* (2011) and Jia *et al.* (2018) found that the seasonal selective feeding on Chinese pennisetum (*Pennisetum alopecuroides*) by the Milu greatly affected the composition of the habitat of the plant community and the proportion of plant biomass with seasons. The burning and killing of ticks may be the primary reason for the vegetation status of the habitat. The results provide a theoretical basis for ecological restoration of the habitat in the Dafeng Milu reserve. Jing (1992) conducted research on whether to introduce the deer in Hainan Island from the aspects of climate and precipitation. The results show that Hainan Island has a humid climate and enough precipitation to facilitate the survival and development of the Milu. The release of the deer in this area is feasible.

Yang *et al.* (2002) sought to determine if the Milu could be returned to the ancient habitat of the Dongting Lake and conducted research on whether the natural population of the deer could be restored. Ding *et al.* (2005) has conducted a large amount of scientific research and experiments on Yellow Sea beaches, providing a theoretical basis for the introduction of the wild deer population in this area. Fang *et al.* (2009) conducted a comprehensive assessment of the indicators in the living environment of Dafeng Milu. Zhang *et al.* (2010) compared the environment and water quality of the deer court before and after the wetland restoration project in Beijing and concluded that the wetland environment recovery in the region was relative. In addition, the analysis of the births and deaths of the deer in 2006-2009 demonstrated that the survival status of the Milu has improved, and the use of reclaimed water to repair the wetland environment has achieved initially promising results in protecting the deer.

Li *et al.* (2012) analyzed the reasons for the decline of the quality of the wetlands habitat: its degradation, environmental resistance, and slowing of the growth rate of the Milu population due to human factors, natural environment changes and population growth and took measures to improve the ecological environment and restoration. Corresponding countermeasures and suggestions were discussed in the qualities of wetland function, increasing the carrying capacity per unit area and expanding the area of wild stocking. Ding *et al.* (2015) faced a series of ecological degradation problems in the habitats of the Dafeng Milu National Nature Reserve in Jiangsu Province, such as a poor water environment, a single habitat, insufficient food resources and parasite threats, that helped to analyze the ecological problems of the protected areas. As a result, measures and projects, such as water source improvement, rotational grazing and vegetation conservation, forage base, parasite prevention and control, and the translocation of the Milu, were proposed.

Simultaneously with the expansion of the population of the Milu, the demand for food is increasing, since the original habitat cannot meet the needs of its growth and development. This has resulted in an expansion of the lands on which the deer are active. Its feeding and trampling behavior not only destroyed the ecological environment of the semi-stocked area but also changed the physicochemical properties of the plant community structure and soil in the area. To this end, personnel and researchers that seek to protect the Milu have conducted in-depth research and extensive discussion and proposed solutions that meet the needs for environmental ecology and sustainable development strategies, such as expanding the stocking area of the deer, artificially improving the ecological environment of the stocking area or trans locating the Milu.

ADVANCES IN RESEARCH ON THE PREVENTION AND CONTROL OF DISEASES OF THE MILU

During the research on the prevention and treatment of the Milu diseases in 2010, a large number of deaths of the deer of the two populations of Shishou Milu National Nature Reserve and Sanheyuan in Hubei Province occurred. This situation exposed many problems in the prevention and control of diseases of the deer. Pathogens, such as *Clostridium welchii*, enteropathogenic *E. coli*, *Clostridium putrificum*, *Pasteurella* and *Haemaphysalis* spp., threaten the survival of the deer. These pathogens are usually spread by mixed infection, which greatly increase the mortality of the deer. Zhang *et al.* (2012) analyzed the law of the death of the Milu in captivity. Winter and spring are the peak seasons of the deer deaths. It was believed that digestive tract disease was the primary factor for death in captivity, followed by surgical disease. In March 2010, more than 90 Milu died in Sanheyuan. In addition to infection with *Clostridium welchii*, their death was aggravated by the infection of *Aeromonas hydrophila* due to the feeding of dead fish to the deer (Zhang *et al.*, 2011). It was found that malignant catarrhal fever and hemorrhagic enteritis were the two most serious diseases of the Milu. Hemorrhagic enteritis is one of the most frequently reported diseases of the Milu in China. It is caused primarily by *Clostridium welchii* and enteropathogenic *E. coli* (Khan, 2011). These diseases are highly effective at killing the deer because of their sudden onset, rapid death and poor treatment options. These pathogens are common bacteria in the natural environment and animal intestines, which are easily induced to become pathogenic by weather, stress,

decreased physical shape or improper feeding (Wang *et al.*, 2018). Local researchers have performed epidemiological investigations, determined the clinical symptoms, and performed pathological necropsies and laboratory tests on the deaths of the Sanheyuan wild Milu in the south bank of the Shishou River. The results show that the death of the deer population was caused by infection with *Clostridium perfringens* and *Aeromonas hydrophila*. (Zhang *et al.*, 2011). Due to the combined effects of over-feeding on reed shoots, drinking polluted water and being subjected to large weather changes, the deer with poor immunity died. Zhang *et al.*(2012) tested the incidence of Milu diseases and confirmed that the incidence was caused by *Clostridium welchii* type C. *Clostridium* is an opportunistic pathogen, and its effects are primarily due to toxins. It was recommended to adjust the structure of high-energy feed and fiber to provide a wholesome environment to ensure the health of the animals and to reduce the amount of disease.

Regarding the population dynamics and estrus behavior after the Milu disease outbreak, Xu (2013) continuously monitored the dynamic changes of the population of the wild reindeer *Rangifer tarandus* population outside the protected area and the population of the deer in the protected area after the outbreak to recover and provided basic data and a scientific basis for the Shishou population and the protection of the Milu resources. From December 2011 to January 2013, based on the long-term ecological monitoring of the Shishou Milu population in Hubei Province, the natural estrus period and the difference in the effort between the wild Milu population and the scattered population was studied. Xu (2013) suggested improving the wetland habitat and restoring the wetland function and water circulation to help improve the habitat, population growth and disease control.

Many studies have been conducted on the living environment, genetic inheritance and behavior of the captive Milu. Diseases to which the captive Milu are susceptible include malignant catarrhal fever, bovine viral diarrhea (BVDV)-like strains, hemangiosarcoma, endocarditis, *Schistosoma japonicum* and a variety of parasitic antibodies. The relationship between the control of the histological basal hormone release and the reproductive cycle and initiation is the content of the reproductive biology research (Flach *et al.*, 2002; Sedlak and Bartova, 2006). The research on the physiological, biochemical and genetic aspects of the deer primarily include the existing genetic status and its protection and the corresponding physiological and chemical components of the Milu blood. Simultaneously, it also includes research on the disease prevention and control of the stag beetles that cause anemia in the deer, the medicinal effects of staghorn antlers, the extraction of fecal DNA, the disease occurrence analysis of the Beijing and Shishou wild Milu population, and the investigation of the cause of death.

OTHER RESEARCH PROGRESS ON THE MILU

Local researchers have conducted more basic research on the history of the Milu, its population behavior, disease prevention and habitat in relation to worldwide research. The research on the history of the deer is primarily focused on the reasons that the wild population went extinct under natural conditions, the distribution of the deer in different regions and the resources of the ancient Chinese Milu (Bai *et al.*, 2012; Ding *et al.*, 2014). Behavioral research on the growth habits, physiological and biochemical processes, reproductive behavior and population phenomena includes the growth and reproduction behavior in different seasons under semi-natural conditions, the spatial distribution frequency of winter manure and fecal piles, the behavioral study of eating the invasive plant *Spartina alterniflora* (Yu *et al.*, 1996; Ding, 2009), the behavioral analysis of the spectrum of the behavior of the deer and the PAE coding system (Jiang *et al.*, 2000), the behavior of the same-sex groups and the synchronous activity of the deer, rutting call behavior and sequence level affecting reproductive behavior, and the relationship between the effective population of the deer and the mating behavior (Li *et al.*, 2001; Zeng, 2007; Jiang *et al.*, 2012). Studies on the physiology of the Milu include the observation of anatomy and histology in infant Milu hearts (Zhong *et al.*, 2018) and observations on antler shedding in the summer (Ding *et al.*, 2018).

The research on the population behavior and habitat of the Milu primarily examines the interaction between the environment and the population in different areas, including the study of the deer habitat in the Dafeng National Nature Reserve (Xu and Ding, 1997), the change of the population of Milu (Su *et al.*, 2003), the extension of the habitat of the Shishou Milu in Hubei (Liu *et al.*, 2007), the choice of the winter habitat (He *et al.*, 2007), the relationship between the living environment of the semi-wild Milu, the vegetation and the plant species that they feed upon, deleterious factors, the habitat affected by the population, herbaceous plant composition and biomass structure gradient (Zhang *et al.*, 2019), to inhibit the growth factors of the deer population and the impact of heavy metals on the habitat environment (He *et al.*, 2012).

In addition, other studies cover the investigation of animal resources in Swan Island and surrounding small areas (Wang *et al.*, 2001), including a survey on the

herbaceous flora and species diversity (Zhang *et al.*, 2019), the joint research of the Shishou Milu Conservation Area and the community (Feng, 2005), the ability of the wild deer to survive, extinction risk assessment (Li and Yu, 2009), the relationship between the population density and birth rate of the Milu in Shishou (Song *et al.*, 2015) and development prospects and management methods for the captive Milu population (Zhang *et al.*, 2011).

In other respects, the physiological behaviors of foraging and the trampling of the terrestrial vegetation inhibited the growth of the plants in the short-term and had an important impact on the community structure and dynamic changes of the plants. The herbivore effect on food specialization was more significant. The deer are primarily herbivorous. Their principal area of activity is swamp wetland, which is effective for both resisting natural enemies and feeding. The deer are fond of the young leaves of common plants in marshy wetlands. With the expansion of the population of the deer, the demand for food is increasing, since the original habitat cannot meet the needs of the growth and development of the population. The Milu have expanded the areas in which they are active for this reason. The behavior, particularly selective feeding, not only affects the habitat environment and its containment but also causes partial changes in the biological and abiotic characteristics of the area. To this end, Milu protection workers and researchers in China have conducted in-depth research and extensive discussion and proposed to improve the ecological environment quality of the stocking area, the artificial restoration of multiple habitats, increase the environmental capacity of the deer stocking area and the stocking type of the deer. Improvements, such as solutions to environmental ecology and sustainable development strategies, will be implemented. For the deer population and its management, Chinese researchers have long-term monitoring and research programs on the population dynamics, immigration rate, migration rate and structure of the ages of the deer population to predict the population changes. The deer have been re-introduced and developed for a long time in the country. For example, Yang *et al*. (2007) compared the population dynamics of Shishou, Dongting Lake and Dafeng by studying the population dynamics of the deer in the Shishou protection area and found that the population dynamics in different habitats are noticeable. The difference is that the growth rate of the wild Milu in Dongting Lake is higher than that in Shishou, and the dynamic index of each population in Shishou is higher than that in Dafeng. Statistics indicate that the number of Milu populations in China had reached more than 2,500 at the end of 2010. Many studies suggest that lower genetic diversity, habitat factors, disease and human disturbance are the principal factors limiting the development of Chinese free-ranging Milu populations (Wang *et al.*, 2009; Zhang *et al.*, 2011; Song *et al.*, 2015). To this end, the Chinese government, animal protection agencies and other institutions have proposed a variety of practical solutions through a large number of research experiments. The increasing number of the deer populations has led to the problem of saturated environmental capacity in Beijing and the Dafeng and Shishou Milu Conservation Areas, limiting the development of the population. In this regard, Jiang *et al.* (2001) proposed measures to remove some Milu individuals from the protected area and reduce the fertility of the female deer by chemical or other methods, aiming to temporarily reduce the population density and solve the problem of the saturation of environmental capacity. Dispersing and releasing the deer in different places is the principal measure China is currently using to maintain the population within carrying capacity levels.

The genetic study of the Milu population indicates that it has lower genetic diversity in China. Only a single mtDNA D-loop haplotype was found in the deer. In addition, only five polymorphic microsatellite loci were screened out from 84 pairs of deer-transferred primers (Zeng *et al.*, 2007). The genetic makeup in the three deer populations was significantly different ($P < 0.01$). He (2012) indicates that the allelic richness in the Tianezhou population was the highest, and it is the most appropriate source population in China for the establishment of new Milu populations in the wild.

SUMMARY AND PROSPECTS

The current management status of the deer is still facing severe challenges. All types of deteriorating environmental factors are potential threats to the deer, and under certain conditions, disease will be induced, causing deaths in a large number of them. After the habitat is destroyed, the self-purification function of the water body declines, and eventually the water ecosystem deteriorates. The large-scale reproduction of pathogenic bacteria is believed to be directly related to this. It is worth noting that bacteria and viruses in the unsuitable environment will cause natural decay and death, and plants will absorb some organic pollutants through their metabolism. Plant root exudates have an inactivating effect on *E. coli* and other pathogens. Therefore, improving the habitat of the deer through ecological measures will undoubtedly contribute to prevention and disease control. Obviously, using the ecological regulation of the habitat as the entry point, the ecological control technology based on the prevention and control of disease is of substantial significance to the healthy development of the Milu population. The genetic diversity of Milu is also worthy of attention. The genetic

background of a species with low genetic diversity should be prioritized in management.

Currently, the comprehensive research data on the Milu is relatively insufficient, and the systematic research on the deer habitat also merits further research. The characteristics and distribution of habitats of Père David's deer and their relationship with the water environment and wetland ecosystems in the active area are still unclear. How to effectively rehabilitate the degraded habitat to be suitable for the living habits of the deer is still not clear. These factors need to be studied to form a theoretical basis for the management of the Milu.

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Statement of Conflict of Interest

The authors declare no conflicts of interest.

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