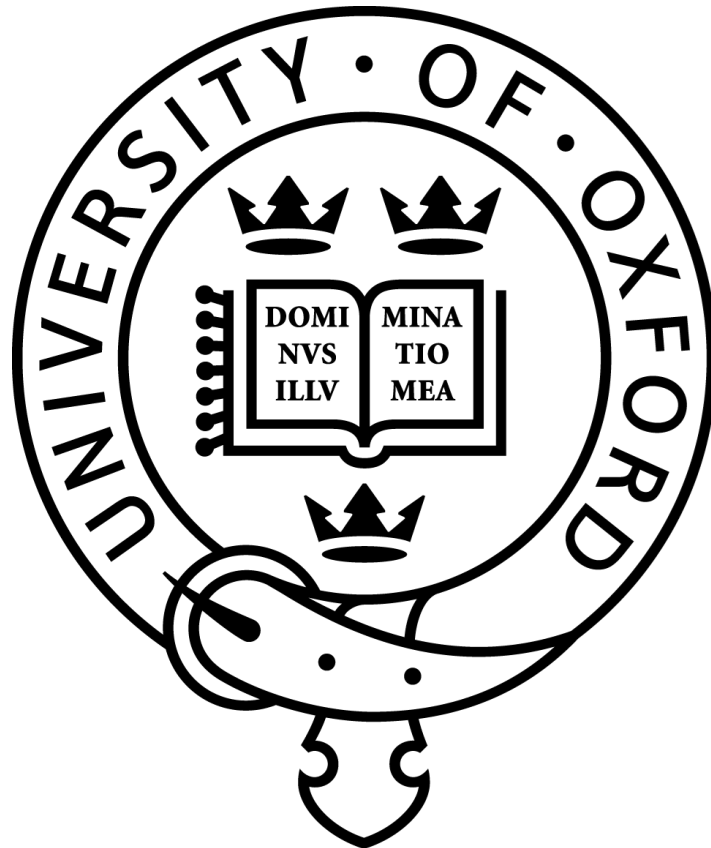


**Human Influences on Animals from an Ancient DNA Perspective:
Case Studies on Chinese Domestic Cats (*Felis catus*), Chinese Leopard
Cats (*Prionailurus bengalensis*), Fallow Deer (*Dama dama*), and
Chinese Domestic Pigs (*Sus scrofa*)**



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Thesis submitted for the degree of
Doctor of Philosophy
Michaelmas Term 2023

Declaration

I hereby declare that this thesis is based on the research conducted at the School of Archaeology, University of Oxford, UK, home of the Wellcome Trust Palaeogenomics & Bio-Archaeology Research Network, and the Laboratory of Genomic Diversity and Evolution, School of Life Sciences, Peking University. The outcomes of this paper-based thesis are offered in the form of individual manuscripts. No parts of this thesis have been submitted elsewhere for any other degree or qualification, and the whole document is my own work under the supervision of Professor Greger Larson, unless otherwise stated in the author contributions.

Yu HAN
Oxford, January 2023

**Human Influences on Animals from an Ancient DNA Perspective:
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Pigs (*Sus scrofa*)**

Abstract

Human-animal interactions since the late Pleistocene have exerted long-lasting and significant influences on animals. With the advance of ancient DNA techniques, the availability of genomic data from ancient samples has profoundly increased, and now with great possibilities we are able to investigate the past and gain knowledge of the phased genetic changes in history. Applying predominantly ancient genomic methods, I have conducted case studies on four different species under the states of domestication, semi-domestication, and commensalism respectively to explore how the anthropogenic exercises have affected the evolutionary trajectories of animals.

The first case involves Chinese domestic cats (*Felis silvestris catus*) by examining 4 nuclear genomes and 22 mitochondrial genomes from 22 ancient Chinese samples spanning 5,500 years. Chinese domestic cats are descended from African wild cats (*Felis silvestris lybica*) and were likely brought to China via the Silk Road during the 7th and 9th centuries AD. Leopard cats (*Prionailurus bengalensis*) might have been enjoying a commensal relationship alongside humans in the Late Neolithic before domestic cats arrived in China (6,000-5,500 BP). However, this commensal link did not survive the Han Dynasty (202 BC - 220 AD). Small felids and humans did not cohabit again until the Tang Dynasty (618-907 AD) when domestic cats were imported. Since then, domestic cats have not mixed with any Chinese native wild cat species, including the Chinese mountain cat (*Felis silvestries bieti*) and Asiatic wild cat (*Felis silvestries ornata*).

The second case involves the Chinese leopard cat (*Prionailurus bengalensis*), who were in a commensal relationship with humans since the Chinese Neolithic period (5,500 BP). This study collected seven ancient leopard cats from the Middle Reach of the Yellow River Basin. Ancient genome study shows that the leopard cats from the Middle Reach of the Yellow River Basin and Far East Russia were closely related, albeit the former was receiving introgression with Indochina leopard cats. According to archaeological, chronological, and literary analysis, leopard cats and humans ceased their commensal connection when the Han Dynasty (202 BC - 220 AD) fell, and never recovered. Domestic cats replaced leopard cats in the human niche, when they were introduced around the Tang Dynasty (618-907 AD).

The third case involves the Persian and European fallow deer (*Dama dama mesopotamica* and *Dama dama dama*). The post-glacial dispersal of the fallow deer was nearly entirely mediated by people. We generated and analysed 29 partial or mitochondrial genomes of ancient fallow deer to gain insights into their evolution and range changing. Ancient DNA analysis indicates that the two fallow deer subspecies diverged 0.57 million years ago and might have interbred in Southwestern Anatolia. An ancient maritime commercial route brought Persian fallow deer from the Levant to Cyprus and westward to the Mediterranean, while European fallow deer from Anatolia were dispersed to continental Europe.

The fourth case involves the Chinese domestic pigs (*Sus scrofa*). The Middle Reach of the Yellow River Basin was one of the domestication hubs of pigs. We have conducted ancient genomic investigation of five nuclear and nine mitochondrial genomes of ancient domestic pigs from two archaeological sites in the Middle Reach of the Yellow River Basin spanning at least 3,000 years, and zooarchaeological analysis on 1,400 pieces faunal remains excavated from the Zheng-Han City (2,500 BP). I suggest that the populations represented by the ancient domestic pigs from the Middle Reach of the Yellow River Basin had contributed to the formation of

modern Chinese breeds and issued new hypothesis of the origin of the Chinese domestic pigs. Zooarchaeological approaches showed a reduction in pigs' prominence in archaeological sites and potential intensified pig husbandry in the MYR from the late Neolithic to the Iron Age.

Combining the results of the four case studies, we have addressed the importance of anthropogenic forces in altering the evolutionary trajectories of animals: 1) The human-mediated dispersal can dramatically change the range of the species, even for the animals who had lost most of their genetic diversities, accompanying the migration of human, they were still able to be spread globally. 2) By establishing affinities or cultural connections with human, the population dynamics of animals would closely correlate with the development of human societies. 3) Importing new species would lead to the admixtures between exotic animals and its related local counterparts. Such admixtures could in one hand introduce new genetic diversities, while in another hand, offset the adaptability of the native species to local environment.

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1. Introduction

1.1 Motivation of the thesis

Recent global environmental changes suggest that Earth may have entered a human-dominated geological epoch, referred to as the Anthropocene (Crutzen, 2006; Lewis and Maslin, 2015a, 2015b; Zalasiewicz et al., 2010). While the precise definition, onset, and necessity of the Anthropocene as a distinct geological period remain debated (Hamilton, 2015; Head, 2014; Lorimer, 2017; Zalasiewicz et al., 2008), it is indisputable that human activities now exert a global impact, resulting in significant alterations to the Earth's land surface and atmospheric composition (Capinha et al., 2015; Dean et al., 2014; Smith and Zeder, 2013; Summerhayes and Zalasiewicz, 2018).

A key global change during the Anthropocene is the transformation of Earth's fauna due to human influence (Steffen et al., 2006). Numerous species have formed biological or social bonds with humans, consequently altering their evolutionary trajectories to adapt to anthropogenic environments (Beck and Katcher, 2003; Hemsworth, 1987; Zeder, 2012a, 2012b). This highlights the pervasive impact of human activities on the natural world and further underscores the importance of understanding and addressing the consequences of these changes.

The influence of humans on animals should not be solely considered as an observable outcome in modern fauna but as an ongoing process that gained momentum at least 10,000 years ago and continues to this day (Hemsworth, 1987; Larson et al., 2014; Zeder, 2012b, 2012b). Human activities have persistently impacted animal populations, beginning in the late Pleistocene with

the domestication of dogs (Galibert et al., 2011; Larson et al., 2012). However, a more significant shift occurred during the Neolithic Revolution in the Holocene, when agriculture and sedentary lifestyles became predominant, leading to the domestication of various animals across Eurasia (Larson et al., 2010; Ottoni et al., 2013; Zhang et al., 2020), Africa (Epstein, 1971; Marshall, 2000), and America (Anderson, 2006). Consequently, natural landscapes have been continuously encroached upon by farmland expansion.

Current estimates indicate that 43% of habitable land is occupied by agriculture, in stark contrast to the mere 0.5% utilized around 7,000 years ago, shortly after the emergence of agricultural societies (Klein Goldewijk et al., 2017; Poore and Nemecek, 2018). Of this 43%, 83% is dedicated to livestock production, while the remaining 17% is used for direct human food production (Poore and Nemecek, 2018).

Given that human influence on animals began long ago and continues to the present day, relying solely on modern samples would be inadequate for grasping the full scope of this impact, as various trajectories might yield similar results. Thus, investigating historical human influences on animals is vital for deepening our comprehension of humanity's role in natural systems and elucidating the dynamics of how anthropogenic forces have shaped natural environments (Frantz et al., 2020, 2020; Larson and Burger, 2013). Fortunately, advancements in ancient DNA technology have enabled researchers to reconstruct past climates (Pedersen et al., 2015), examine ancient population dynamics (Frantz et al., 2019), and identify historical pathogens (Stone and Ozga, 2019). These breakthroughs have considerably enhanced our understanding of the extent to which humans have influenced animals throughout history.

In recent years, the field of DNA research has experienced remarkable advancements. New methodologies targeting aged samples have significantly enhanced the efficiency of recovering highly degraded ancient DNA molecules (Carøe et al., 2018; Dabney et al., 2013a; Llamas et al., 2017). High-throughput techniques have been developed to expedite sequencing, substantially increasing the quantity of genetic information while reducing cost and time (Goodman et al., 2016). As a result, our ability to interpret human-animal interactions at a molecular level has improved, providing us with high-resolution information.

The anthropogenic influence on animals is both profound and enduring (Beck and Katcher, 2003; Hemsworth, 1987; Zeder, 2012a). If it is desirable to enrich our knowledge of this ongoing process and inform future resource utilization and conservation efforts, this thesis predominantly employs ancient genomic methods to examine four distinct species living in Eurasia sharing affinities with humans. These species include: 1) the ancient Chinese domestic cat (*Felis catus*), which was domesticated in the Near East and reached East Asia through human-mediated dispersal; 2) the ancient Chinese leopard cat (*Prionailurus bengalensis*) from the Middle Reach of the Yellow River Basin, which established a commensal relationship with ancient Chinese people; 3) the Eurasian fallow deer (*Dama Dama*), which were semi-domesticated, with their post-glacial translocation being predominantly human-mediated; and 4) the Chinese domestic pig (*Sus scrofa*), one of the major indigenous domestic animals in East Asia.

1.2 Human influence on domestic, semi-domestic, and commensal animals

As the case studies in this thesis will concentrate on domesticated cats and pigs, semi-domesticated fallow deer, and commensal leopard cats, our discussion will primarily explore human influences on animals in the contexts of domestication, semi-domestication, and

commensalism.

1.2.1 Domestic animals

1.2.1.1 Definition of domestication

Domestication as a scientific concept first emerged as an academic concern in the 19th century, with Charles Darwin playing a prominent role in its development. Although domestic animals are ubiquitous worldwide, and our lives heavily rely on their products and companionship, defining domestication remains a challenge. The various definitions of domestication often depend on the degree of emphasis placed on human subjectivity within these relationships.

When humans are viewed as the leading partner in relationships with other organisms, domestication is often characterized in economic terms. At one extreme, researchers tend to focus on human intentionality, assuming that actions such as moving, feeding, breeding, or controlling animals serve a clear purpose in domestication. Conversely, other researchers portray domestication as a mutualistic relationship, emphasizing the balance and benefits for both humans and domestic animals or plants.

Zeder (2012) strongly argued for a reconciliation between these two perspectives and proposed a definition of domestication that encompasses both human intentionality and mutualistic relationships:

A sustained, multigenerational, mutualistic relationship in which humans assume some significant level of control over the reproduction and care of a plant/animal in order to secure a more predictable supply of a resource of interest and by which the plant/animal is able to

increase its reproductive success over individuals not participating in this relationship, thereby enhancing the fitness of both humans and target domesticates.

Zeder's definition of domestication emphasizes the mutual association between humans and animals or plants, with both parties benefiting from the relationship. This approach highlights human initiative while also recognizing the ecological significance of domestication as a form of mutualism. It strikes a balance between human intentionality and the inherent biological aspects of the process. Both cultural and biological mechanisms of domestication are considered, with the concept being treated as a sustainable process rather than a singular event.

Larson and Burger (2013) discussed the dynamics of domestication from a population genetics perspective, describing animal domestication as a long-term and ongoing process involving multiple stages. This view offers a more comprehensive understanding of domestication by incorporating both the cultural and biological factors that contribute to the complex relationships between humans and domesticated organisms.

1.2.1.2 Human influence on domestic animals

While various conceptual definitions of domestication exist, some researchers have taken a more practical approach by identifying a series of phenotypic or genetic traits that distinguish domesticated animals from their wild progenitors. These traits, collectively referred to as "domestication syndrome," are associated with endocrine alterations, docility, fertility, product output, coat colour, floppy ears, facial neoteny, and changes in body size and proportions (Hammer, 1984; Lord et al., 2020; Wilkins et al., 2014). Domestication syndrome traits are useful in determining domesticated animals, while also suggesting the significant role played by anthropogenic forces in modifying the evolutionary trajectory of these organisms.

Indeed, domestication, as a long-term process, continuously imparts human influence on animals. Intensive artificial management and selective breeding lead to changes in various aspects of domestic animals, including behaviour, morphology, and habitat. Moreover, the relatively enclosed environment of captivity can also impact well-being of animals. This perspective emphasizes the complex and multifaceted nature of domestication, as well as the need to consider its long-term implications for the organisms involved.

1.2.1.2.1 Physical changes to domestic animals under human influence

The initial stage of domestication typically involves the transformation of animal behaviour. In most cases, populations or individuals suitable for domestication already possess certain behavioural characteristics in their natural state. These include: 1) a social structure characterized by large group sizes, hierarchies, and male affiliations; 2) a promiscuous, male-dominated mating system with sexual signals; 3) well-developed maternal traits and precocial offspring; 4) generalist diets, strong adaptive capacity to environmental changes, and a lack of reliance on shelters; and 5) reduced resistance to or even an affinity for humans. These characteristics serve as preconditions for domestication from a behavioural perspective and are further intensified through selective breeding or captive environments.

One of the most important behavioural changes in domestic animals is the development of docility, which is considered a key aspect of domestication syndrome (Hammer, 1984; Larson et al., 2014). This change results in animals becoming less aggressive compared to their wild counterparts, making them easier to tame. Research on docility has been applied to various domestic taxa, including chickens (Li et al., 2019), cattle (Grignard et al., 2001; Le Neindre et al., 1995), and dogs (Arata et al., 2010). An experimental domestication study on red foxes has

even been conducted to gain insights into the processes and mechanisms underlying docility (Lord et al., 2020).

Considering human intentionality, the primary purpose of domestication often involves securing a consistent supply of animal products or labour to support human livelihood. As a result, phenotypic traits associated with characteristics such as fertility, fur, milk, or strength are emphasized during domestication. For example, Moghani and Ghezel sheep have played crucial roles in the native subsistence of north-western Iran and north-eastern Turkey due to their high-quality meat and carpet-grade wool. Studies have shown that selection of BMP15 (bone morphogenetic protein 15) and GDF9 (growth differentiation factor 9) genes both contribute to improvements in ovulation rate (Barzegari et al., 2010). Similar cases include the selection of the DGAT1 (diacylglycerol O-acyltransferase 1) gene for milk yield in cattle (Dudášová et al., 2021; Grisart et al., 2002; Samuel et al., 2022); the GGA (Golgi associated, gamma adapt in ear containing, ARF binding protein) gene family for chicken meat production (Nadaf et al., 2007); and the COX4I2 gene for trotting and racing abilities in horses (Dall'Olio et al., 2021).

The selection of these genes in certain species or breeds demonstrates how humans, in pursuit of animal products, have actively influenced the evolution of domestic animals. However, it should be noted that these genes are typically associated with the later stages of domestication and may not have been present during the early phases.

Aside from traits selected to meet humans' direct subsistence needs, some characteristics have been selected for other reasons. The transformation of coat colour is one such change, recognized as one of the most significant domestication syndrome (Hammer, 1984; Larson et

al., 2014). Domestic animals usually exhibit greater variation or marked distinctions compared to their wild progenitors. In natural environment, wild boars, the ancestors of domestic pigs, typically have a camouflage coat that protects them from predation. However, the most common coat colour of domestic pigs is black. Research indicated that black coat colour in domestic pigs was commonly favoured by people in the ancient world, although the genetic mechanisms behind this preference differ, with mutations in the MC1R gene occurring in Western and Eastern Eurasia, respectively. Similarly, the prevalence of white coat colour in domestic cats in East and Southeast Asian countries may also indicate a cultural preference. This characteristic does not relate to any functional aspects of subsistence and likely experienced selection to fulfil the cultural preferences of local people.

1.2.1.2.2 Disease resistance

The advent of agriculture and animal farming has been accompanied by the emergence of various significant diseases within the human niche, many of which are thought to be associated with animal farming. Factors such as relatively confined spaces, denser populations, modified diets, poorer sanitation, and more frequent contact with humans for domestic animals (Zeder, 2012a) have led to an increased risk of pathogens, particularly zoonotic diseases such as measles, pertussis, smallpox, tuberculosis, taeniid worms, and falciparum malaria. (Morand et al., 2014; Pearce-Duvet, 2006).

In response, the immune systems of domestic animals have also evolved. Salmonella, an infectious disease causing significant health issues in various domestic animals, has prompted the evolution of salmonella resistance genes in domestic chickens, pigs, and cattle, mitigating the serious impact of the disease (Morand et al., 2014; Wigley, 2004). Notably, salmonella is responsible for a significant proportion of the global infectious disease burden, with most cases

being food- or water-borne. To ensure the safety of meat supply, humans continue to improve the resistance to salmonella in domestic populations through selective breeding (Andres and Davies, 2015; van Winsen et al., 2001).

1.2.1.2.3 Range changing

The life history of domestic animals are inextricably linked to humans, including their dispersal, which always occurs alongside human movement. Cattle (Bollongino et al., 2012; Loftus et al., 1999) and sheep (Pedrosa et al., 2005), thought to be domesticated in the Fertile Crescent around 10,000 BP, are now raised globally. Pigs, domesticated in Anatolia 9,500 years ago (Frantz et al., 2019; Larson et al., 2007; Ottoni et al., 2013), were dispersed to Europe at least 8,000 years ago following early farmer migrations. East Asian pig breeds were transported to Europe in the 19th century to improve local breeds and increase meat production efficiency (Frantz et al., 2019). African wild cats, originated in the Near East up to 9,500 BP, have become a globally significant species for controlling rodents and a popular companion animal following domestication (Driscoll et al., 2007; Ottoni et al., 2017; Vigne et al., 2004).

In general, human-mediated dispersal of domestic animals can be rapid and far-reaching, exceeding the natural dispersal limits of the transported species. Humans have helped domestic animals colonize more geographical regions and significantly expand their populations. Many regional species have become global. However, it is undeniable that the sudden arrival of a new population can potentially threaten native ecosystems. On one hand, the invasion of exotic domestic animals can accelerate local declines in wildlife abundance, population extinctions, and range contractions (Ceballos et al., 2015; Morand et al., 2014). On the other hand, the importation of advantaged foreign domestic animals challenges the conservation of local breeds, impacting global biodiversity. Although East Asia has its own domesticated dog, the

arrival of other domestic dogs, likely from Western Eurasia, nearly completely replaced the original population approximately 2,500 years ago in the East Asian hinterland (Zhang et al., 2020). Similarly, Chinese domestic pigs, with an independent local origin outside the Near East, have diverged into many breeds with various merits. However, with the introduction of Western commercial breeds, local pigs have gradually retreated from the Chinese meat industry, facing rigorous conservation conditions (Y. Huang et al., 2020; Quan et al., 2020; Yang et al., 2003).

1.2.1.2.4 Domestication as a bottleneck event?

The traditional model of domestication posits that a small group of animals was brought into a captive environment, where gene flow with their wild counterparts was halted. Concurrently, selective breeding would amplify the frequency of certain phenotypic or genetic traits within a population, while range changes would isolate a subset of the original diversity, leading to the founder effect. Under this model, domestic animals would likely experience a genetic bottleneck effect and a decline in genetic variation. However, recent research has revealed that domestication does not necessarily entail a bottleneck effect (Fuller et al., 2022; Frantz et al., 2020; Larson et al., 2014; Larson and Burger, 2013).

In fact, admixture and introgression have played, and continue to play, a crucial role in animal domestication (Frantz et al., 2020; Larson et al., 2014; Larson and Burger, 2013). On one hand, during the initial stages of domestication, animal captivity was presumably less intensive, allowing domestic animals to interact with wild populations more frequently. Studies of mitochondrial DNA have shown that Chinese domestic pigs possess high genetic diversity and are indistinguishable from local wild boars (Cai et al., 2019; Larson et al., 2010; Xiang et al., 2017; Zhang et al., 2022). On the other hand, while long-distance translocation of domestic

animals may separate a portion of the original gene pool, the translocated populations can still interbreed with native interfertile wild or domestic species that share a relatively close genetic relationship with their progenitors. For example, domestic sheep from the Near East have been found to interbreed with Tibetan argali sheep (*Ovis ammon hodgsoni*), with modern Tibetan domestic sheep breeds containing approximately 3% Tibetan mouflon sheep ancestry in their nuclear genomes (Zhao et al., 2017). Similar post-translocation admixture or introgression events have been observed in African cattle (*Bos taurus* and *Bos indicus*) (Edea et al., 2015; Ibeagha-Awemu et al., 2004), Western Chinese domestic cats that interbred with local *Felis silvestris bieti* (Driscoll et al., 2007; Yu et al., 2022), and Near Eastern domestic pigs that interbred with European wild boars after arriving in Europe (Frantz et al., 2019; Larson et al., 2007; Ottoni et al., 2013). These findings demonstrate that range changes can indeed facilitate genetic interactions with local animals, introducing new diversity to translocated populations.

It is important to note that the absence of a genetic bottleneck in domestic animals may not be applicable to all domestic species, such as cattle. Genetic studies have indicated that modern taurine cattle populations originated from a small number of Near Eastern founders, as evidenced by the analysis of mitochondrial DNA (Bollongino et al., 2012).

In summary, animal domestication embodies a long-term mutualism that encompasses both biological and cultural dimensions. Humans, as agents in this process, have persistently exerted influence on domestic animals, significantly altering their evolutionary trajectories. Consequently, the natural characteristics of these animals have been modified, resulting in the development of new adaptations tailored for anthropogenic environments. Additionally, as humans migrated, domestic animals expanded beyond their original habitats to colonize new regions. Despite the pressures of selection and translocation, domestic animals do not

inevitably experience bottleneck events.

1.2.2 Semi-domestic animals

1.2.2.1 Definition of semi-domestication

In contrast to domestic livestock, semi-domestic animals are subject to lighter management, with partial control over their mortality, food supply, and living spaces, and typically no artificial selection. Exploitation of such animals includes primary or secondary products, labour, and most frequently, trophies or ornaments. Morphological traits of most semi-domestic animals are less affected (Clutton-Brock, 1989), which is attributed to the low level or absence of selective breeding, one of the major criteria distinguishing domestications from semi-domestication (Mysterud, 2010). A variety of taxa have achieved semi-domestication status, including insects (Curry, 1980; Yen, 2015), birds (Lukanov, 2019; Zann & Runciman, 2003), and most commonly, large or medium-sized ungulates, such as reindeer (*Rangifer tarandus*) (Suominen & Olofsson, 2000), sambar deer (*Cervus unicolor swinhoei*) (Chan et al., 2009) (Scherf & Loftus, 1993). Research tend to view semi-domestication as a state characterized by less artificial management. Direct selective breeding in this process is minimal or even absent. However, under human influence, changes in the genetics, population structure, behaviours, and natural range of semi-domestic animals still occur in various ways (Allendorf et al., 2008).

1.2.2.2 Human influence on semi-domestic animals

1.2.2.2.1 Low genetic diversity

One phenomenon observed in semi-domestic animals is low genetic diversity and strong population structure. On the one hand, a confined region, such as a deer garden, typically maintains a small, fragmented population, which tends to reduce gene flow frequency, promote

strong population isolation, and increase the risk of inbreeding (Baker et al., 2017; Blanchong et al., 2013; Kharzinova et al., 2018). On the other hand, for animals used in gaming, intense harvesting can also alter population subdivision and reduce genetic variation (Allendorf et al., 2008). To date, relatively low genetic diversity or strongly structured populations have been identified in semi-domestic animals such as reindeer (Ju et al., 2019), silkworms (*Bombyx mori*) (Kar et al., 2005), and guinea fowl (*Numida meleagris*) (Weimann et al., 2016). These populations are thought to be more susceptible to drift, and consequently, their potential for adapting to changing environments is limited (Hayward & Kerley, 2009).

1.2.2.2 Selections associated with human behaviour

In the absence of selective breeding, human-induced selective pressure can still be exerted on semi-domestic animals. One of the primary exploitations of semi-domestic taxa is gaming. It has been suggested that, in a population regularly harvested by humans, the frequency of certain behaviours that make animals less sensitive and more easily exposed to threats, or phenotypes favoured by hunters, may decrease. This is because individuals with these characteristics are more likely to be hunted and removed from the population (Allendorf et al., 2008; Harris et al., 2002; Law, 2007).

For example, a study on game animals in Germanic countries identified a strong preference for specific phenotypic traits, such as the presence of large antlers in cervids and horns in bovids. If a trophy characteristic is heritable, selective hunting may lead to a decline in that feature over time, resulting in long-term unintended consequences for the animals' evolution. To address this issue and promote more sustainable hunting, compensatory culling of low-quality males was implemented (Myrsterud and Bischof, 2010). Notably, due to regulations limiting the hunting of female animals and human preference for male products, the selection pressures

induced by hunting are often sexually biased and tend to be more intense for males. Interestingly, the decline of desirable traits in semi-domesticated animals used for hunting stands in stark contrast to domestication, where the frequency of preferred traits is increased through selective breeding.

1.2.2.2.3 Reduced strength of natural selection

As the influence of human-induced selection pressures increases, the impact of natural selection also changes. Human behaviour can counteract the force of natural selection. A prime example of this is the consequence of artificial feeding. To increase trophy size or mitigate the impact of extreme climatic conditions, humans may employ management strategies such as providing food to semi-domestic populations during periods of harsh environmental conditions. However, these challenging conditions could also serve as a time when natural selection functions at a high level, filtering out less adapted individuals. Consequently, the genetic traits associated with lower adaptation may be maintained.

In addition, anthropogenic factors can alter the mode of natural selection. For instance, the demographic structure of semi-domestic animals controlled by humans can influence the prey selection of natural predators. In northern Scandinavia, human harvests cause seasonal variations in semi-domestic reindeer abundance. The population size peaks in summer and declines after harvest, driving male lynx to prey significantly more on adult male reindeer during winter. In contrast, during periods before harvest or in purely natural conditions, calves are typically the main targets and are more intensely selected (Mattisson et al., 2013).

1.2.2.2.4 Behavioural changes

Under the influence of human interactions, the behaviors of semi-domestic animals can also

change, with foraging strategy being a prime example. Research on the gut microbiomes of wild and semi-domestic Père David's Deer (*Elaphurus davidianus*) has suggested that there are considerable differences in the microbial community structures between confined and free-ranging populations. These differences are primarily characterized by variations in the relative abundance of 39 bacterial species. It is likely that human intervention has influenced the diet of the deer in semi-captive environments (Sun et al., 2019).

1.2.2.2.5 Range changing

Studies have shown that humans can directly modify the range of semi-domestic animals to meet specific needs. Père David's deer, which originated in East Asia, is believed to have been semi-domesticated and used for harvest and ornamentation. Following the global cooling of the climate in the middle Holocene, the ideal habitat for Père David's deer receded to the Yangtze River Basin. However, due to increased demand for gaming and ornamentation among the royal class, some populations of Père David's deer were artificially relocated to and established in Northern China. In the 19th century, the species was further moved to Europe (Dayuan et al., 2022; Zhang et al., 2019). Similar cases can also be observed in semi-domestic Eurasian fallow deer, whose post-glacial range was limited to the Near East and suffered from low genetic diversity. For the purposes of securing food resources, entertainment, or conservation, these deer were artificially translocated worldwide (Baker et al., 2017; Chapman and Chapman, 1980).

In summary, semi-domestication primarily differs from domestication in terms of selective breeding, with semi-domestic animals experiencing less management and direct artificial selection. Nevertheless, human influence still plays a role in several aspects. Under semi-domestication, animals may face population isolation and low genetic diversity. For

populations utilized for gaming, male-biased selective pressures can lead to a decline in the frequency of certain traits preferred by hunters. Human behaviours, such as providing food, also play a significant role in counterbalancing the strength of natural selection and reshaping the behavioural responses of animals. Most notably, compared to wild animals, there is a stronger social and cultural bond between humans and semi-domestic animals. To meet our needs, the range of semi-domestic animals may be significantly altered through long-distance translocation.

1.2.3 Commensal animals

1.2.3.1 Definition of commensalism

Commensalism is a long-term symbiotic relationship in which individuals of one species benefit from another, while those of the other species experience neither harm nor benefit. This relationship can be contrasted with several other symbiotic patterns, such as mutualism, where both parties benefit from each other; amensalism, in which one organism is harmed while the other remains unaffected; parasitism, where one organism benefits at the expense of the other; and parasitoidism, which is similar to parasitism, but the parasitoid has a free-living stage and ultimately kills its host (Leung and Poulin, 2008; Mathis and Bronstein, 2020; Wilson, 1975).

Commensalism plays a critical role in a range of ecological processes and evolutionary outcomes. A classic example of commensalism is the relationship between *Escherichia coli* (*E. coli*) and its vertebrate hosts. Strains of *E. coli* residing in the guts of vertebrates rely on their host for a consistent nutrient supply, a stable habitat, protection against certain stresses, as well as transport and dispersal. At the same time, these strains neither provoke the host's immune system nor provide direct benefits, resulting in a commensal relationship.

1.2.3.2 Human-animal commensal relationship

Commensal associations can be established between various types of organisms, such as birds and mammals (Mikula et al., 2018), marine life (Norman et al., 2021), or insects (Zhu et al., 2022), among others. Notably, anthropogenic activities have led to the formation of commensal relationships between humans and several mammal species. Unlike domestication or semi-domestication, humans do not manage or intentionally exert direct influence on animals in commensal relationships. In such associations, animals typically benefit from living in human niches by acquiring additional stable food sources or shelter.

Human-animal commensalism has played a pivotal role in influencing the evolutionary trajectories of various mammalian or bird species. One of the most significant outcomes of commensal relationships is the potential path toward domestication. Commensalism is now understood to be a typical pathway taken by animals when making their first contact with humans (Zeder, 2012a, 2012b). A common example is the domestication of dogs (*Canis familiaris*) from wolves (*Canis lupus*). As the first domesticated species to integrate into human environments during the late Palaeolithic Age, dogs were attracted to human settlements and their food waste. Over time, stronger social and ecological bonds developed between the species. The advent of agriculture, or the Neolithic transition, significantly increased the accumulation of trophic resources in human settlements, thereby accelerating and broadening the process of commensal relationships between humans and animals. Several taxa, such as cats, pigs, and fowl, first came into contact with agricultural settlements by feeding on refuse or preying on other animals attracted to human niches. Through their commensal relationships with human environments, these animals developed stronger social or economic ties with their human hosts than other commensals inhabiting the same niche. These ties eventually led to their domestication.

It is important to note that domestication is not the inevitable outcome of commensal relationships with humans, and many animals in commensal relationships have not been domesticated historically or to this day. For example, the desert hare (*Lepus capensis*) in Western China has never been domesticated nor is it related to modern domestic rabbits, which originated from the wild European rabbit (*Oryctolagus cuniculus*) (Irving-Pease et al., 2018). However, stable isotopic analysis has shown that 4,200 years ago, some desert hares living in the Loess Plateau incorporated millet, a human-planted crop, into their diets. This finding suggests that undomesticated desert shares gained additional food resources from agricultural settlements, implying a commensal relationship with humans (Sheng et al., 2020). Similar examples can be found in modern cities, where red foxes, mice, or rodents have all been observed exploiting urban environments and feeding on human-generated trophic sources (Banks and Smith, 2015; Scholz et al., 2020; Stenseth et al., 2003; Vrbanec et al., 2021).

1.2.3.3 Human influence on commensal animals

1.2.3.3.1 Behaviour changes

During the process of commensalism, undomesticated animals are also subjected to human-associated influences. One of the most important and frequently studied changes is the behaviour of commensal animals. To cope with anthropogenic environments, animals have developed new behavioural adaptations. Studies on small mammals have found that urban populations exhibit greater daring and exploratory behaviours than their rural counterparts in their respective locales. However, when evaluated under common environmental settings, rural individuals displayed minimal change in their behavioural responses, whereas urban individuals significantly altered their behaviour, becoming more timid and less exploratory

than when tested in their original environment. This combination of increased risk-taking and high behavioural flexibility may enable urban inhabitants to successfully adapt to rapidly changing environments induced by humans (Mazza et al., 2020).

In addition to the general shift in behaviours, one of the most noticeable behavioural changes is related to dietary niches. Research has demonstrated that the food preferences of red foxes (*Vulpes vulpes*) living in distinct ecosystems vary significantly, with large differences observed in the variance of urban and rural populations for both ^{13}C and ^{15}N isotopic values. This suggests a foraging strategy that is specific to the environment. Urban foxes typically exhibit higher ^{13}C and lower ^{15}N values, indicating a significant intake of anthropogenic food sources (Scholz et al., 2020).

1.2.3.3.2 synchronization with the development of human societies

Interestingly, the population dynamics of some commensal animals have shown a synchronization with changes in human societies. Research has demonstrated that the body size of wolves, golden jackals (*Canis aureus*), red foxes, European badgers (*Meles meles*), and striped hyenas (*Hyaena hyaena*) increased over a 50-year period since the foundation of the State of Israel in 1948. During this time, there was a significant increase in the amount of waste and agricultural crops available to commensal animals in the region. By inhabiting human niches, these carnivores consistently fed on refuse or preyed on other animals associated with villages or urban areas. The improvement of human societies provided them with a more stable and richer nutritional environment, accelerating their growth (Yom-Tov, 2003).

A similar correlation between commensal animals and human societies was observed in ancient Europe. Genomic investigations of ancient commensal rats revealed a population transition in

temperate Europe between the sixth and tenth centuries, coinciding with archaeological evidence of a population reduction in the black rat (*Rattus rattus*). The near extinction and subsequent reappearance of black rats in Europe may have resulted from the collapse of the Roman Empire and the First Plague Pandemic, both of which induced significant economic recessions (Yu et al., 2022).

In summary, human-animal commensal relationships have been established since the late Pleistocene (Galibert et al., 2011; Larson et al., 2012; Zeder, 2012b), gaining momentum following the advent of the Neolithic transition (Wirtz and Lemmen, 2003; Zeder, 2012b, 2012a). Animals within these relationships initially came into contact with humans by being attracted to the abundant trophic resources in human settlements. Throughout the process of commensalism, numerous animals were domesticated. However, those that remained in a wild state were still significantly influenced by humans, particularly in terms of their behavioural adaptations (Mazza et al., 2020; Scholz et al., 2020). Moreover, the population of some commensal species responded to the progress of human societies (Yom-Tov, 2003; Yu et al., 2022).

1.3 Ancient genomics to explore the human influence on animals in the past

1.3.1 Why using ancient DNA

The evolutionary trajectories of animals may converge on the same destination, even though their paths differ significantly. A comprehensive understanding of this process necessitates examining not only contemporary results but also the critical events obscured by history. For example, an investigation of the genetic structure of modern domestic pigs in Europe might lead one to conclude that they originated locally, given their similar ancestries to native wild

boar. However, ancient DNA research has revealed a far more complex story.

Approximately 8,000 years ago, the first domestic pigs were introduced to Europe from the Near East. Upon their arrival, they immediately began to interbreed with native wild boar. By 5,500 BP, the Near East ancestry had been almost entirely replaced by that of Europe's native wild boar. Today, only trace amounts of Near East ancestry persist in Southern European pig populations. Furthermore, some commercial European pig breeds exhibit East Asian ancestry, which can be attributed to breeding programs initiated in the 19th century (Frantz et al., 2019; Larson et al., 2007; Ottoni et al., 2013).

To fully comprehend the ways in which human influences have shaped animal evolution and their subsequent responses, it is crucial to not only examine present-day interactions but also investigate the historical events that have occurred in different phases. Thanks to advancements in ancient DNA technology, we are now better equipped to delve into the past and piece together the puzzles of evolutionary history. This enhanced perspective allows for a more nuanced understanding of the complex interplay between humans and animals over time.

1.3.2 A brief history of ancient DNA research

Approximately four decades ago, the first ancient DNA sample was retrieved from the soft tissues of a female human excavated from the Mawangdui Han tomb (2,100 BP) in China. However, at that time, the sample did not undergo nucleotide sequence analysis (Huang et al., 2020). In 1984, the first ancient DNA research was conducted, in which DNA molecules were obtained from two pieces of 140-year-old dried muscle tissue of the quagga (*Equus quagga*), an extinct species of zebra. Although only a small fragment of 229 bp was recovered, this

research undeniably marked the establishment of ancient DNA as a discipline (Higuchi et al., 1984).

The development of polymerase chain reaction (PCR) technology (Schochetman et al., 1988), Sanger sequencing (Sanger et al., 1977), and the utilization of ancient bones and teeth (Merriwether et al., 1994; Tuross, 1994) alongside soft tissues contributed to the rapid growth of ancient DNA research (Béraud-Colomb, 1997; Pääbo et al., 1989). PCR is particularly well-suited to aged samples, as it can amplify targeted DNA segments to a level sufficient for direct sequencing, even when starting with minimal amounts of DNA or single molecules (Pääbo et al., 1989; Woide et al., 2010). Using PCR and Sanger sequencing, researchers gained valuable insights into the origins and dispersal of domestic or semi-domestic animals, making significant progress in the field (Béraud-Colomb, 1997; Cai et al., 2011; Larson et al., 2007; Leonard et al., 2002; Madgwick et al., 2013).

Nevertheless, due to the damaged nature of ancient DNA molecules (Dabney et al., 2013b) and the low throughput of Sanger sequencing (Sanger et al., 1977; Schuster, 2008; Sikkema-Raddatz et al., 2013), research was primarily focused on small fractions of maternal-inherited mitochondrial genomes, such as the Cyt b gene or control region (CR), usually with sequences less than 300 bp. This limitation on data volume significantly impacted the resolution and scope of ancient DNA studies (Larson et al., 2010; Xiang et al., 2017). Furthermore, the degraded nature and low endogenous rate of ancient DNA made it more susceptible to contamination (Dabney et al., 2013b; Handt et al., 1994; Richards et al., 1995), causing confusion and scepticism within the discipline. For instance, the recovery of mitochondrial DNA from a dinosaur (Woodward et al., 1994) was later proven to be a NUMT gene from a human (Hedges and Schweitzer, 1995). The uncertainty surrounding the authenticity and difficulty in data

acquisition raised concerns about the discipline's reliability and necessity (Handt et al., 1994; Poinar and Cooper, 2000; Wayne et al., 1999).

Concerns over the authenticity and necessity of ancient DNA studies persisted from its inception until the early 21st century. However, advances in decontamination methods and the advent of next-generation sequencing (NGS) have resolved many of these concerns and propelled the discipline forward.

Since the 2000s, numerous studies on the mechanisms and forms of ancient DNA damage have broadened our understanding of its characteristics (Dabney et al., 2013b; Hofreiter et al., 2001a; Karni et al., 2013; Smith et al., 2003). As a result, targeted decontamination methods have flourished, and pipelines have been established for field sampling and laboratory work (Kampmann et al., 2017; Llamas et al., 2017; Yang and Watt, 2005). The use of dedicated ancient DNA laboratories and sample treatment methods, such as bleaching (Kemp and Smith, 2005) and pre-digestion (Damgaard et al., 2015), has significantly reduced contamination and increased the rate of endogenous molecule recovery. Additionally, bioinformatic methods have been developed to distinguish contamination from sequencing data (Ginolhac et al., 2011; Jónsson et al., 2013; Peyrégne and Peter, 2020). These combined efforts have greatly improved the reliability of ancient DNA studies.

NGS has revolutionized genetic research by significantly reducing the time and cost associated with obtaining whole genomes. For example, the Human Genome Project, launched in 1990 and involving organizations from six countries, took a decade to publish its first draft. In contrast, as of 2014, Illumina's new sequencing machine can process over 45 human genomes in a single day for approximately \$1,000 each (Goodman et al., 2016). In the context of ancient

DNA, studies based on the Sanger method primarily focused on short sequences ranging from dozens to hundreds of base pairs. With the advent of NGS, researchers can now extend their studies to a genomic scale, uncovering more detailed information on the evolutionary history of ancient animals (Chen et al., 2020; Frantz et al., 2020; Larson and Burger, 2013).

Over three decades later, progress in ancient DNA technology has enabled the recovery of ancient DNA from samples as old as 1.3 million years, such as mammoth bones excavated from Siberian permafrost (Callaway, 2021). As demonstrated by Frantz et al. (2020), we are now in an era of ancient genomics, marked by high data output efficiency. By analysing ancient data, researchers can address a broader range of questions and enhance our understanding of the long-term interactions between humans and animals (Frantz et al., 2020; Larson and Burger, 2013; Schuster, 2008).

1.3.3 The development of ancient DNA laboratory methods

Many variety of ancient genomic methods have been developed and widely applied, progressively becoming powerful and even essential tools for uncovering the processes through which human influences have affected animals. In the following section, we will briefly introduce the progress made in ancient genomic wet laboratory methods.

1.3.3.1 Extraction

Since 1984, various extraction methods have been established for obtaining ancient DNA from diverse substrates, including fur (Sinding et al., 2015), teeth, bones (Rohland and Hofreiter, 2007), plants (Kistler, 2012), and even sediments (Epp et al., 2019). In this research, we will utilize Dabney's method, which involves an overnight proteinase K digestion, a chaotropic guanidium-based salt to bind DNA fragments and limit inhibitors, silica spin columns, and a

guanidine hydrochloride binding buffer (Dabney et al., 2013a). Additionally, a minor modification to Dabney's method will be applied, primarily involving the addition of a pre-digestion step using EDTA and proteinase K to reduce exogenous DNA and potential contamination, thus enriching the concentration of endogenous DNA (Damgaard et al., 2015).

Dabney's method has proven effective in extracting ancient DNA from Pleistocene substrates, excelling particularly at recovering highly degraded DNA fragments with lengths down to 30 bp. This makes it highly suitable for obtaining ancient DNA from samples found in challenging preservation environments (Dabney et al., 2013a). Most ancient bones in this study were sampled in the Middle East of the Yellow River Basin or the Mediterranean region, which are considered relatively humid and warm climates that may not provide ideal conditions for preserving DNA molecules. Furthermore, the ages of many samples exceed 5,000 years, which can intensify the degradation of exogenous DNA (Dabney et al., 2013b; Haynes et al., 2002; Hofreiter et al., 2001b; Karni et al., 2013). Therefore, the application of Dabney's method could be an optimal choice, increasing the efficiency of extraction.

1.3.3.2 Library building

The process of library preparation involves ligating a pair of adapters to the ends of randomly fragmented DNA to anchor them onto the surface of the lanes on the flow cell of the sequencing machine.

In recent years, two major methods have been widely employed for building libraries, targeting single-stranded (Gansauge et al., 2020) and double-stranded (Carøe et al., 2018) DNA, respectively. The single-stranded method is believed to recover more endogenous ancient DNA fragments. However, it also includes endogenous environmental DNA and molecules shorter

than 28 bp, which are too short to be informatively mapped to the reference sequence. Such fragments can account for 5% to 27% of all reads, potentially limiting the efficiency of the sequencing process. Additionally, this method increases costs and time, and is restricted to the Illumina sequencing platform (Carøe et al., 2018; Kapp et al., 2021). In contrast, the blunt-end method for double-stranded DNA may result in a 50% loss of molecules due to the ligation of non-distinct adapters by chance, but it is significantly more cost-effective and timesaving, yielding a more ideal DNA size distribution (Bassi et al., 2020; Bennett et al., 2014). This research will adopt the blunt-end method. Furthermore, to save costs, a screening procedure is necessary before deciding which samples will undergo deeper sequencing. Consequently, indexes are also incorporated, in addition to adapters. Different indexes are added to the samples, allowing them to be pooled together and sequenced in the same lane while remaining distinguishable from one another (Carøe et al., 2018; Kircher et al., 2012).

1.3.3.3 The Next-generation Sequencing

The rapid expansion of ancient DNA research can be attributed to the advent of next-generation sequencing (NGS) in the 2000s. Following this development, many companies and research communities have created a series of methods aimed at achieving parallel sequencing and processing large quantities of data in a short time. NGS now refers to a group of sequencing methods known for their high throughput and widespread use in recent years. Currently, there are three primary NGS methods: pyrosequencing, reversible termination sequencing, and Proton/PGM sequencing. Among these, reversible termination, adopted by Illumina, is the most popular method, contributing 70% of the market share (Goodman et al., 2016; Jarvie, 2005; Schuster, 2008; Sikkema-Raddatz et al., 2013).

Despite the advantages of NGS, it is important to acknowledge that error rates are higher compared to Sanger sequencing. To address this issue, efforts should be made to improve the depth of sequencing data, thereby ensuring the authenticity of the results (Stoler and Nekrutenko, 2021).

1.3.3.4 Targeted capture

With the advent of NGS, a new technology called targeted capture has been developed to maximize its potential. This method selectively enriches specific regions of the genome, reducing the cost and data analysis burden compared to whole genome sequencing, while still enabling researchers to study high-value regions of interest (Carpenter et al., 2013; Fu et al., 2013). For ancient DNA studies, where DNA molecules may be highly degraded due to their age (Dabney et al., 2013b; Karni et al., 2013), complete genomes may not be directly obtainable through NGS. Therefore, targeted capture has become an important tool for studying specific regions of interest in ancient genomes.

While targeted capture is an effective approach to enrich specific regions of the genome and reduce the cost of sequencing, it also has several drawbacks. One major limitation is the bias introduced during the selection process, which can result in non-uniform coverage and missing data in certain regions. This can be particularly problematic for ancient DNA samples, which are often highly degraded and have lower endogenous DNA content. Additionally, the design of the capture probes can be time-consuming and expensive, particularly for non-model organisms or poorly studied regions of the genome. Overall, while targeted capture is a valuable tool in genetic research, it is important to carefully consider its limitations and potential biases in study design and data analysis.

1.4 Overview of the thesis

My thesis comprises four case studies that utilize ancient DNA to explore the evolution of animals under human influence.

The first paper investigates the origin of domestic cats in China, focusing on when and how they were introduced to East Asia, how the population was established, and the role of admixture in this process.

The second paper explores the evolutionary history of leopard cats in the Middle Reach of the Yellow River Basin, investigating their genetic association with other populations in the Far East, Indochina, Malay, and Sunda. It also examines the relationship between leopard cats and humans in ancient China, including the duration of their commensal relationship and whether it progressed to domestication.

The third paper aims to shed light on the ancient human-induced range expansion of the Persian and European fallow deer, focusing on the origins of translocated populations across the Mediterranean and England, and the connection between the dispersal of fallow deer and ancient trade routes. The paper also examines the possibility of admixture between European and Persian fallow deer.

The fourth paper focuses on the evolution of domestic pigs in the Middle Reach of the Yellow River Basin. It combines ancient genomic and zooarchaeological evidence to explore the management and utilization of Chinese domestic pigs from 5,500 to 2,500 BP, and their contributions to the formation of modern pig breeds in China. The paper also outlines future research directions for the study of the origin and evolution of Chinese domestic pigs.

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2 An Ancient DNA perspective on the origin of the Chinese domestic cats (*Felis silvestris catus*)

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Abstract

Domestic cat (*Felis catus*) serves as an a highly popular pet and an effective rodenticide in China. Modern Chinese domestic cats are derived from African wild cats (*Felis silvestris lybica*). Nevertheless, we remain limited knowledge about their first occurrence and the development of this populations. To investigate the timing of theirr arrival, as well as the evolutionary history, we conducted ancient genomic research on a dataset consisting of 4 nuclear genomes and 22 mitochondrial genomes from 22 ancient Chinese samples spanning 5,500 years. Through predominantly ancient DNA analysis and radiocarbon dating, we find that, before the arrival of domestic cat leopard cats (*Prionailurus bengalensis*) might have established a commensal relationship with humans from the Late Neolithic period (6,000-5,500 BP) to the Han Dynasty (202 BC - 220 AD). Domestic cats, originated from African wild cats, were introduced to China during the 7th to 9th centuries AD, possibly through the Silk Road.

After their introduction, the population structure of domestic cats in China has remained stable for over 1,200 years, with minimal influence from other lineages of domestic cats and wildcats.

Keywords: ancient DNA; Chinese domestic cats; origin; evolutionary history

2.1 Introduction

Domestication as an enduring process involves a strong association between humans and animals, in which anthropogenic forces consistently shape the participating species. Domestic cat (*Felis catus*) serves as a prime example to comprehensively understand this process. As one of the most important companion animals and a crucial agent controlling rodent, its evolutionary trajectory has been markedly influenced by humans.

2.1.1 Cats domestication from a zooarchaeological perspective

For decades, the origin and evolution of domestic cats have captured the attention of scholars from a wide range of academic fields, including archaeology and biology. Zooarchaeological records suggest that cats were domesticated in the Near East approximately 10,000 years ago (Driscoll et al. 2007; Turner et al. 2013; Ottoni et al. 2017), likely following a commensal pathway (Zeder 2012). Commensalism, a type of symbiosis, refers to the coexistence of two or more species where only one benefits while the others remain unaffected (Zeder 2012). Commensal pathway is usually walked by species that capitalize on their proximity to humans, frequently feeding on scraps or small animals associated with human settlements (e.g., rodents, pigs). Over time, these species establish social and economic relationships with humans through frequent interaction in human habitats, eventually leading to domestication.

Cats are thought to be initially drawn to human settlements by the presence of mice or rats

feeding on early farmers' grain stores, ultimately leading to domestication (Zeder 2012;). According to archaeological evidence, the earliest remains of a domestic cat were discovered at the Khirokiitia site in Cyprus, where a cat skeleton was found in a human cemetery dating back to approximately 9,500 BP. Zooarchaeological research demonstrated that the cat's morphology closely resembled that of domestic cats (Vigne et al. 2004). As there were no wildcats on the island, it is believed that this ancient individual was brought to the island from the Near East mainland via human-mediated maritime routes, which indicates a close relationship with humans and even domestication (Vigne et al. 2004).

2.1.2 Cats domestication from a genetic perspective

Genetic evidence suggests that domestic cats originated from African wild cats (*Felis silvestris lybica*). Based on ND5&ND6 genes of mitochondrial genome, and nuclear STR, modern domestic and wild cats can be classified into at least five clades, representing five geographically distinct, interfertile subspecies (Driscoll et al., 2007). These clades correspond to European wild cats (*Felis silvestris silvestris*), Asiatic wild cats (*Felis silvestris ornata*), Chinese mountain cats (*Felis silvestris bieti*), Southern African wild cats (*Felis silvestris cafra*), African wild cats (*Felis silvestris lybica*). Lybica clade also contains all present domestic cats. According to which, African wild cats are considered the progenitors of domestic cats, with the Near East likely serving as the domestication centre. The result is consistent with archaeological findings. Five haplogroups (A-E) have diverged within the clade of lybica, suggesting that domestication may have involved at least five maternal lineages (Driscoll et al. 2007).

A study using ancient DNA techniques verified the Near Eastern origin of domestic cats and delineated the initial geographic distribution of wild cats from the Neolithic period to 800 BP.

The findings indicate that European wild cats predominated in Western Europe; both European wild cats and the lybica A-1 haplogroup were equally distributed in South-eastern Europe; the lybica A* haplogroup was prevalent in Anatolia; the lybica B haplogroup was widespread in the Levant; and the lybica C haplogroup was extensively distributed across Egypt (Ottoni et al. 2017).

2.1.3 The origin of the Chinese domestic cat

A recent study demonstrated that all modern domestic cats in China are descended from African wild cats (Yu et al. 2021). However, the timing of their initial appearance in China remains unknown. Two major hypotheses have been proposed regarding the introduction of domestic cats to China: the first posits their first occurrence during the late Neolithic period (6,000-5,000 B.P.), while the second suggests their occurrence during the Han Dynasty (approximately 2,000 BP).

The first hypothesis is based on the discovery of three small Felidae remains at Quanhucun (6,000-5,000 B.P.), a site located in the middle Yellow River Basin. The cat bones were thought to be morphologically similar to domestic cats, and stable isotopic analysis revealed that they had consumed a significant amount of C4 food, potentially originating from rodents that fed on millet, the dominant crop planted at this site. Consequently, researchers posited that these individuals may have been domestic cats (Hu Y et al. 2014). However, a subsequent study employing Geometric Morphometric (GMM) techniques suggested that the small felids at the Quanhucun Site were leopard cats (*Prionailurus bengalensis*), a species genetically distinct from both wild and domestic cats (Vigne et al. 2016).

In contrast, the Han Dynasty origin hypothesis appears more convincing, as it is supported by

both archaeological and ancient literary evidence. Zooarchaeology suggests that the earliest domestic cats in China occurred during the Han Dynasty, as revealed by cat skeletons found from two sites. (Wang. Z. 2016; Wang. Z. 2010; Hu, S. et al. 2006; Wang 1986). At the Dabaotai Tomb (80BC-45BC), at least fourteen pieces remains of cats were unearthed (Wang. Z. 2010; Wang 1986).

Another site is the Chang'an City in Shannxi Province, the capital of the Western Han Dynasty (202 BC to 8 AD). A cat's right tibia was excavated at the southwest corner of the city wall. Although the length of the tibia of the Chang'an cat is significantly greater than that of modern domestic cats, researchers insisted on identifying it as a domestic cat, as its morphology was believed to be strikingly similar to that of domestic cats. (Hu, S. et al. 2006). .

In addition to zooarchaeological evidence, ancient literature provides descriptions supporting the Han origin hypothesis. According to *Solutions to the Problems of Fine Horses*: "*While Qiji, Lver, Fei Hong, and Hualiu (renowned horse names) are the most magnificent horses. If they must capture rodents in the palace, they are not even equal to a crippled cat*". The "cat" in this context is associated with rodent hunting in a human-associated environment (the palace). Given this, it is likely a domestic cat.

Nonetheless, distinguishing small felids based on skeletal morphology proves to be challenging, and the Chinese term for "cat" does not differentiate among various species of small felids. Consequently, the proposition of a Han Dynasty origin should be approached with caution.

1.2.4 Admixtures with wild cats

Hybridization between domestic cats and wildcats is not uncommon.(Yu et al. 2021; Germain

et al. 2008; Pierpaoli et al. 2003), and. it is noteworthy that two interfertile wild cat subspecies, *Felis silvestris bieti* and *Felis silvestris ornata*, inhabit western China. According to Yu et al. (2021), modern domestic cats in Southwest China have experienced minor introgression from Chinese mountain cats. Although the research (Yu et al. 2008) mentioned that the ancestry of Chinese mountains cats in domestic cats reflected a recent admixture events, it is difficult to rule out the possibility that similar instances of hybridization have occurred multiple times throughout history in various regions of China.

2.1.4 Research questions

The modern population of domestic cats in China is derived from the African wild cat; however, due to the lack of in-depth exploration and, in particular, the absence of ancient genomic analysis, the first occurrence and population history of domestic cats in China remain obscure. This study will use ancient genomic method in conjunction with radiocarbon dating and literary analysis, to:

- 1) find out when and where domestic cats were first introduced into China.
- 2) investigate the population dynamics of Chinese domestic cats over time.
- 3) assess the possibility of admixture between Chinese domestic cats and Asiatic wild cats (*Felis silvestris ornata*) or Chinese mountain cats (*Felis silvestris bieti*) in ancient periods.

2.2 Materials

A total of 22 cat bone specimens, representing 15 sites from the Late Chinese Neolithic Age (6,000 to 5,000 BP) to the Late Qing Dynasty (150 B.P.), were collected (Table 1). These sites are situated in Western, Northern, and Central China (Figure 1). To maximize the sample set, the Minimum Number of Individuals (MNI) was chosen from each stratigraphic layer,

wherever possible. All samples were obtained either directly from excavators and zooarchaeologists or through participation in excavations. In addition to the ancient samples, modern mitochondrial genomes of 53 Chinese domestic cats, 5 Chinese mountain cats, and 4 Asiatic wild cats were provided by the Laboratory of Evolutionary Genomics (LUO Lab), School of Life Sciences, Peking University for comparison (Table S1). Ancient and modern datasets from Western Eurasia, obtained from Driscoll et al. (2007) and Jamieson et al. (2021), were also utilized in this research (Table S1).

Table 1 Sample information of ancient Chinese cats.

Sample	Site	Location	Type of Site	Context	Period	Date
FS1	Xitucheng City	Northern China	City	Ash pit	Jin	800BP
FS2	Xitucheng City	Northern China	City	Ash pit	Jin	800BP
FS3	Xitucheng City	Northern China	City	Ash pit	Jin	800BP
FS4	Xitucheng City	Northern China	City	Ash pit	Jin	800BP
FS5	Xitucheng City	Northern China	City	Ash pit	Jin	800BP
FS6	Xinxiang Song	Central China	Cemetery	Tomb	Song	900BP
FS7	Huoshiliang	Western China	Neolithic Settlement	Sediment	Neolithic	4500BP
FS8	Wuzhuangguoliang	Western China	Neolithic Settlement	Sediment	Neolithic	4000BP
FS10	Laoniupo	Central China	Settlement	Sediment	Shang	3200BP
FS12	Tongwan City	Western China	Capital City	Ashpit	Song	1000BP
FS13	Yangling Mausoleum	Central China	Mausoleum	Burial Pit	Han	2150BP
FS14	Xinjie	Central China	Neolithic Settlement	Sediment	Neolithic	5000BP
FS15	Quanhucun	Central China	Neolithic Settlement	Ashpit	Neolithic	5500BP
FS16	ZhengzhouShangcheng	Central China	Village	Ashpit	Ming	500BP
FS17	Yunzhongjun	Nothern China	City	Foundation	Qing	150BP
FS18	Zhengzhou Song Tomb	Central China	Cemetery	Tomb	Song	900BP

FS19	Huangdiguli(Han)	Central China	City/Cemetery	Tomb	Han	1750BP
FS20	Huangdiguli(Song/Jin)	Central China	City	Ashpit	Song/Jin	900-800BP
FS21	Dingxinlu	Central China	City	Ashpit	Song/Jin	900-800BP
FS22	Dingxinlu	Central China	City	Ashpit	Song/Jin	900-800BP
FS23	Dingxinlu	Central China	City	Ashpit	Song/Jin	900-800BP
FS24	Chang'an City	Central China	Capital City	Ashpit	Han	2000BP
Total	22					

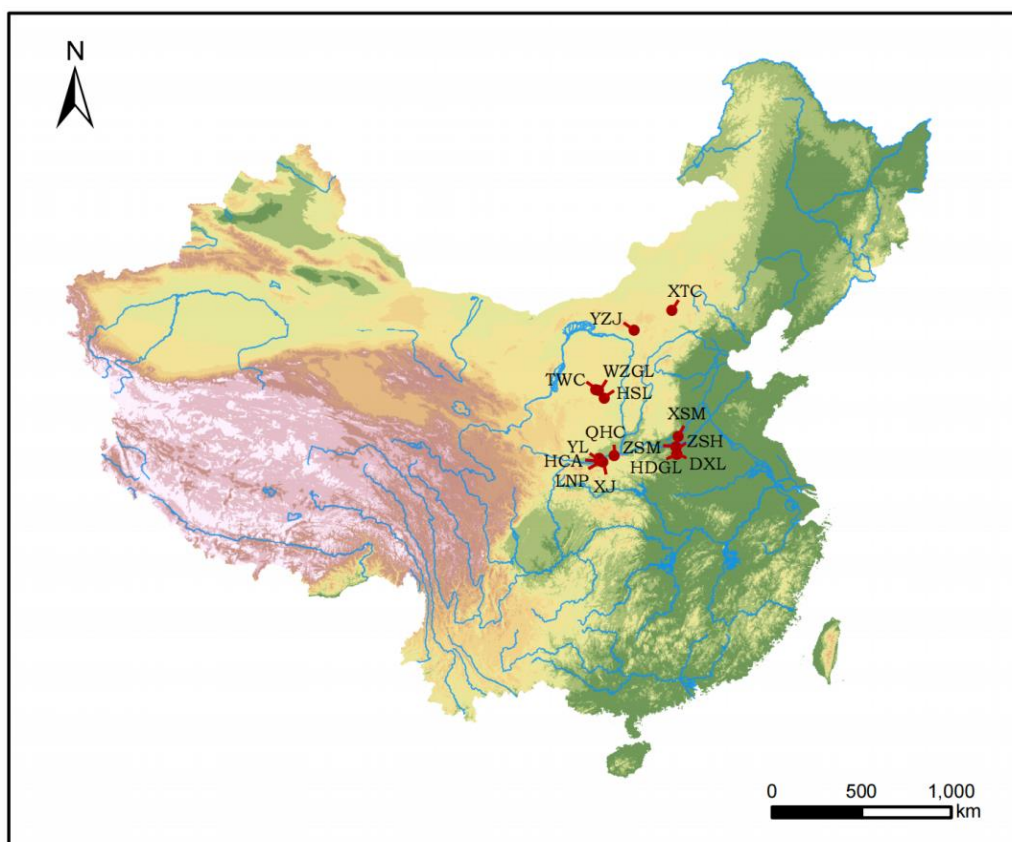


Figure 1 Sampling locations of ancient Chinese cats. Xitucheng City Site (XTC), Xinxiang Song Tomb (XSM), Huoshiliang Site (HSL), Wuzhuangguoliang Site (WZGL), Laoniupo Site (LNP), Tongwan City Site (TWC), Yangling Mausoleum (YL), Xinjie Site (XJ), Quanhucun Site (QHC), Zhengzhoushangcheng Site (ZSH), Zhengzhou Song Tomb (ZSM), Huangdiguli Site (HDGL).

2.3 Methodology (Figure 2)

2.3.1 Ancient DNA Laboratory Methods

For the ancient laboratory work, all samples were prepared in a dedicated paleogenetic

laboratory at the School of Life Sciences, Peking University. Protective clothing (3M) and a series of decontamination protocols were employed to minimize the risk of DNA contamination.

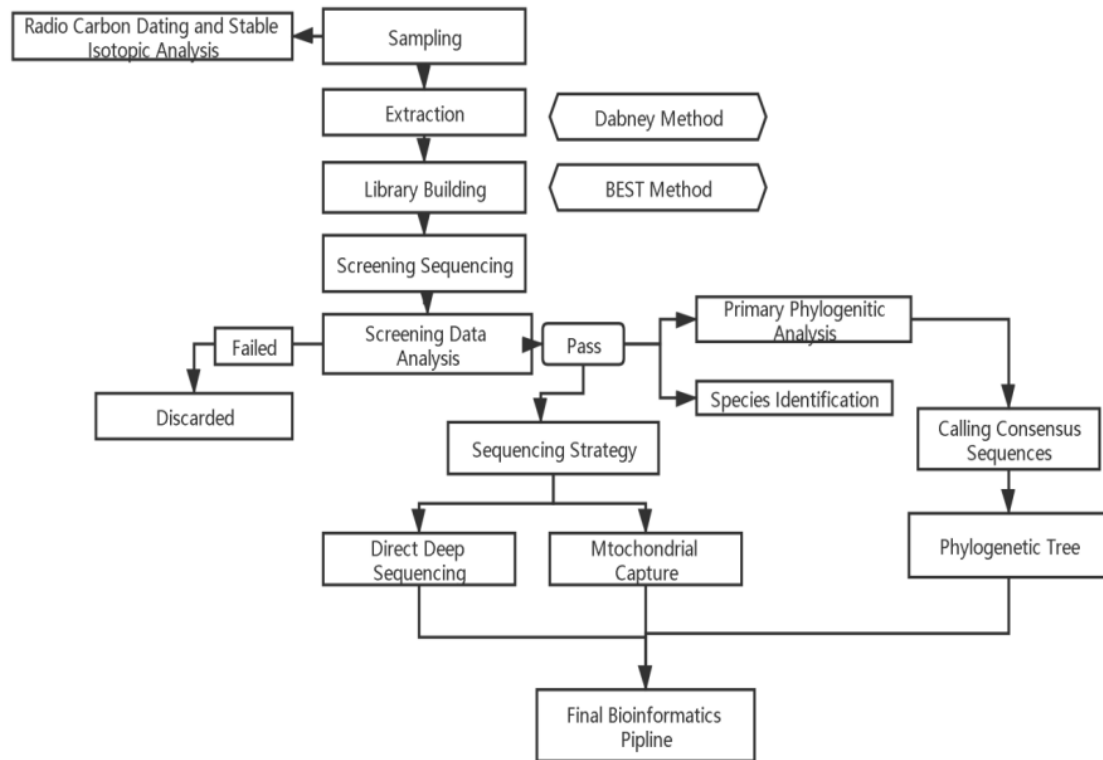


Figure 2 Methods and workflow of this research.

2.3.1.1 Sample preparation

Bone samples, weighing between 100–250 mg, were prepared using a Dremel multi-tool drill. The bone surface was carefully removed with a circular cutting disk to eliminate any exogenous DNA. To minimize the risk of cross-contamination, the drill was cleaned with a 10% bleach solution, and the spindle and disposable disk were replaced after processing each sample. The benchtop was also cleaned with 10% bleach and 75% ethanol between each sample, and gloves were changed frequently. Negative controls were employed as blanks for each batch of tested samples at every stage of the laboratory procedures, from extraction to capture. The bones were

pulverized using a hammer, and any equipment that came into contact with the samples was thoroughly cleaned between samples using 10% bleach and 75% ethanol.

2.3.1.2 Extraction

DNA was extracted using a protocol based on Dabney et al. (2013), incorporating a pre-digestion step as outlined by Damgaard et al. (2015). Bone powder was pre-digested at 37°C in 1 ml of extraction buffer containing water, 10 mg/ml Proteinase K, TWEEN 20, and 0.5 M EDTA (pH 8.0) for 30 minutes to further eliminate exogenous DNA from the bone particle surfaces. Samples were then centrifuged for one minute (Hettich Universal 320R), and the supernatants were discarded. Bone powders were incubated with the same buffer used in pre-digestion at 55°C overnight to thoroughly release DNA. Samples were subsequently centrifuged to pellet bone powders, and supernatants were collected and mixed with a binding buffer containing PB (QIAGEN) mixed with 5M NaCl, 3M NaAc, and 19% HCl (pH 4-4.5). DNA in the supernatants was filtered by spinning and passing the samples mixed with binding buffer through MinElute spin columns. Products were washed with PE buffer (QIAGEN) and ultimately eluted with 32 µl of EB (QIAGEN) into a 1.5 ml fresh Lo-band collection tube.

2.3.1.3 Library building

Double-stranded libraries for next-generation sequencing were prepared using 16 µl of DNA extract and the BEST (blunt end single tube) method (Carøe et al. 2018). SPRI (Solid Phase Reversible Immobilization) beads (Beckman Coulter) were employed for library purification, as specified in the protocol.

2.3.1.4 qPCR

Libraries were then assessed for optimal PCR (polymerase chain reaction) cycle numbers

(Schochetman et al. 1988) by performing qPCR (quantitative PCR) (Kubista et al. 2006) using the StepOne Real-Time PCR System (Applied Biosystems) per the manufacturer's instructions, and a pair of qPCR-specific primers. Thermocycling conditions included an initial denaturation step of 2 minutes at 95°C, followed by cycles of 15 seconds denaturation at 95°C, 30 seconds annealing at 60°C, and 30 seconds extension at 68°C for the specified number of cycles for each sample. A final extension step of 7 minutes at 68°C was conducted.

2.3.1.5 Library amplification

Libraries was subsequently amplified and externally indexed using standard Illumina P5 and P7 primers with double indexes in a 50 µl PCR reaction (Schochetman et al. 1988), consisting of 15 µl of library, 25 µl Gold Taq 360 Master Mix, 5 µl Reaction Enhancer, 3 µl H₂O, 1 µl of P5 primer, and 1 µl of P7 primer (10nM). Thermocycling conditions included an initial denaturation step of 2 minutes at 95°C, followed by cycles of 15 seconds denaturation at 95°C, 30 seconds annealing at 60°C, and 30 seconds extension at 72°C for the specified number of cycles for each sample. A final extension step of 7 minutes at 72°C was conducted.

The amplified libraries were purified using SPRI beads (Beckman Coulter), and quality control was performed on a Fragment Analyzer (Spectro Scientific) prior to sequencing.

2.3.1.6 Sample screening

All samples underwent screening sequencing on NovaSeq 6000 instrument at Novogene, China. Approximately 3G of raw data were obtained for each sample. Following the screening sequencing, the ratio of nucleotides mapped to the domestic cat whole nuclear reference genome (Felis_catus_9.0 GCA_000181335.4) (endogenous DNA) or the number of reads mapped to the mitochondrial genome (NC_001700.1) was calculated. Further sequencing

strategies were developed based on these results (Figure S1 & Table S7).

2.3.1.7 Whole-genome deep sequencing

Samples representing the spatial and temporal contexts or possessing more than 10% endogenous DNA in screening were selected for deeper sequencing (Figure S1A & Table S7). In total, four samples were collected (FS1, FS2, FS12, FS21) (Table S7). The amount of raw sequencing data required for each sample was determined by the ratio of endogenous DNA. All samples were sequenced on a NovaSeq 6000 instrument at Novogene, Beijing.

2.3.1.8 Mitochondrial genome sequencing

An additional 4G of sequencing data were obtained to acquire full mitochondrial genomes for samples that did not qualify for whole nuclear genome sequencing but had more than 2,000 reads mapped to the mitochondrial reference genome (NC_001700.1) (Figure S1). Samples FS3, FS4, FS5, and FS17 followed this strategy (Table S7) and were sequenced on an Illumina NovaSeq 6000 instrument at Novogene, China.

2.3.1.9 Ancient mitochondrial DNA capture sequencing

In-solution targeted capture of the cat mitochondrial genome was conducted on 14 samples using the myBaits (Arbor Biosciences) Hybridization Capture for Targeted Next-Generation Sequencing protocol, with a hybridization temperature of 55°C and a hybridization time of 48 hours.

The *Felis catus* mitochondrial reference genome (NC_001700.1) was employed to synthesize baits for capture. Due to the degraded nature of ancient DNA, the amount of endogenous DNA

per sample is considerably lower than that of modern samples, resulting in the potential for probes in a single capture reaction to be significantly overwhelmed for each sample. To achieve optimal cost-effectiveness, more than one sample was pooled per reaction. Consequently, three reactions, each containing 2-3 ancient libraries pooled equimolarly, were prepared. The libraries combined in a single pool had distinct indexes. The three reactions were then sequenced on an Illumina NovaSeq 6000 instrument at Novogene, China.

2.3.2 Bioinformatics methods

2.3.2.1 Data filtering and mapping

Following sequencing, raw data were processed by trimming adapters and collapsing reads using AdapterRemoval v2.3.0 (Schubert, M., Lindgreen, S., & Orlando, L., 2016). Subsequently, Cutadapt v3.5 (Martin, 2011) was employed to remove 5 bases from both the 5' and 3' ends of the reads to minimize the impact of deamination (Dabney, J., Meyer, M., & Pääbo, S., 2013; Hofreiter et al., 2001). The same software was also utilized to trim low-quality reads shorter than 25 bp, containing over 10% Ns, or with a mapping quality lower than 25.

2.3.2.2 Mitochondrial DNA analysis

2.3.2.2.1 Mapping

The processed data were then mapped to the domestic cat mitochondrial reference genome (NC_001700.1) using the aln function in BWA v0.1.17 (Li et al., 2013) with default settings. SAMtools v1.14 (Danecek et al., 2021) were subsequently used to sort the BAM files, filter out PCR duplicates, unmapped reads, or reads with a mapping quality lower than 30. HTSbox was employed to call consensus mitochondrial sequences using default settings. Control regions of the consensus sequences were then excluded, and the remaining sequences were

aligned to each other using MUSCLE v5 (Edgar, 2021).

2.3.2.2.2 Phylogeny

A Maximum Clade Credibility (MCC) phylogenetic tree was constructed using BEAST v2.6.7 (Bouckaert et al., 2019) to identify the species of the samples and calculate the time of divergence. This analysis included 22 ancient samples, and modern mitochondrial genomes of domestic cats, wild cats, and leopard cats, with the tiger (NC_010642.1) chosen as the outgroup. The analysis was conducted utilizing the dates of the tips to calibrate divergence times. We implemented the Tamura-Nei 93 (TN93) nucleotide substitution model, incorporating both a gamma distribution (G) and a proportion of invariant sites (I). A strict molecular clock and constant population size were assumed, with uniform priors applied. The BEAST analysis was run for 100 million generations, with parameters and trees sampled every 10,000 generations.

A Maximum Likelihood (ML) phylogenetic tree of whole mitochondrial genomes was generated using IQ-TREE v2 (Minh et al., 2020; Kalyaanamoorthy et al., 2017; Nguyen et al., 2015), following the TN93+I+G model with 1,000 replicates. The analysis included 14 Chinese ancient domestic cats, 37 Chinese modern domestic cats, and the dataset from Jamieson et al. (2021) (Figure 10). The missing data for each Chinese sample was less than 2%, with a depth greater than 30-fold (Table S2.2). The missing data for European or Central Asian samples was generally less than 10%, with a depth greater than 10-fold.

A Maximum Likelihood (ML) phylogenetic tree of the ND5 and ND6 genes on mitochondrial genomes was constructed using IQ-TREE v2 (Minh et al., 2020; Kalyaanamoorthy et al., 2017; Nguyen et al., 2015) following the TN93+I+G model with 1,000 replicates. The analysis included 14 Chinese ancient domestic cats, 37 modern domestic cats, and the dataset from

Driscoll et al. (2007) and Jamieson et al. (2020). To incorporate the sequences from Driscoll et al. (2007) and Jamieson et al. (2021) into the dataset, the samples were trimmed to obtain sequences with a length of 286 bp. The sand cat (*Felis margarita*) was chosen as the outgroup.

2.3.2.3 Nuclear Genome Analysis

2.3.2.3.1 Mapping

Cleaned data were mapped to the domestic cat reference genomes (*Felis_catus*_9.0 GCA_000181335.4) using the "aln" function in BWA v.0.1.17 (Li et al., 2013) with the settings of -n 0.01 -o 2 -l 1024. Subsequently, SAMtools v.1.14 (Danecek et al., 2021) were utilized to sort the bam files and filter out PCR duplicates, unmapped reads, or reads with a mapping quality less than 30.

2.3.2.3.2 Calling SNPs

Single nucleotide polymorphisms (SNPs) were identified and filtered using GATK v.4.2 (McKenna et al., 2010), BCFtools v.1.14 (Danecek et al., 2021), and VCFtools v.0.1.16 (Danecek et al., 2011). All transitions were removed to mitigate the influence of deamination (Dabney et al., 2013; Hofreiter et al., 2001). BEDtools v.2.30.0 (Quinlan et al., 2010) was employed to exclude all non-neutral loci, and PLINK v.1.9 (Purcell, 2014; Purcell et al., 2007) was used to filter out all SNPs located in linkage disequilibrium regions and on sex chromosomes. Through these steps, a total of 470,747 SNPs were generated for downstream analysis.

2.3.2.3.3 Population analysis

Using 470,747 SNPS, two rounds of Principal Component Analyses (PCA) were performed with EIGENSTRAT SmartPCA (Patterson et al., 2006; Price et al., 2006). The first PCA incorporated a total of 71 samples, including 4 Chinese domestic cats, 13 European ancient cats, 1 ancient Central Asian domestic cat (Dhzankent), several modern domestic cats across Eurasia, and wild cats. The second PCA aimed to elucidate high-resolution geographic patterns among different domestic cat populations; therefore, wild cats, except for African wild cats, and all domestic cats extensively admixed with wild cats, were excluded from the analysis. As African wild cats are the progenitors of domestic cats, they were retained. Since ancient samples may contain a considerable amount of missing data, potentially impacting PCA results, the "LsqProject: Yes" function was adopted to project all ancient data onto the PCA initially defined by high-quality modern data.

ADMIXTURE analysis employed 470,747 SNPS generated from 95 samples, including 4 ancient Chinese domestic cats, 13 European ancient cats, 1 ancient Central Asian domestic cat (Dhzankent), various modern domestic cats across Eurasia, wild cats, and Sand cats (Table S2.1). The assay was conducted using ADMIXTURE 1.3.0 (Alexander and Lange, 2011). The optimal number of subpopulations (K) was estimated to be 6.

The f3 and f4-statistics analyses were conducted with EIGENSTRAT AdmixTools v.7.0 (Patterson et al., 2012). The qp3Pop method was utilized to inform the f3-statistics. The Sand cat (*Felis margarita*) was selected as the outgroup, and this study tested various paired populations within our sample set, including 4 Chinese ancient domestic cats, the ancient Dhzankent cat, 13 ancient European cats, modern domestic cats from different regions of Eurasia, and wild cats. The Chinese ancient domestic cats were initially tested individually.

Subsequently, we incorporated geographic information into the analysis. Since both FS1 and FS2 were obtained from Northern China, they were merged into a single group Catus.

Four ancient Chinese samples were assigned to 3 populations from Western China, Central China, and Northern China. Domestic cats from Europe and wild cats, excluding African wild cats, were excluded from the analysis. The qpDstat method was employed to inform the *f4-statistics* to further explore potential admixtures between ancient Chinese individuals and wild cats. The population combination of (Outgroup, Wild cat, Ancient European domestic cat, ancient Chinese domestic cat) was adopted where sand cat (*Felis margarita*) was chosen as the outgroup.

2.3.3 Radiocarbon dating

Six samples extracted from disturbed sedimentary layers were submitted for accelerator mass spectrometry (AMS) dating. Three samples (FS16, FS17, and FS19) were dated at Beta Analytic in the United States, while the remaining samples (FS12, FS13, and FS24) were processed at the School of Archaeology, Peking University, China. All radiocarbon dates reported were calibrated using the INTCAL20 calibration curve (Reimer et al., 2020)

2.4 Results

2.4.1 Radiocarbon dating

The radiocarbon dating results for the six samples provide important chronological context for understanding their origins (Table 2). The radiocarbon dating results of three samples are consistent with archaeological context. Sample FS13, from Yangling in Central China, was dated to the Han Dynasty with an uncalibrated date of 2095 ± 20 BP, corresponding to a

calibrated date range of 168 BC to 48 BC. Sample FS19, from Huangdiguli in Central China and associated with the Han Dynasty, has an uncalibrated date of 1760 ± 30 BP and a calibrated date range of 234 to 380 AD. Finally, sample FS24, originating from Han Chang'an in Central China and also linked to the Han Dynasty, presented an uncalibrated date of 1995 ± 20 BP, with a calibrated date range of 44 BC to 76 AD.

The results of the dating analyses conducted on three samples, namely FS12, FS16, and FS17, have yielded unexpected outcomes, which are not consistent with the assumed archaeological contexts. Specifically, sample FS12, which was obtained from Tongwan City in Western China and believed to belong to the Song Dynasty, was dated to the Tang Dynasty. The uncalibrated date for this sample was found to be 1220 ± 20 B.P, with a calibrated date range between 706 and 883 AD, with a 95.4% probability. Similarly, sample FS16, which was thought to belong to the Tang Dynasty, was dated to the Ming Dynasty. The uncalibrated date for this sample was 450 ± 30 BP, with a calibrated date range between 1413 and 1480 AD. Lastly, sample FS17, which was assumed to belong to the Han Dynasty, was found to fall within the range of the Qing Dynasty. The uncalibrated date for this sample was 140 ± 30 BP, with two calibrated date ranges: 1797 to 1944 AD and 1671 to 1779 AD, with probabilities of 58.2% and 37.2%, respectively (Table 2).

Table 2 Radiocarbon dating results of ancient Chinese cats

Sample	Site	Location	Context date	Uncalibrated date (BP)	Date cal. BCE/CE (95.4%, overall range IntCal 20)
FS12	Tongwan City	Western China	Song Dynasty	1220 ± 20	706AD (7.5%) 728AD 772AD (88.0%) 883AD
FS13	Yangling	Central China	Han Dynasty	2095 ± 20	168BC (95.4%) 48BC
FS16	Zhengzhou Shang City	Central China	Tang Dynasty	450 ± 30	1413 - 1480 Cal AD (537 - 470 Cal BP)
FS17	Yunzhong	Northern China	Han Dynasty	140 ± 30	1797 - 1944 Cal AD (153 - 6 Cal BP) (58.2%); 1671 - 1779 Cal AD ((279 - 171 Cal BP) (37.2%))
FS19	Huangdiguli	Central China	Han Dynasty	1760 ± 30	234 - 380 Cal AD (1716 - 1570 Cal BP)
FS24	Han Chang'an	Central China	Han Dynasty	1995 ± 20	44BC (95.4%) 76AD

2.4.2 Genetic results

A total of four ancient nuclear genomes and 22 mitochondrial genomes were generated as part of this study. The coverage depth of the four domestic cat nuclear genomes ranges from 2 to 3.5X. The coverage depth of the 22 mitochondrial genomes ranges from 60 to 304X, with less than 5% of missing data (Table 3).

Table 3 Sequencing result. Sample FS8, FS10, FS13, FS14, FS15, FS19, FS24 were mapped to the leopard cat reference genomes (see chapter 3). The rest of the samples were mapped to the domestic cat reference genomes.

Sam ple	Endogen ous%	Num_rea ds(mt)	Length (mt)	Coverage(mt)%	Mean_dept h(mt)X	Num_read s(Nuc)	MEAN_DEP TH(Nuc)	SNPs_use d(Nuc)
FS1	12.5	24336	16732	98.3789	86.5136	128005822	3.22737	95470
FS2	13.82	21736	16865	99.158	82.8478	101903410	2.43784	99631
FS3	11.4	11063	16473	96.8487	34.7035	—	—	—
FS4	10.69	15936	16570	97.419	59.8712	—	—	—
FS5	11.2	29916	16769	98.589	39.2182	—	—	—
FS6	0.34	15982	16961	99.7178	112.491	—	—	—
FS7	0.91	17321	16730	99.53	16.9127	—	—	—
FS8	8.76	9870	16416	98.2935	47.3244	153959749	5.96269	17194519
FS1 0	0.45	24215	16338	97.8265	11.5208			
FS1 2	13.21	23207	16742	98.4377	78.8921	111504420	3.52959	107849
FS1 3	0.06	4117	15792	94.5572	26.2551	—	—	—
FS1 4	0.06	12377	15584	93.3118	20.1013	—	—	—
FS1 5	2.3	2826	16114	96.4852	15.6682	162299570	3.32325	14322059
FS1 6	4.04	6512	16991	99.8942	207.187	—	—	—
FS1 7	17.89	5274	16871	99.1887	45.9693	—	—	—
FS1 8	0.05	4969	16975	99.8001	145.439	—	—	—
FS1 9	6.48	27643	16518	98.9043	51.2599	175356056	4.70431	15747266
FS2 0	0.05	10271	16694	98.148	99.1598	—	—	—
FS2 1	10.24	19562	16708	98.237	77.6107	115273634	3.23206	95137
FS2 2	0.1	25761	16593	97.5542	59.5793	—	—	—
FS2 3	3.42	13341	17009	100	304.291	—	—	—
FS2 4	2.7	20836	16617	99.497	90.8493	—	—	—

2.4.2.1 Mitochondrial analysis

2.4.2.1.1 Mitochondrial species identification

The ancient samples analysed in this study were classified into three distinct clades: leopard cats, Asiatic wild cats, and domestic cats. Sample FS7 (3,800 BP) was found to be closely related to the Asiatic wild cat lineage. A majority of samples from the Neolithic Age to the Han Dynasty (5,500 - 1,760 BP), including FS8, FS10, FS13, FS14, FS15, FS19, and FS14, were assigned to the leopard cat clade. In contrast, all samples dating from or after the Tang Dynasty (N=14), comprising FS1, FS2, FS3, FS4, FS5, FS6, FS12, FS16, FS17, FS18, FS20, FS21, FS22, and FS23, were clustered within the domestic cat clade (Figure 3). Leopard cats share a common ancestor with Asiatic wild cats and domestic cats, dating back to approximately 3.0 to 3.6 million years ago (Ma). Domestic cats and Asiatic wild cats diverged around 0.32 to 0.44 Ma (Figure 3).

Based on the findings, the earliest domestic cat in China, as identified in our study, dates back to approximately 1,220 BP (Table 2), corresponding to the Tang Dynasty period. Furthermore, the individuals predating the Han Dynasty (before 1,760 BP) (Table 2) were found to belong to other species or subspecies, exhibiting a deep divergence from *Felis silvestris lybica*, having no direct relevance to the domestication of cats.

For the purpose of this chapter, which focuses on the domestic cat, we have excluded specimens FS7, FS8, FS10, FS13, FS14, FS15, FS19, and FS24 from subsequent analyses, as their mitochondrial genomes were found to be related to leopard cats or Asiatic wild cats.

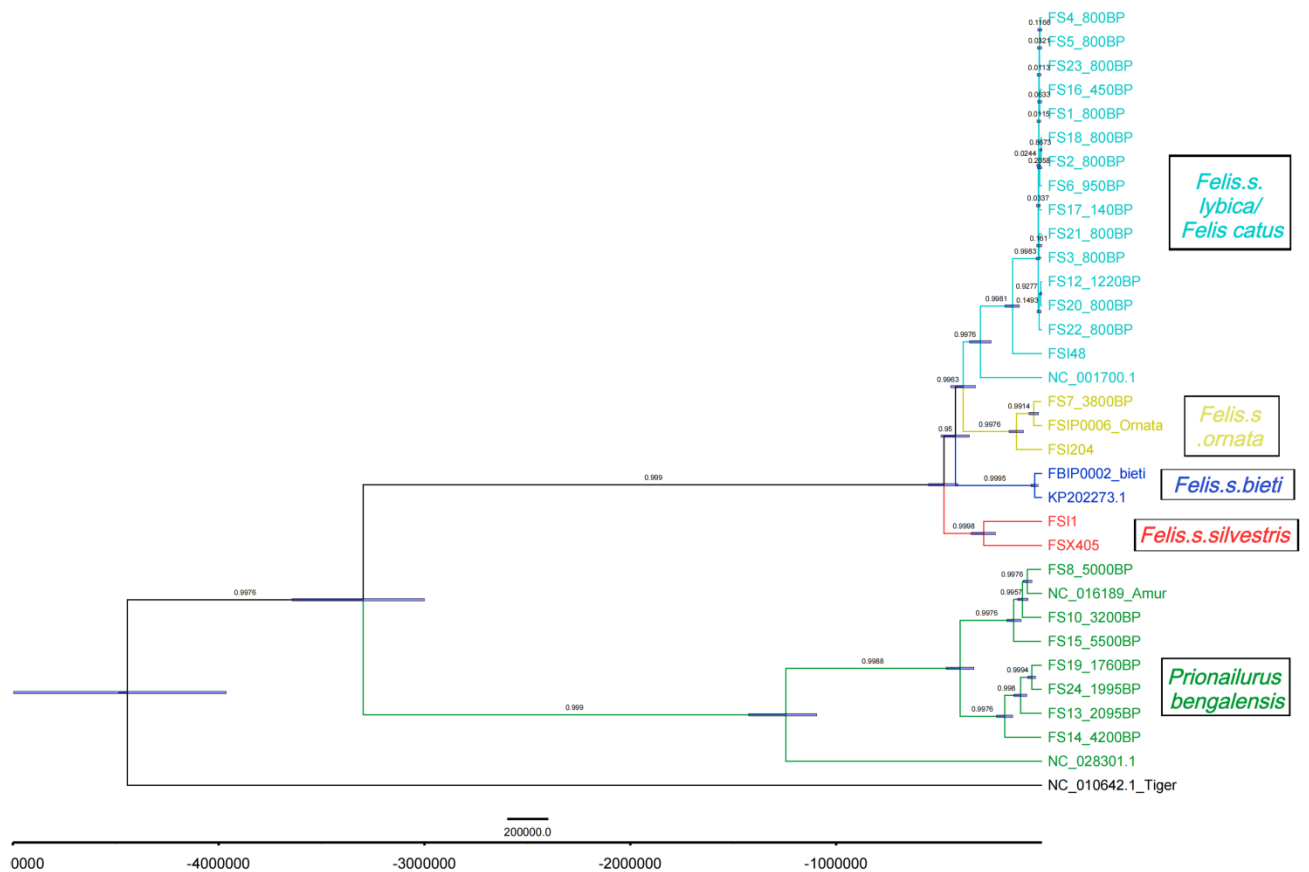


Figure 3 MCC tree constructed using 22 ancient Chinese samples with tiger as outgroup.

The identification of species based on mitochondrial genome data could be complicated in cases where an individual is the offspring of hybridization between two different but interfertile species, as nuclear and mitochondrial genomes are independently inherited. However, in the context of this study, it is unlikely that hybridization occurred between domestic cats and leopard cats, which could result in contradictory species identification results. This is due to the low success rate of hybridization between these two species, as previously reported by Gershony et al. (2014) and YosIDA (1982). Therefore, we believe that our species identification results based on mitochondrial genome data are reliable and accurate.

2.4.2.1.2 Whole mitochondrial genome phylogenetic analysis

To understand the maternal lineages of ancient Chinese domestic cats, we constructed a Maximum Likelihood (ML) phylogenetic tree using whole mitochondrial genome data (Figure

4; Figure S1).

The topology of the tree was similar to that inferred by Jamieson et al. (2021), where the European samples clustered in two major clades representing two haplogroups, namely D1 and D2. The D2 clade was further split into two sub-clades, D2a and D2b. Our results showed that all ancient Chinese domestic cats and the same 36 modern domestic cat samples, which accounted for 68% of the total Chinese modern domestic cats, formed a monophyletic clade, which we named "D2c". This clade also included the ancient Dhzankent cats from Central Asia (Haruda et al. 2020). In addition, FSI48, a modern African wild cat from Israel, was basal to D2c (Figure 4; Figure S1)..

For Chinese samples that were not in D2c, four of them (7.5%) formed a sister clade with all the other modern or ancient samples from China, Central Asia, or Europe. One of them (1.9%) clustered with D1. Three of them (5.7%) clustered with D2a, and nine samples (17%) were within D2b. The whole mitochondrial genome phylogeny also demonstrated the relatively higher maternal diversity of ancient European domestic cats, as the haplogroups of the ancient European populations were more diverse compared to the Chinese ancient domestic cats (Figure 4; Figure S1).

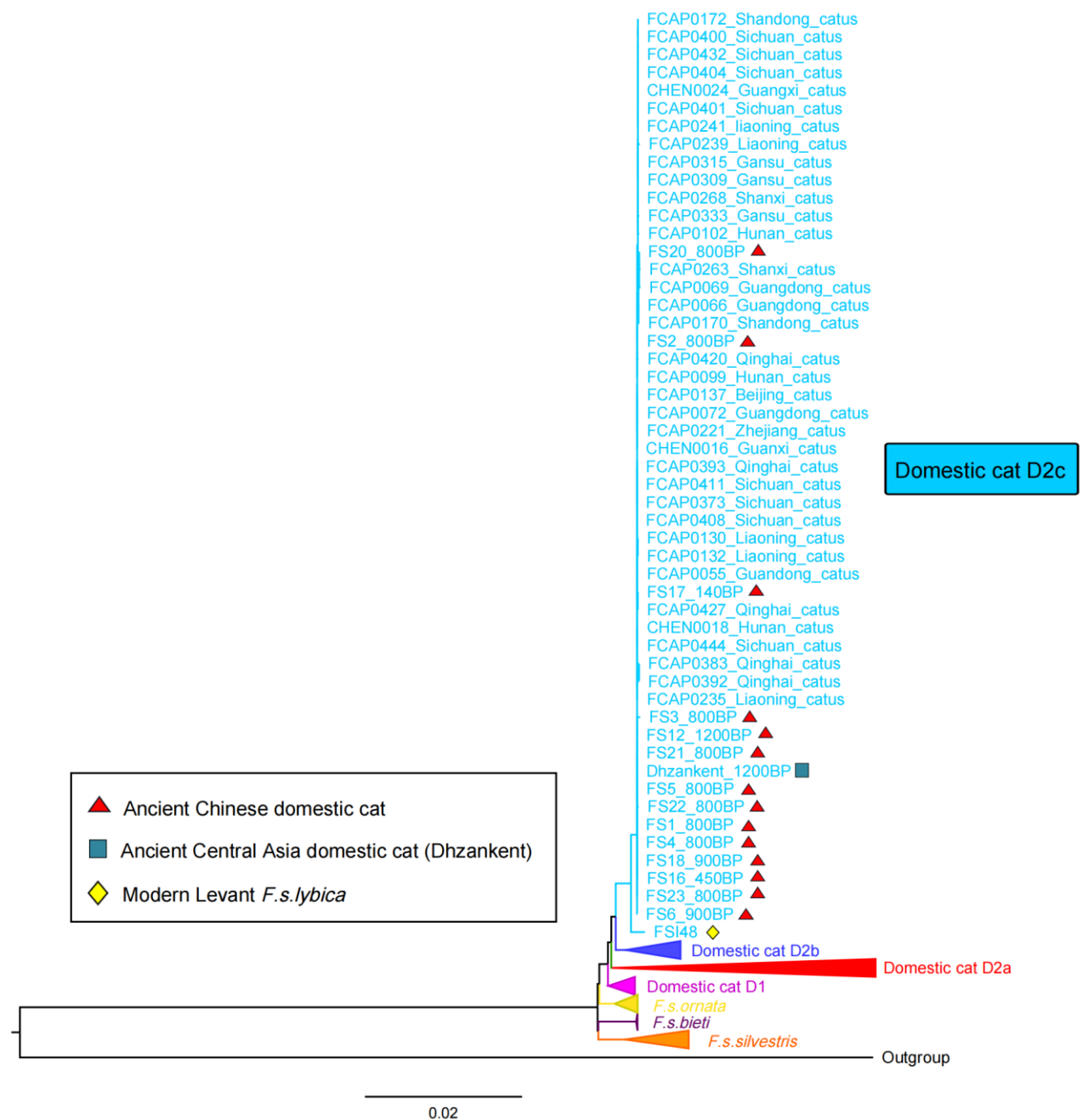


Figure 4 ML tree constructed using the whole mitochondrial genomes of domestic cats and wild cats, with leopard cat being outgroup. . Clades of *F.s.silvestris*, *F.s.bieti*, *F.s.ornata*, domestic cat D1, domestic D2a and domestic cat D2b are collapsed in the plot. See Figure S 1 for a detailed tree showing all samples.

2.4.2.1.3 ND5&ND6 phylogenetic analysis

Our analysis revealed that all ancient Chinese domestic cats (N=14) and approximately 68% of Chinese modern domestic cats (36 out of 53) formed a monophyletic clade, which also included samples assigned to haplogroup IV-B in Ottoni et al. (2007). Haplogroup IV-B is

known to be the predominant haplogroup in the Levant. The Dhzankent ancient cat from Central Asia (Haruda et al. 2020) was also integrated into this clade. Chinese samples that clustered outside of haplogroup IV-B were typically located in the coastal region of China (Figure 5).

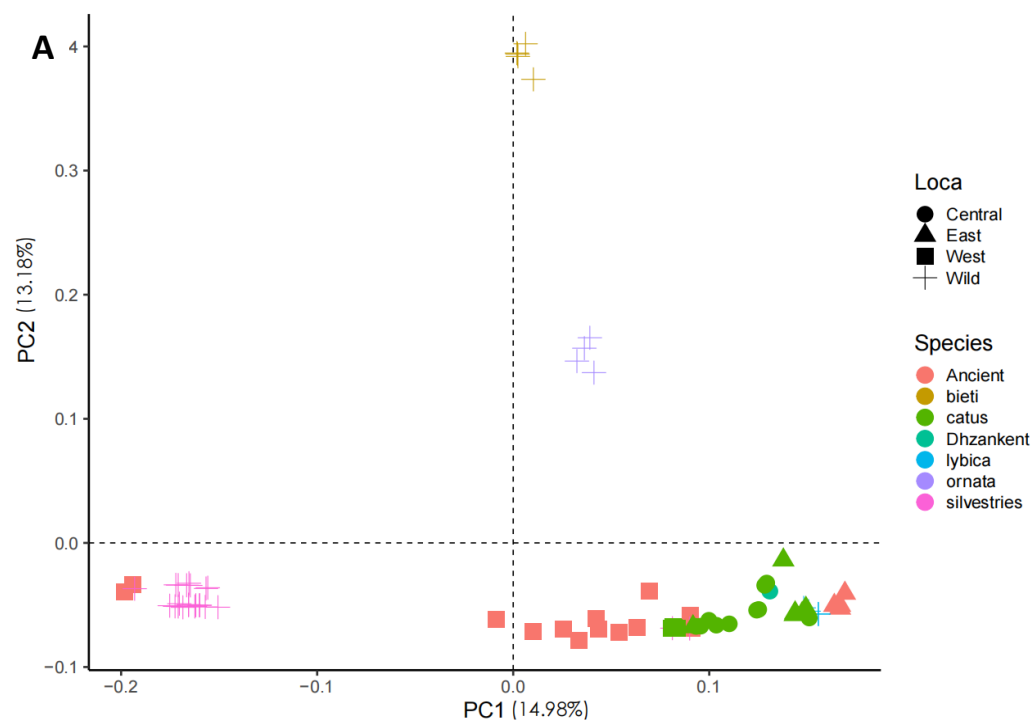
Comparatively, the populations of ancient European domestic cats were found to be more diversified, as they were assigned to all five haplogroups previously identified (Ottoni et al. 2017; Driscoll et al. 2007) (Figure 5).

2.4.2.2 Nuclear DNA analysis

2.4.2.2.1 Principal Component Analysis

To understand the genetic relationships and infer the population structure across our dataset, we conducted two rounds of Principal Component Analysis (PCA) (Figure 6).

The first PCA (Figure 6A) incorporated four Chinese ancient domestic cats and the sample sets of Jamieson et al. (2021). PC1, which explained 14.98% of the variation, appeared to distinguish between domestic cats (*Felis catus*) and European wild cats (*Felis silvestris silvestris*), while PC2 (which explained 13.18% of the variance) split Asiatic wild cats (*Felis silvestris ornata*) and Chinese mountain cats (*Felis silvestris bieti*) from other populations. The four ancient Chinese samples fell within the range of domestic cats, which further confirmed the results of species identification based on mitochondrial DNA. The African wild cat samples could not be isolated from domestic cats, which was expected as they are the progenitors of domestic cats (Figure 6A).



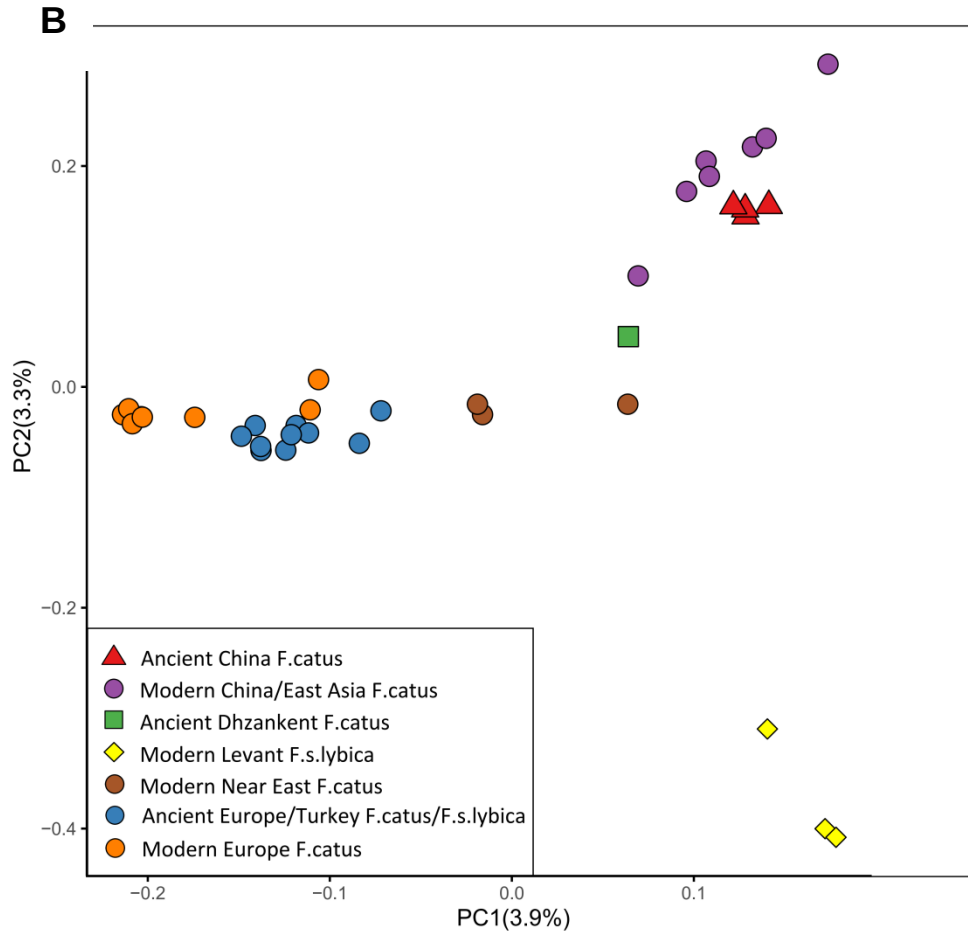


Figure 6 Principal component analysis (PCA) using 470,747 SNPS. Plot A is the first PCA that have incorporated the *F.s.bieti*, *F.s.ornata*, *F.s.silvestris*, *F.s.lybica* and domestic cats. B is the second PCA that excluded wild cats except *F.s.lybica*.

To further investigate the population structure of domestic cats from diverse locations, we conducted an additional PCA after excluding modern Chinese mountain cats, European, and Asiatic wild cats. This approach facilitated the emergence of a plot with increased geographic resolution, unveiling more intricate relationships among various domestic cat populations. The samples from Europe and China formed two distinct groups, mirroring their genetic differentiation. Furthermore, the domestic cats in Central Asia and the Near East appeared to be subdivided, with some individuals exhibiting a closer relationship to the European group, while others, including the Dhzankent cat (Haruda et al., 2020), displayed a stronger affinity with the Chinese group. PC2 separated African wild cats from domestic cats, although they maintained an affinity with the Chinese population along PC1 (Figure 6B).

2.4.2.2.2 ADMIXTURE analysis

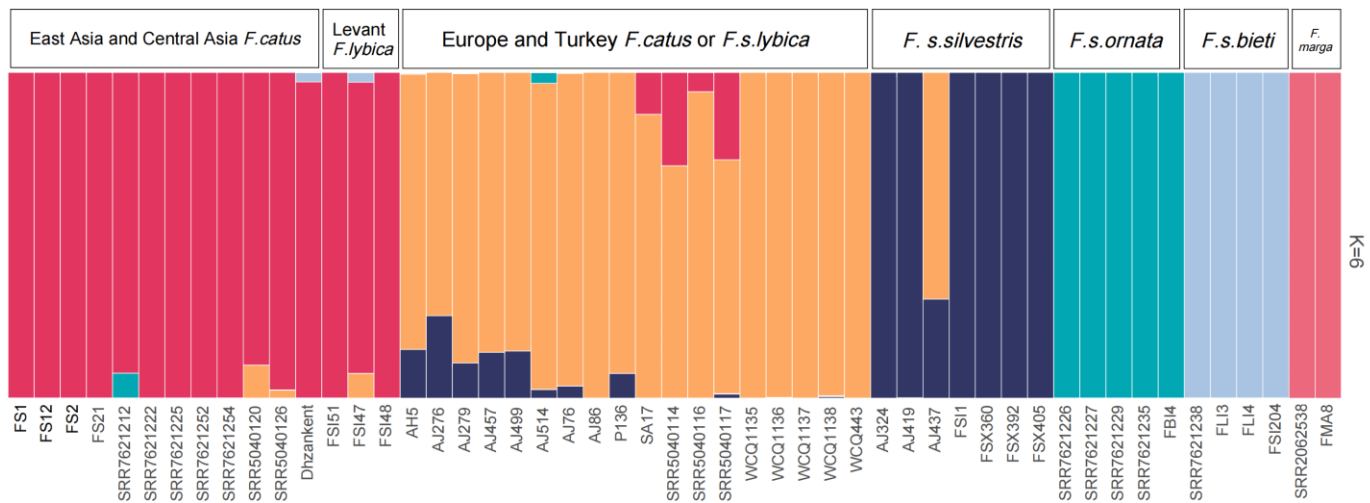


Figure 7 ADMIXTURE analysis using 470,747 SNPs, K=6. FS1, FS2, FS12, and FS21 are the ancient Chinese domestic cats included in this study. Dhzankent represents the ancient Central Asia domestic cat.

We conducted an ADMIXTURE analysis to investigate the population structure of our cat samples. When K=6, different species or subspecies of cats, as well as the domestic cats from Eastern and Western Eurasia, were clearly distinguished (Figure 7, Figure S2).

Chinese ancient and modern domestic cats predominantly share similar ancestral components, with the exception of a modern Chinese sample (SRR7621212), which exhibited a minor degree of ancestry from Chinese mountain cats. In contrast, the Near Eastern and Central Asian domestic cats displayed a more intricate ancestry profile, with three of them (SRR5040120, SS5040126, Dhzankent) containing ancestries from both East Asian and European or Turkish domestic cats. Furthermore, the Asiatic wild cat ancestry was detected in the ancient Dhzankent sample and two Levantine African wild cats, indicating admixture between these species or subspecies in Central Asia and the Near East. However, the contribution of European and Turkish domestic cat, or wild cat ancestries were minor (less than 10%). Thus ancient and

modern domestic cats from China and Central Asia, as well as the African wild cats, generally exhibited a uniform population structure (Figure 7, Figure S2).

The population structure of European wild cats was frequently detected in European domestic cats, indicating extensive admixture between the two species (or subspecies) in Western Eurasia.

2.4.2.2.3 Out-group f_3 analysis

We conducted an out-group f_3 analysis (Patterson et al. 2012) to test the genetic affinities among different populations.

Results of the out-group f_3 analysis (Figure 8; Table S3) showed that, in contrast to domestic cats from Western Eurasia, such as Europe and Turkey, domestic cats from China and Central Asia exhibit higher f_3 values with Levantine *Felis.s.lybica*. This suggests a close genetic relationship between Levantine and Asian cat populations. Notably, all four ancient Chinese domestic cats display significant f_3 values when compared to modern Chinese domestic cats, highlighting a strong genetic connection between ancient and contemporary populations in China.

Interestingly, among all ancient Chinese domestic cat populations, the ancient Chinese cat (FS12) from western China exhibits the closest genetic affinity to the ancient Central Asian domestic cat (Dhzankent), followed by ancient cats from Northern and Central China (FS1, FS2, FS21), suggesting that there may have been significant gene flow between cat populations in western China and Central Asia during ancient times. (Figure 8; Table S3).

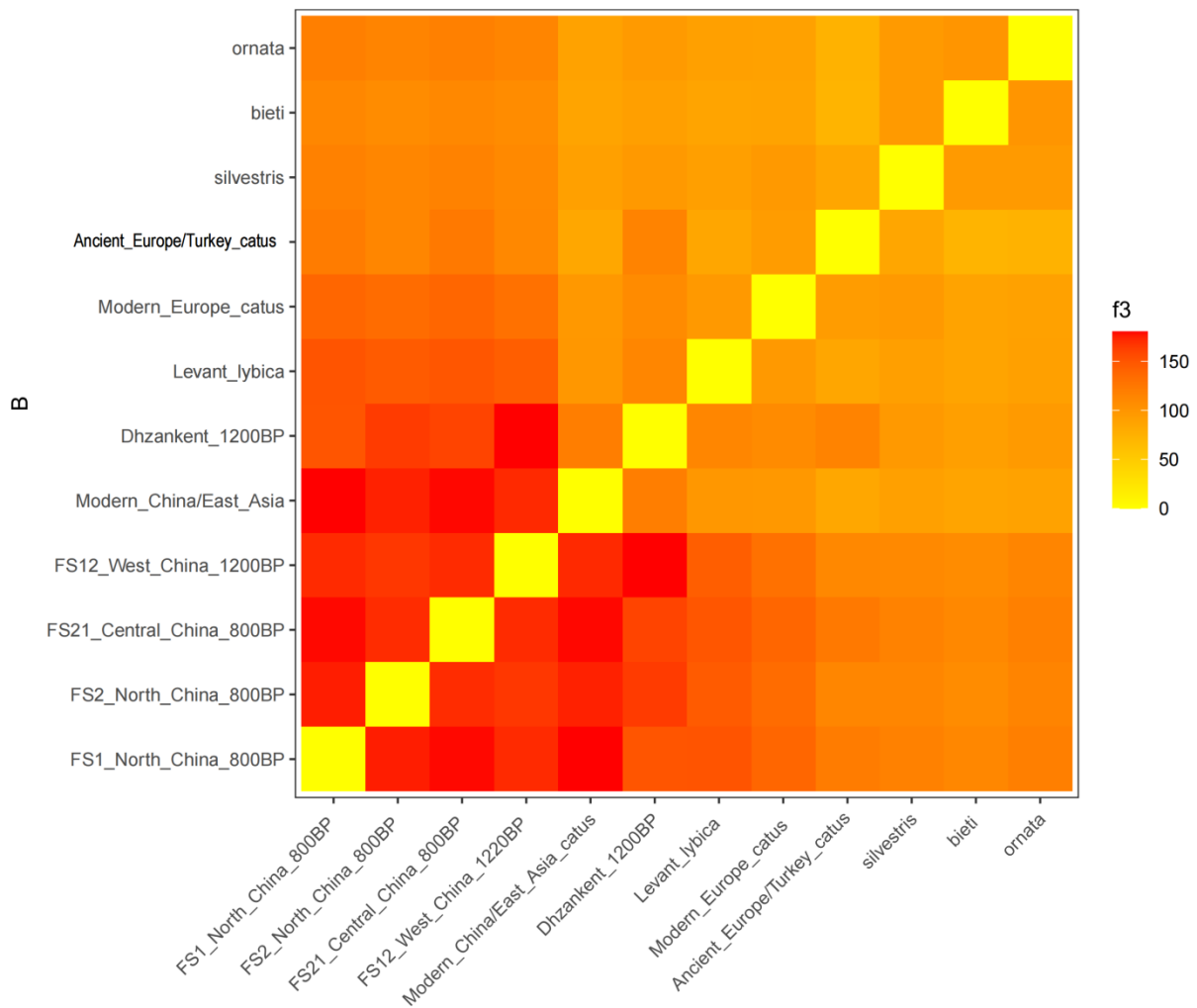


Figure 8 Heatmap presents the outgroup f_3 results.

2.4.2.2.4 *F4-statistics* analysis

F4-statistics analysis was conducted to explore the potential admixture events between the Chinese ancient domestic cats and wild cats. By adopting the population combination of (Outgroup, Wild cat, Ancient European domestic cat, ancient Chinese domestic cat), all the Z-scores were either negative or insignificant, indicating that there was no significant introgression between the wild cats and the Chinese ancient domestic cats (Table 4, Table S4).

Table 4 Results of f4-statistics. Population combination was set as (Outgroup, Wild cat , Ancient European domestic cat, Ancient Chinese domestic cat).

Outgroup	Wild cat	Ancient European domestic cat	Ancient Chinese domestic cat	D value	Z-score
Margarita	Ornate	Ancient Europe/Anatolia	FS1	-0.000061	-0.029
Margarita	Ornate	Ancient Europe/Anatolia	FS2	-0.001553	-0.743
Margarita	Ornate	Ancient Europe	FS12	-0.00215	-1.069
Margarita	Ornate	Ancient Europe/Anatolia	FS21	-0.000966	-0.44
Margarita	Bieti	Ancient Europe/Anatolia	FS1	-0.001363	-0.643
Margarita	Bieti	Ancient Europe/Anatolia	FS2	-0.002389	-1.124
Margarita	Bieti	Ancient Europe/Anatolia	FS12	-0.002717	-1.316
Margarita	Bieti	Ancient Europe/Anatolia	FS21	-0.002793	-1.263
Margarita	Silvestris	Ancient Europe/Anatolia	FS1	-0.019096	-9.084
Margarita	Silvestris	Ancient Europe/Anatolia	FS2	-0.018119	-8.02
Margarita	Silvestris	Ancient Europe/Anatolia	FS12	-0.018434	-8.896
Margarita	Silvestris	Ancient Europe/Anatolia	FS21	-0.019452	-8.598

2.5 Discussion

2.5.1 The origin of the Chinese domestic cats

It has been demonstrated that all current domestic cats in China are descendants of African wild cats (*Felis silvestris lybica*) native to the Near East (Yu et al. 2021). This discovery raises the question of when domestic cats were first introduced to China. Two commonly accepted hypotheses have been proposed: the Neolithic origin and the Han Dynasty origin.

Nevertheless, our new findings challenge both previous hypotheses. The ancient DNA and radiocarbon results presented in this study suggest that domestic cats did not exist in China before the Tang Dynasty (618-907 AD). As illustrated in the phylogeny (Figure 3), Neolithic and Bronze Age samples, including FS8, FS10, FS14, and FS15, have been classified within the leopard cat clade. Despite leopard cats having a broad distribution that encompasses the entirety of East Asia, they are biologically unrelated to modern domestic cats in China (Yu et al. 2021).

Sample FS7 stands out as the only specimen from the period prior to the Tang Dynasty that is not a leopard cat. However, it is not a domestic cat either; rather, it has been identified as an Asiatic wild cat (*Felis silvestris ornata*), a species or subspecies of wild cat indigenous to Central Asia and unrelated to cat domestication (Driscoll et al. 2007). Limited ancestry of *ornata* has been detected in some modern domestic cats (Figure 7, Figure S2). This may just be attributed to minor introgression into the domestic cat population, and it would be inappropriate to regard *ornata* as the ancestor of contemporary Chinese domestic cats.

Taken together, these results provide evidence that domestic cats were not present in China during the Han Dynasty or earlier periods, such as the Neolithic Age. Furthermore, all Han Dynasty samples were genetically identified as leopard cats, including FS24, which had previously been considered one of the oldest domestic cats in China (Hu, S. et al. 2006). Based on these findings, we recommend exercising extra caution when interpreting archaeological materials, images, or literary records associated with cats from the Han Dynasty or earlier periods (Hu, Y. et al. 2014; Wang, Z. 2010; Hu Songmei et al. 2006; Wang, Z. 2010; Wang 1986). It is possible that these cats were, in fact, leopard cats rather than domestic cats.

Utilizing radiocarbon dating, we dated the earliest domestic cat in China to 1,220 BP, which corresponds to the Tang Dynasty period (Sample FS12, Figure 3 Table 2). Thus first occurrence of domestic cats in China could be traced back to about the Tang Dynasty. In fact, from this point onward, cat remains are more frequently discovered at archaeological sites. All our post-Tang period samples are identified as domestic cats, originating from five different archaeological sites located in four provinces, indicating a widespread diffusion of domestic cats (Table 1 & Figure 2).

Interestingly, the first textual reference to cats following the Han Dynasty also appears during the Tang Dynasty (618-907 AD) in Zhang Zhuo's "*The Palace Story*" (张鷟 【唐】《朝野僉载》). Subsequently, literature pertaining to cats increased significantly, and the "cats" mentioned in these records likely referred to domestic cats, as they were associated with catching mice or rats and were described as companion animals. This dual role of cats as both pest control and companions are more characteristic of modern domestic cats. Consequently, we believe that the cats mentioned in literature after the Tang Dynasty were indeed domestic cats.

Furthermore, based on the analysis of genetic data, we propose that Chinese domestic cats were potentially imported from the Levant in the Near East. As evidenced by both mitochondrial and nuclear DNA analysis Chinese domestic cats and Levantine African wild cats consistently assigned same phylogenetic group and remains much higher similarities than that of others, indicating a more extended shared similarity or evolutionary history between them (Figure 8, Table S3). In summary, our analysis demonstrates the affinity between Levantine *lybica* and Chinese domestic cats, it is highly likely that Chinese domestic cats originated from the Levant.

2.5.2 A potential route of dispersal

The current results suggest that domestic cats in China may have been imported from the Levant through Central Asia. They might have initially reached the Northwest and then dispersed into the Chinese hinterland.

To date, the earliest domestic cats unearthed in the eastern part of Eurasia were discovered in Dzankent, Kazakhstan (1225-1060 BP) (Haruda et al. 2020). It is highly probable that this sample is related to the ancestral population of domestic cats in China. As depicted in the phylogeny, the Dzankent cats clustered with ancient and most modern domestic cats in China (Figure 4 & Figure 5). These results imply that domestic cats in ancient Central Asia and China share a common maternal ancestral population.

Genetic similarities are also supported by the analysis of nuclear genomes. In PCA, the Dzankent cat is positioned close to the cluster containing ancient and modern domestic cats in China. Additionally, the ADMIXTURE analysis inferred a similar ancestral combination in the Dzankent cat and its Chinese counterparts (Figure 7; Figure S2), suggesting that domestic cats in China and Dzankent could have derived from the same population. Interestingly, as indicated by the outgroup f_3 analysis (Figure 8, Table S3), the highest value is between the ancient Dzankent cat and FS12, implying a longer shared evolutionary history or a closer affinity between them (Table 1 & Figure 1). Geographically, the location of FS12 also borders Central Asia, and the dates of the two samples are similar, both earlier than other ancient Chinese samples. Integrating this information, we deduce that domestic cats were introduced to East Asia from the Near East, mediated by Central Asia.

However, it should be noted that our research only presents the possibility that Chinese

domestic cats may have been imported along the Silk Road. At this point, we prefer to maintain this as a hypothesis, considering that other possible routes, not analysed in this study due to a lack of samples, may exist. In fact, a maritime route could also be plausible, as some modern domestic cats belonging to haplogroups such as D1 or D2 reside in the coastal regions of China (Figure 4 & Figure S1). Future research on the origins of domestic cats in Chinese coastal regions should be conducted to further explore this possibility.

2.5.3 Long-term population continuity

Our analysis suggests that from the oldest sample (1200 BP) to the modern Chinese domestic cats, both ancient and most modern Chinese domestic cats consistently exhibit similar mitochondrial haplogroups or population structures, sharing high genetic affinities. This indicates that the population of Chinese domestic cats has remained stable for over 1,200 years. In other words, the domestic cat population in China may have been established through a few introduction events, possessing a relatively singular ancestral population.

In contrast to the more diversified European domestic cats, as indicated by both mitochondrial and nuclear DNA analyses, domestic cats in China have been relatively more homogeneous. It is not unique to domestic cats, and similar patterns have been observed in other imported domestic animals in China, such as sheep (Cai et al. 2007) or cattle (Cai et al. 2014). After being domesticated in the Near East, only a small portion of the whole variants of these animals were transported to China, and the subsequent founder effects made them the dominant lineage locally.

We attribute the long-term stability of the population structures of domestic cats and other imported animals to the relatively closed geographical environment in East Asia and the

subsequent restrictions on cultural exchanges. The natural barrier between the Near East and East Asia, especially in ancient periods when efficient communication media were lacking, could significantly reduce the possibility of novel populations arriving. Consequently, the initially introduced domestic cats in China remained less influenced. By comparison, the relatively shorter distance and Mediterranean Sea-lane facilitated the transportation of cultural materials between the Near East and Europe, resulting in domestic cats being imported to Europe much earlier than East Asia and exhibiting higher diversity (Jamieson et al. 2021).

2.5.5 No admixture between the Chinese ancient domestic cats and wild cats

The admixture between domestic animals and their wild counterparts is a long-standing topic (Yu et al. 2021; Germain et al. 2008; Pierpaoli et al. 2003). Their genetic interaction may significantly influence the evolution of both parties, which is relevant to conservation (Cao et al. 2021; Smith et al. 2021; Frantz et al. 2019; Røed et al. 2014; Hedrick et al. 2013). In ancient times, the management of domestic animals might have been less intense compared to today, providing more opportunities for interaction with wild cats. Thus, it is worthwhile to discuss whether admixture occurred in ancient times.

We initially hypothesized that ancient Chinese domestic cats could have admixed with Chinese mountain cats, as Chinese mountain cats are indigenous to China and their ancestry has been found in some modern individuals of Chinese domestic cats (Yu et al. 2021; Abdukadir et al. 2010; Ottoni et al. 2007; He et al. 2004). However, our results do not find significant evidence of ancient Chinese domestic cats admixing with any wild cats.

Admixtures appear less common in China compared to Europe, where hybridizations between domestic cats and European wild cats are relatively more intensive (Jamieson et al. 2021;

Germain et al. 2008; Pierpaoli et al. 2003). We believe that the absence of ancient admixtures can be attributed to the low probability of contact between domestic cats and Chinese mountain cats in history. Chinese mountain cats inhabit southwestern China and the eastern part of the Tibetan Plateau. Under the nomadic subsistence pattern of ancient times, residents of southwestern China may have lacked a mature, stable agricultural economy, resulting in no significant demand for rodent control. Domestic cats, which are highly associated with settled agriculture, were not extensively introduced to the area. Consequently, in ancient times, the habitats of domestic cats and Chinese mountain cats did not overlap, eliminating the possibility of interbreeding.

2.6 Conclusions

In conclusion, our comprehensive analysis of ancient and modern Chinese domestic cats sheds light on their origins, dispersal routes, and population continuity, while also addressing the issue of potential admixture with wild cat species. We have demonstrated that domestic cats were introduced to China around the Tang Dynasty, likely originating from the Levant in the Near East. The dispersal route possibly involved Central Asia and the Silk Road, although other routes, such as maritime connections, may also have played a role.

The population of domestic cats in China has remained stable and relatively homogeneous for over 1,200 years, with limited evidence of admixture with wild cat species. This continuity can be attributed to the geographical barriers between the Near East and East Asia, which restricted the influx of new genetic variants and resulted in a dominant lineage of domestic cats in China. The long-term stability of the Chinese domestic cat population can also be related to the minimal contact between domestic cats and Chinese mountain cats, whose habitats did not significantly overlap in ancient times.

This study highlights the importance of integrating archaeological, genetic, and historical data to better understand the origins and dispersal patterns of domestic animals. Our findings on the history of domestic cats in China provide a rich context for understanding the process of human-animal interactions and their impact on the distribution, adaptation, and cultural significance of animals.

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Figure S1 ML tree constructed using mitochondrial genomes of ancient domestic cat, modern domestic cats and wild cats, with leopard cat as outgroup.



Table S1 Information of modern Chinese cat samples provided by Peking University

Sample	Species	Location	MT Haplotype
CHEN0016	<i>F.catus</i>	Guangxi	D2c
CHEN0018	<i>F.catus</i>	Hunan	D2c
CHEN0024	<i>F.catus</i>	Guangxi	D2c
FCAP0055	<i>F.catus</i>	Guangdong	D2c
FCAP0066	<i>F.catus</i>	Guangdong	D2c
FCAP0069	<i>F.catus</i>	Guangdong	D2c
FCAP0072	<i>F.catus</i>	Guangdong	D2c
FCAP0099	<i>F.catus</i>	Hunan	D2c
FCAP0102	<i>F.catus</i>	Hunan	D2c
FCAP0130	<i>F.catus</i>	Liaoning	D2c
FCAP0132	<i>F.catus</i>	Liaoning	D2c
FCAP0137	<i>F.catus</i>	Beijing	D2c
FCAP0170	<i>F.catus</i>	Shandong	D2c
FCAP0172	<i>F.catus</i>	Shandong	D2c
FCAP0221	<i>F.catus</i>	Zhejiang	D2c
FCAP0235	<i>F.catus</i>	Liaoning	D2c
FCAP0239	<i>F.catus</i>	Liaoning	D2c
FCAP0241	<i>F.catus</i>	Liaoning	D2c
FCAP0263	<i>F.catus</i>	Shaanxi	D2c
FCAP0268	<i>F.catus</i>	Shaanxi	D2c
FCAP0309	<i>F.catus</i>	Gansu	D2c
FCAP0315	<i>F.catus</i>	Gansu	D2c
FCAP0333	<i>F.catus</i>	Gansu	D2c
FCAP0373	<i>F.catus</i>	Sichuan	D2c
FCAP0383	<i>F.catus</i>	Qinghai	D2c
FCAP0392	<i>F.catus</i>	Qinghai	D2c
FCAP0393	<i>F.catus</i>	Qinghai	D2c
FCAP0400	<i>F.catus</i>	Sichuan	D2c
FCAP0401	<i>F.catus</i>	Sichuan	D2c
FCAP0404	<i>F.catus</i>	Sichuan	D2c
FCAP0408	<i>F.catus</i>	Sichuan	D2c
FCAP0411	<i>F.catus</i>	Sichuan	D2c
FCAP0420	<i>F.catus</i>	Qinghai	D2c
FCAP0427	<i>F.catus</i>	Qinghai	D2c
FCAP0432	<i>F.catus</i>	Sichuan	D2c
FCAP0444	<i>F.catus</i>	Sichuan	D2c
FCAP0135	<i>F.catus</i>	Liaoning	D
FCAP0062	<i>F.catus</i>	Guangdong	D
FCAP0216	<i>F.catus</i>	Zhejiang	D
FCAP0228	<i>F.catus</i>	Jiangxi	D
CHEN0027	<i>F.catus</i>	Guangxi	D1
FCAP0203	<i>F.catus</i>	Jiangsu	D2a
FCAP0056	<i>F.catus</i>	Guangdong	D2a

FCAP0219	<i>F.catus</i>	Zhejiang	D2a
FCAP0230	<i>F.catus</i>	Jiangxi	D2b
FCAP0067	<i>F.catus</i>	Guangdong	D2b
FCAP0104	<i>F.catus</i>	Hunan	D2b
FCAP0022	<i>F.catus</i>	Guangdong	D2b
FCAP0375	<i>F.catus</i>	Sichuan	D2b
FCAP0103	<i>F.catus</i>	Hunan	D2b
FCAP0059	<i>F.catus</i>	Guangdong	D2b
FCAP0202	<i>F.catus</i>	Jiangsu	D2b
FCAP0313	<i>F.catus</i>	Gansu	D2b
FSIP0006	<i>F.s.ornata</i>	Xinjiang	—
FBIP0006	<i>F.s.ornata</i>	Qinghai	—
FBIP0007	<i>F.s.ornata</i>	Gansu	—
FBIP0026	<i>F.s.ornata</i>	Gansu	—
FBIP0002	<i>F.s.bieti</i>	Qinghai	—
FBIP0003	<i>F.s.bieti</i>	Qinghai	—
FBIP0004	<i>F.s.bieti</i>	Gansu	—
FBIP0008	<i>F.s.bieti</i>	Sichuan	—
FBIP0025	<i>F.s.bieti</i>	Gansu	—



Figure S2 ADMIXTURE analysis using 95 ancient and modern cat samples. K=2 to 6.

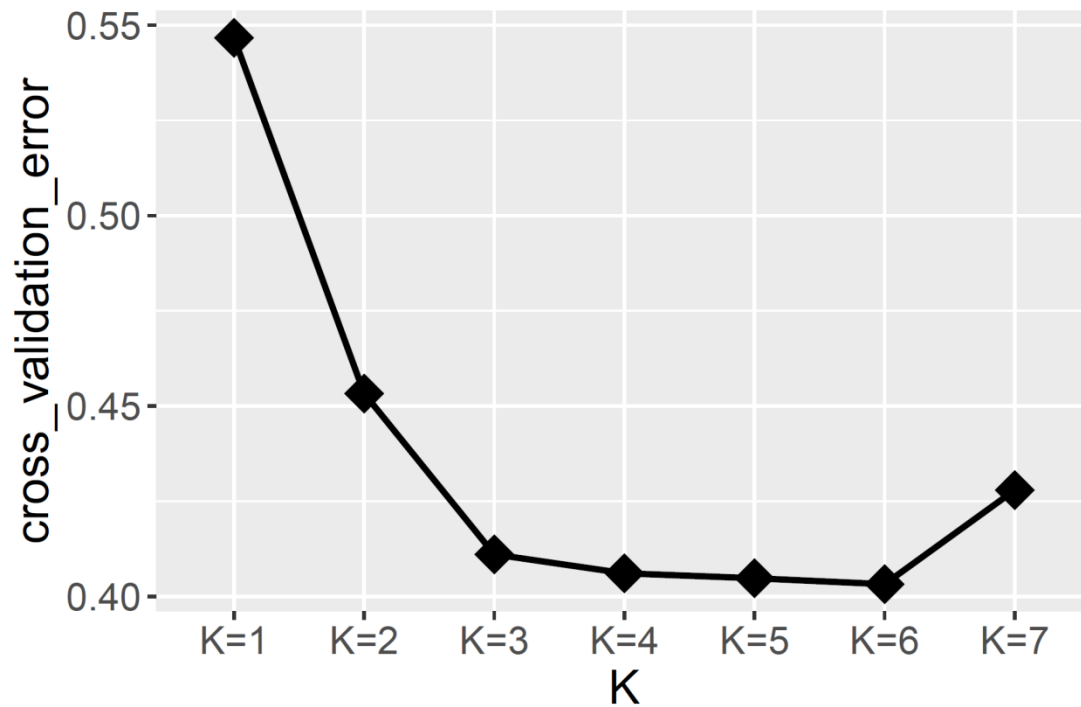


Figure S3 Cross Validation plot for ADMIXTURE analysis of cats.

Table S2 Analysis of cat samples.

Sample	Time	Location	Species	Source	PCA	ADMIXTURE	f3	f4
AH5	ancient	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
AJ276	ancient	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
AJ279	ancient	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
AJ324	ancient	Europe	?	Jamieson et al.2021	Yes	Yes	Yes	Yes
AJ419	ancient	Europe	?	Jamieson et al.2021	Yes	Yes	Yes	Yes
AJ437	ancient	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
AJ457	ancient	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
AJ499	ancient	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
AJ514	ancient	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
AJ76	ancient	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
AJ86	ancient	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
Dhzankent	ancient	Central Aisa	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
P136	ancient	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SA17	ancient	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FS1	ancient	China	<i>F.catus</i>	This paper	Yes	Yes	Yes	Yes
FS12	ancient	China	<i>F.catus</i>	This paper	Yes	Yes	Yes	Yes
FS2	ancient	China	<i>F.catus</i>	This paper	Yes	Yes	Yes	Yes
FS21	ancient	China	<i>F.catus</i>	This paper	Yes	Yes	Yes	Yes
SRR2062187	Modern	—	<i>F. margarita</i>	Jamieson et al.2021	No	Yes	Yes	Yes
SRR2062538	Modern	—	<i>F. margarita</i>	Jamieson et al.2021	No	Yes	Yes	Yes
FMA8	Modern	—	<i>F. margarita</i>	Jamieson et al.2021	No	Yes	Yes	Yes
SRR7621226	Modern	China	<i>F.s.bieti</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes

SRR7621227	Modern	China	<i>F.s.bieti</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR7621229	Modern	China	<i>F.s.bieti</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR7621235	Modern	China	<i>F.s.bieti</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FBI4	Modern	China	<i>F.s.bieti</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FLI25	Modern	—	<i>F.s.cafra</i>	Jamieson et al.2021	No	Yes	Yes	Yes
SRR7621238	Modern	Cental Asia	<i>F.s.ornata</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FLI3	Modern	Cental Asia	<i>F.s.ornata</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FLI4	Modern	Cental Asia	<i>F.s.ornata</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FSI204	Modern	Cental Asia	<i>F.s.ornata</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FSI194	Modern	Cental Asia	<i>F.s.ornata</i>	Jamieson et al.2021	No	Yes	Yes	Yes
FSI51	Modern	Near East(Levant)	<i>F.s.lybica</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FSI47	Modern	Near East(Levant)	<i>F.s.lybica</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FSI48	Modern	Near East(Levant)	<i>F.s.lybica</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FSI7	Modern	Near East(UAE)	<i>F.s.lybica</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FSI1	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FA661	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
ex19	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
ex38	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
ex9	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
ex21	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
ex40	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FSX360	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FSX392	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
FSX405	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ243	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ340	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ343	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ344	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ402	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ427	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ428	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ550	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ553	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ564	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ047	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ052	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ099	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ100	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ158	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ165	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ168	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ209	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ211	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ211	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ212	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes

WCQ213	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ214	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ216	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ218	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ224	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ227	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ230	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ231	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ234	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ236	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ246	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ248	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ249	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ252	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ255	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ515	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ613	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ903	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ904	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ915	Modern	Europe	<i>F.s.silvestris</i>	Jamieson et al.2021	No	Yes	Yes	Yes
WCQ1135	Modern	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ1136	Modern	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ1137	Modern	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ1138	Modern	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
WCQ443	Modern	Europe	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR5040107	Modern	Neareast	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR5040110	Modern	Neareast	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR5040111	Modern	Neareast	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR5040113	Modern	Neareast	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR5040114	Modern	Neareast	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR5040116	Modern	Neareast	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR5040117	Modern	Neareast	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR5040118	Modern	Neareast	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR5040120	Modern	Neareast	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR5040125	Modern	Neareast	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR5040126	Modern	East Aisa	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR7621212	Modern	China	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR7621222	Modern	China	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR7621225	Modern	China	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR7621252	Modern	China	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes
SRR7621254	Modern	China	<i>F.catus</i>	Jamieson et al.2021	Yes	Yes	Yes	Yes

Table S3 Results of outgroup f3 analysis of cats.

Source 1	Source 2	Out-group	f_3	std.err	Z	SNPs
FS1	catus_East	sand_cat	180.217857	11.322142	15.917	81007
FS1	FS21	sand_cat	179.386971	11.588262	15.48	50863
FS1	FS2	sand_cat	175.838276	11.48101	15.316	53082
FS1	FS12	sand_cat	172.016607	11.573873	14.862	58323
FS1	lybica	sand_cat	151.322506	9.438672	16.032	77866
FS1	Dhzankent	sand_cat	150.717017	55.144777	2.733	1878
FS1	catus_Central	sand_cat	150.108511	9.376213	16.01	81611
FS1	catus_West	sand_cat	139.112562	8.677047	16.032	81937
FS1	Ancient_West	sand_cat	122.010783	17.430569	7	8738
FS1	ornata	sand_cat	119.739959	7.444453	16.084	66125
FS1	silvestries	sand_cat	118.399198	7.359948	16.087	70271
FS1	bieti	sand_cat	112.936007	7.047862	16.024	60043
FS2	FS1	sand_cat	175.838276	11.48101	15.316	53082
FS2	catus_East	sand_cat	174.721107	11.255582	15.523	84297
FS2	FS21	sand_cat	171.422822	11.359815	15.09	53303
FS2	FS12	sand_cat	167.054076	11.598646	14.403	60457
FS2	Dhzankent	sand_cat	165.109074	58.683773	2.814	1966
FS2	lybica	sand_cat	146.892821	9.435001	15.569	81263
FS2	catus_Central	sand_cat	144.985004	9.324507	15.549	85180
FS2	catus_West	sand_cat	134.72156	8.629847	15.611	85434
FS2	ornata	sand_cat	115.6054	7.403732	15.614	69362
FS2	Ancient_West	sand_cat	114.059218	14.730644	7.743	9158
FS2	silvestries	sand_cat	113.908786	7.306579	15.59	73504
FS2	bieti	sand_cat	109.283011	7.022039	15.563	63007
FS12	Dhzankent	sand_cat	180.251636	77.922663	2.313	2125
FS12	catus_East	sand_cat	172.257181	11.165351	15.428	91443
FS12	FS1	sand_cat	172.016607	11.573873	14.862	58323
FS12	FS21	sand_cat	171.49798	11.460161	14.965	57531
FS12	FS2	sand_cat	167.054076	11.598646	14.403	60457
FS12	lybica	sand_cat	145.002262	9.403209	15.421	88355
FS12	catus_Central	sand_cat	143.110243	9.282915	15.417	92433
FS12	catus_West	sand_cat	131.447904	8.489932	15.483	93524
FS12	ornata	sand_cat	114.954985	7.393877	15.547	75907
FS12	Ancient_West	sand_cat	112.614972	15.776276	7.138	10099
FS12	silvestries	sand_cat	111.982976	7.232932	15.482	80847
FS12	bieti	sand_cat	108.959044	7.022332	15.516	69196
FS21	catus_East	sand_cat	179.484622	11.363918	15.794	80396
FS21	FS1	sand_cat	179.386971	11.588262	15.48	50863
FS21	FS12	sand_cat	171.49798	11.460161	14.965	57531
FS21	FS2	sand_cat	171.422822	11.359815	15.09	53303

FS21	Dhzankent	sand_cat	160.432691	63.095555	2.543	1855
FS21	lybica	sand_cat	150.354938	9.507685	15.814	77340
FS21	catus_Central	sand_cat	149.460376	9.408975	15.885	80918
FS21	catus_West	sand_cat	138.905252	8.728777	15.913	81089
FS21	Ancient_West	sand_cat	124.682825	17.452172	7.144	8715
FS21	ornata	sand_cat	119.255034	7.460807	15.984	65445
FS21	silvestries	sand_cat	117.792872	7.376722	15.968	69232
FS21	bieti	sand_cat	112.533826	7.053864	15.954	59429
FS21	FS21	sand_cat	0	-1	0	-1
Dhzankent	FS1	sand_cat	150.717017	55.144777	2.733	1878
Dhzankent	FS2	sand_cat	165.109074	58.683773	2.814	1966
Dhzankent	FS12	sand_catsand_cat	180.251636	77.922663	2.313	2125
Dhzankent	FS21	sand_cat	160.432691	63.095555	2.543	1855
Dhzankent	Dhzankent	sand_cat	0	-1	0	-1
Dhzankent	Ancient_West	sand_cat	116.979767	23.262014	5.029	5356
Dhzankent	catus_East	sand_cat	120.932735	23.63015	5.118	7215
Dhzankent	catus_Central	sand_cat	115.243198	22.548891	5.111	7625
Dhzankent	catus_West	sand_cat	109.555393	21.377054	5.125	8111
Dhzankent	lybica	sand_cat	113.876662	22.249337	5.118	6969
Dhzankent	silvestries	sand_cat	97.587149	19.052361	5.122	6541
Dhzankent	ornata	sand_cat	96.563797	18.809416	5.134	5567
Dhzankent	bieti	sand_cat	91.958451	17.888265	5.141	4915
catus_East	FS1	sand_cat	180.217857	11.322142	15.917	81007
catus_East	FS2	sand_cat	174.721107	11.255582	15.523	84297
catus_East	FS12	sand_cat	172.257181	11.165351	15.428	91443
catus_East	FS21	sand_cat	179.484622	11.363918	15.794	80396
catus_East	Dhzankent	sand_cat	120.932735	23.63015	5.118	7215
catus_East	Ancient_West	sand_cat	85.16037	6.992342	12.179	30964
catus_East	catus_East	sand_cat	0	-1	0	-1
catus_East	catus_Central	sand_cat	100.158917	2.801907	35.747	344552
catus_East	catus_West	sand_cat	98.470755	2.751153	35.793	350175
catus_East	lybica	sand_cat	99.70676	2.790807	35.727	342163
catus_East	silvestries	sand_cat	91.460678	2.55638	35.777	350244
catus_East	ornata	sand_cat	89.930103	2.514533	35.764	338484
catus_East	bieti	sand_cat	87.520902	2.442639	35.83	334647

Table S4 Results of f4 analysis of cats.

O	C	B	A	D	Zscore	BABA	ABBA	SNPs
sand_cat	lybica	Dhzankent	FS1	0.003013	0.734	212	200	3796
sand_cat	lybica	Ancient_West	FS1	0.003676	1.734	933	881	14083
sand_cat	lybica	catus_East	FS1	0.002478	3.119	6566	6338	91803
sand_cat	lybica	catus_Central	FS1	0.003511	3.813	7596	7274	91803
sand_cat	lybica	catus_West	FS1	0.012225	11.399	8399	7276	91803
sand_cat	lybica	Dhzankent	FS2	0.009122	2.288	224	188	3967
sand_cat	lybica	Ancient_West	FS2	0.004266	2.039	956	893	14776
sand_cat	lybica	catus_East	FS2	0.002518	3.35	6881	6640	95851
sand_cat	lybica	catus_Central	FS2	0.003331	3.649	7973	7654	95851
sand_cat	lybica	catus_West	FS2	0.01215	12.056	8793	7629	95851
sand_cat	lybica	Dhzankent	FS12	0.003034	0.851	222	209	4367
sand_cat	lybica	Ancient_West	FS12	0.002854	1.436	1032	985	16257
sand_cat	lybica	catus_East	FS12	0.000346	0.424	7434	7398	103613
sand_cat	lybica	catus_Central	FS12	0.001152	1.211	8617	8498	103613
sand_cat	lybica	catus_West	FS12	0.009908	8.888	9590	8563	103613
sand_cat	lybica	Dhzankent	FS21	0.005496	1.441	201	181	3730
sand_cat	lybica	Ancient_West	FS21	0.004433	1.971	928	866	13978
sand_cat	lybica	catus_East	FS21	0.001583	1.998	6463	6319	91356
sand_cat	lybica	catus_Central	FS21	0.002254	2.459	7486	7280	91356
sand_cat	lybica	catus_West	FS21	0.01114	10.838	8242	7224	91356
sand_cat	silvestries	Dhzankent	FS1	-0.001524	-0.389	166	172	3796
sand_cat	silvestries	Ancient_West	FS1	-0.019096	-9.084	608	877	14083
sand_cat	silvestries	catus_East	FS1	-0.002124	-2.925	5099	5294	91803
sand_cat	silvestries	catus_Central	FS1	-0.010663	-12.414	5527	6506	91803
sand_cat	silvestries	catus_West	FS1	-0.029418	-26.872	5179	7880	91803
sand_cat	silvestries	lybica	FS1	-0.010171	-11.269	5607	6541	91803
sand_cat	silvestries	Dhzankent	FS2	0.006861	1.816	183	156	3967
sand_cat	silvestries	Ancient_West	FS2	-0.018119	-8.02	629	896	14776
sand_cat	silvestries	catus_East	FS2	-0.002998	-4.514	5283	5570	95851
sand_cat	silvestries	catus_Central	FS2	-0.011798	-13.711	5741	6872	95851
sand_cat	silvestries	catus_West	FS2	-0.030657	-27.56	5365	8304	95851
sand_cat	silvestries	lybica	FS2	-0.011337	-13.368	5790	6876	95851
sand_cat	silvestries	Dhzankent	FS12	-0.000275	-0.079	176	177	4367
sand_cat	silvestries	Ancient_West	FS12	-0.018434	-8.896	681	981	16257
sand_cat	silvestries	catus_East	FS12	-0.005023	-7.21	5737	6257	103613
sand_cat	silvestries	catus_Central	FS12	-0.013977	-16.642	6212	7660	103613
sand_cat	silvestries	catus_West	FS12	-0.033219	-29.27	5866	9308	103613
sand_cat	silvestries	lybica	FS12	-0.013242	-15.271	6264	7636	103613
sand_cat	silvestries	Dhzankent	FS21	0.000822	0.21	158	155	3730
sand_cat	silvestries	Ancient_West	FS21	-0.019452	-8.598	603	875	13978

sand_cat	silvestries	catus_East	FS21	-0.002508	-3.659	5022	5252	91356
sand_cat	silvestries	catus_Central	FS21	-0.011309	-13.366	5443	6476	91356
sand_cat	silvestries	catus_West	FS21	-0.029775	-27.231	5100	7821	91356
sand_cat	silvestries	lybica	FS21	-0.010591	-12.398	5532	6500	91356
sand_cat	ornata	Dhzankent	FS1	0.001663	0.417	170	164	3796
sand_cat	ornata	Ancient_West	FS1	-0.000061	-0.029	732	733	14083
sand_cat	ornata	catus_East	FS1	0.001762	2.171	5305	5143	91803
sand_cat	ornata	catus_Central	FS1	-0.006667	-6.781	5706	6318	91803
sand_cat	ornata	catus_West	FS1	-0.003241	-2.875	6203	6501	91803
sand_cat	ornata	lybica	FS1	-0.005251	-4.877	5838	6320	91803
sand_cat	ornata	Dhzankent	FS2	0.006649	1.73	182	156	3967
sand_cat	ornata	Ancient_West	FS2	-0.001553	-0.743	735	758	14776
sand_cat	ornata	catus_East	FS2	0.000982	1.315	5485	5391	95851
sand_cat	ornata	catus_Central	FS2	-0.007715	-8.39	5952	6692	95851
sand_cat	ornata	catus_West	FS2	-0.004333	-4.39	6428	6843	95851
sand_cat	ornata	lybica	FS2	-0.006555	-7.014	6022	6650	95851
sand_cat	ornata	Dhzankent	FS12	0.002548	0.678	182	171	4367
sand_cat	ornata	Ancient_West	FS12	-0.00215	-1.069	795	830	16257
sand_cat	ornata	catus_East	FS12	0.000507	0.58	6065	6013	103613
sand_cat	ornata	catus_Central	FS12	-0.008068	-8.275	6517	7353	103613
sand_cat	ornata	catus_West	FS12	-0.004703	-4.285	7141	7628	103613
sand_cat	ornata	lybica	FS12	-0.006619	-6.313	6614	7299	103613
sand_cat	ornata	Dhzankent	FS21	0.004172	1.036	168	153	3730
sand_cat	ornata	Ancient_West	FS21	-0.000966	-0.44	725	738	13978
sand_cat	ornata	catus_East	FS21	0.000976	1.348	5243	5154	91356
sand_cat	ornata	catus_Central	FS21	-0.007871	-8.558	5633	6352	91356
sand_cat	ornata	catus_West	FS21	-0.004127	-4.075	6123	6500	91356
sand_cat	ornata	lybica	FS21	-0.006288	-6.395	5770	6345	91356
sand_cat	bieti	Dhzankent	FS1	0.002792	0.71	161	150	3796
sand_cat	bieti	Ancient_West	FS1	-0.001363	-0.643	668	687	14083
sand_cat	bieti	catus_East	FS1	0.001245	1.636	4901	4787	91803
sand_cat	bieti	catus_Central	FS1	-0.004227	-4.745	5389	5777	91803
sand_cat	bieti	catus_West	FS1	-0.005611	-5.536	5651	6166	91803
sand_cat	bieti	lybica	FS1	-0.0037	-3.901	5463	5803	91803
sand_cat	bieti	Dhzankent	FS2	0.010978	2.852	179	136	3967
sand_cat	bieti	Ancient_West	FS2	-0.002389	-1.124	666	701	14776
sand_cat	bieti	catus_East	FS2	0.000737	1.032	5096	5025	95851
sand_cat	bieti	catus_Central	FS2	-0.004717	-5.467	5637	6089	95851
sand_cat	bieti	catus_West	FS2	-0.006277	-6.65	5884	6486	95851
sand_cat	bieti	lybica	FS2	-0.004587	-4.967	5677	6117	95851
sand_cat	bieti	Dhzankent	FS12	0.007259	1.925	187	155	4367
sand_cat	bieti	Ancient_West	FS12	-0.002717	-1.316	755	799	16257

sand_cat	bieti	catus_East	FS12	0.000648	0.737	5692	5625	103613
sand_cat	bieti	catus_Central	FS12	-0.004794	-4.96	6240	6737	103613
sand_cat	bieti	catus_West	FS12	-0.006542	-5.991	6582	7260	103613
sand_cat	bieti	lybica	FS12	-0.004167	-4.071	6319	6751	103613
sand_cat	bieti	Dhzankent	FS21	0.005241	1.249	156	136	3730
sand_cat	bieti	Ancient_West	FS21	-0.002793	-1.263	658	697	13978
sand_cat	bieti	catus_East	FS21	0.000583	0.833	4843	4790	91356
sand_cat	bieti	catus_Central	FS21	-0.005081	-5.987	5320	5784	91356
sand_cat	bieti	catus_West	FS21	-0.006742	-7.382	5558	6174	91356
sand_cat	bieti	lybica	FS21	-0.004622	-5.343	5421	5843	91356
sand_cat	bieti	Dhzankent	FS1	0.002792	0.71	161	150	3796
sand_cat	bieti	Dhzankent	FS2	0.010978	2.852	179	136	3967
sand_cat	bieti	Dhzankent	FS12	0.007259	1.925	187	155	4367
sand_cat	bieti	Dhzankent	FS21	0.005241	1.249	156	136	3730

Table S5 Sequencing strategies and analysis used ancient Chinese cat samples.

Sample	Site	Period	Date	C14	Nuc_analysis	Mito_analysis	Strategy
FS1	Xitucheng City Site	Jin	800BP	No	Yes	Yes	nuc_deep
FS2	Xitucheng City Site	Jin	800BP	No	Yes	Yes	nuc_deep
FS3	Xitucheng City Site	Jin	800BP	No	No	Yes	mito_deep
FS4	Xitucheng City Site	Jin	800BP	No	No	Yes	mito_deep
FS5	Xitucheng City Site	Jin	800BP	No	No	Yes	mito_deep
FS6	Xinxiang Song Tomb	Song	900BP	No	No	Yes	mito_capture
FS7	Huoshiliang	Neolithic	4500BP	No	No	Yes	mito_capture
FS8	Wuzhuangguoliang	Neolithic	4000BP	No	No	Yes	mito_capture
FS10	Laoniupo	Shang	3200BP	No	No	Yes	mito_capture
FS12	Tongwan City Site	Song	1000BP	Yes	Yes	Yes	nuc_deep
FS13	Yangling	Han	2150BP	Yes	No	Yes	mito_capture
FS14	Xinjie	Neolithic	5000BP	No	No	Yes	mito_capture
FS15	Quanhucun	Neolithic	5500BP	No	No	Yes	mito_capture
FS16	ZhengzhouShangcheng	Ming	500BP	Yes	No	Yes	mito_capture
FS17	Yunzhongjun	Qing	150BP	Yes	No	Yes	mito_capture
FS18	Zhengzhou Song Tomb	Song	900BP	No	No	Yes	mito_capture
FS19	Huangdiguli(Han)	Han	1750BP	Yes	No	Yes	mito_capture
FS20	Huangdiguli(Song/Jin)	Song/Jin	900-800BP	No	No	Yes	mito_capture
FS21	Dingxinlu	Song/Jin	900-800BP	No	Yes	Yes	mito_capture
FS22	Dingxinlu	Song/Jin	900-800BP	No	No	Yes	mito_capture
FS23	Dingxinlu	Song/Jin	900-800BP	No	No	Yes	mito_capture
FS24	Chang'an City Site	Han	2000BP	Yes	No	Yes	nuc_deep

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He YU

Signature(s):



3 Early commensalism between leopard cat (*Prionailurus bengalensis*) and human in ancient China

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Abstract

Leopard cats (*Prionailurus bengalensis*) are a widespread small-sized felid species found across Asia throughout the Holocene. Previous studies have categorized them into two broad groups represented by four populations residing in Far East Russia, the Indochina Peninsula, Malay, and Sunda. However, the genetic background of leopard cats in Central China, the heartland of East Asia, remains poorly understood due to a lack of research. Notably, leopard cats in this region may have established commensal relationships with humans as early as the late Neolithic period (5,500 BP), with some researchers proposing that they were even domesticated in ancient China. Nevertheless, the persistence of this commensal interaction and the potential domestication of leopard cats in ancient China remain unclear. To address these knowledge gaps, we conducted ancient genomic analysis on a total of seven leopard cats from the Middle Reach of the Yellow River Basin. Our results demonstrate that leopard cats in this

region and Far East Russia belong to the same population, although the former was also genetically influenced by the Indochina population. We found evidence to suggest that the commensal relationship between leopard cats and humans ended with the fall of the Han Dynasty and the subsequent disarray of human societies over 500 years, as determined by chronological, archaeological, and literature analysis. After the introduction of domestic cats, the niches previously occupied by leopard cats in human-associated environments were filled by domestic cats. While leopard cats have affinities with humans, there is no evidence to support the hypothesis that they were ever domesticated.

Key Words: ancient genomics; Chinese leopard cats; commensal relationship

3.1 Introduction

Leopard cat (*Prionailurus bengalensis*) is one of the most common and significant small-sized felids in Asia during the Holocene (Kerr, 1792). Thanks to its high adaptability, its natural distribution covers a vast range extending from the islands of Southeast Asia to Far East Russia. These cats inhabit various environments dominated by forests, including tropical rainforests, monsoon forests, coniferous forests, and shrub forests (Ross et al, 2015). As the species is often a major predator in its native ecosystem, research on leopard cats has primarily focused on their diets, which can largely represent the biodiversity of the local environment (Bateman and Fleming, 2012; Choi et al., 2012; Shehzad et al., 2012).

In contrast, the genetics of the leopard cat has received limited attention. A few genetic studies

have classified Asian leopard cats into two groups that diverged 1.5 million years ago and were separated by islands and the mainland. The populations in Far Eastern Russia and Indochina represent the mainland group, while the island group is represented by the Malay and Sunda populations. As the foundation of the Malay population resulted from admixtures between the Indochina and Sunda leopard cats, the Malay Peninsula is considered an important hybridization zone in the species' evolutionary history (Miao, 2017; Luo et al, 2014; Koh et al, 2014; Mukherjee et al, 2010; Tamada et al, 2008) (Figure 9). While previous efforts have clarified the basic biogeography of leopard cats (Miao, 2017; Luo et al, 2014; Koh et al, 2014; Mukherjee et al, 2010; Tamada et al, 2008), some details still need refinement, such as the genetic background and population attribution of leopard cats inhabiting the Middle Reach of the Yellow River Basin, the heartland of China, which remain less understood.

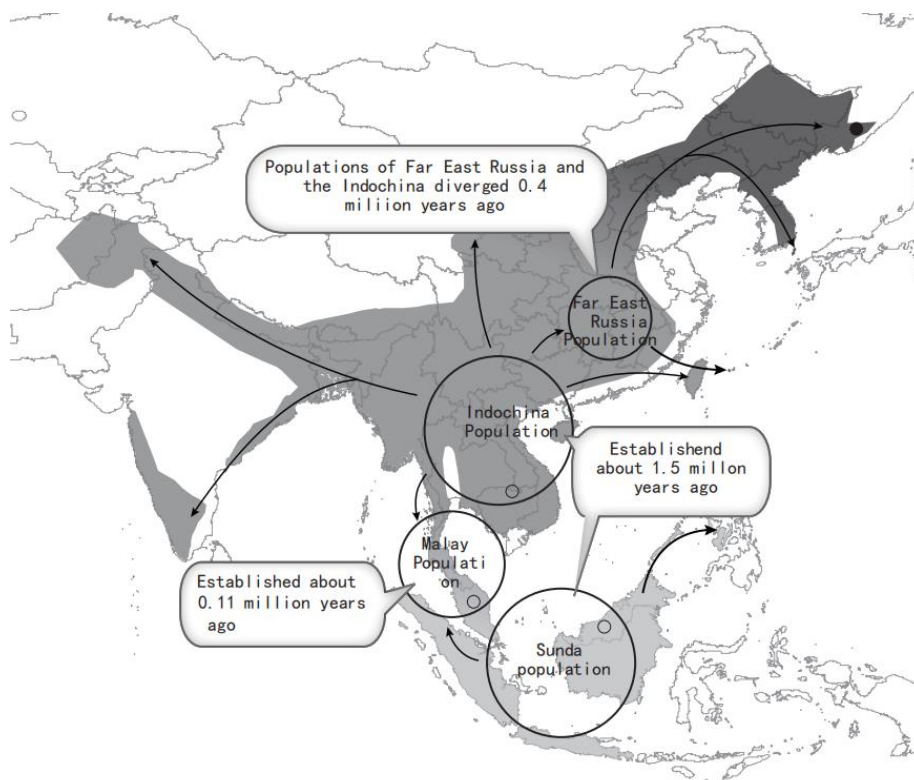


Figure 9 Schematic diagram of leopard cat evolutionary history and phylogeography.

The leopard cat in the Middle Reach of the Yellow River Basin may have played a crucial role in ancient human society. Based on previous research, they were likely the first small felid to establish a commensal relationship with humans in ancient China (Hu et al., 2014a; Vigne et al., 2016). Commensalism is a pattern of symbiosis in which one species benefits unidirectionally from another without harming the host (Lenski Gerhard, 1976). A typical example of commensal interaction among animals is the relationship between birds and herbivorous mammals in Africa, where birds gain food by eating the parasites or insects found on the skin of herbivorous mammals (Mikula et al., 2018).

Commensal relationships have also existed extensively between humans and animals. Such cases are observed in hares (Sheng et al., 2020), red foxes (Scholz et al., 2020), and rats (Aplin et al., 2011; Puckett et al., 2020), which benefit from this relationship by scavenging or stealing food associated with humans. The concept of commensalism has been introduced to zooarchaeology as a pathway leading to domestication by Zeder (2012a), wherein the stage prior to domestication, wild animals were initially attracted to human settlements by food resources or possibly shelter that could protect them from predators. Rats, dogs, pigs, and cats were domesticated along this pathway (Zeder, 2012a; Zeder, 2012b).

Direct evidence of the commensal relationship between leopard cats and humans was provided by the Quanhucun Site (Hu et al., 2014a). This major Neolithic settlement is located in the middle Yellow River Basin reach and primarily relied on agriculture for subsistence (PKU&CASS, 2003). At least three individuals of small felids were discovered at the site.

Stable isotopic research revealed that a certain proportion of agricultural products, such as millet, were included in their diets, causing their ^{13}C values to surpass those of local wild animals while approaching those of domestic animals. Since cats are carnivores and should not directly consume grains, the C_4 contributions presumably originated from rodents that fed on the grains produced by humans (Hu et al., 2014a). Later research using geometric morphometric and ancient genetic approaches identified these individuals as leopard cats (Vigne et al., 2016; Zhu et al., 2022), rather than domestic cats, which were derived from *Felis silvestris lybica* of the Near East (Driscoll et al., 2007).

In some radical claims, leopard cats were even thought to have been domesticated in ancient China (Wang, 2010). However, the commensal relationship between leopard cats and humans does not appear to have persisted over time, and all modern domestic cats are descended from African wild cats (*Felis silvestris lybica*) (Driscoll et al., 2007), which are genetically distinct from leopard cats (Johnson et al, 2006; G. Li et al, 2016). Combined, these aspects raise questions about exactly when and why the commensal connection that began during the Neolithic period in China ended, and whether leopard cats were domesticated in ancient China.

To address these questions and further our understanding of the evolution of leopard cats, this project collected and analysed seven ancient samples from the Middle Reach of the Yellow River Basin, spanning over 3,500 years. We analysed these samples in a broader context, using 23 modern samples of leopard cats from across Asia, from Far East Russia southwards to Island Sunda. By applying genomic methods to these samples, along with radiocarbon dating,

archaeological, and historical approaches, we aimed to investigate the genetic background of leopard cats in the Middle Reach of the Yellow River Basin and gain insights into the relationship between leopard cats and humans in ancient China.

3.2 Materials

In total, we collected 7 ancient leopard cat samples from the Middle Reach of the Yellow River Basin (MYR), spanning over 3,500 years (Figure 10; Table 5). In addition to the ancient samples, 23 modern mitochondrial genomes and raw nuclear DNA shotgun sequencing data from 18 modern samples were provided by Luo from Peking University. The mitochondrial genomes correspond to 20 modern leopard cats from Far East Russia (FER, N=4), Indochina (IND, N=6), Malay (MA=6), Sunda (SU=4), 2 fishing cats, and one flat-headed cat (*Prionailurus planiceps*). The raw NGS data correspond to 15 modern leopard cats collected in Far East Russia (FER, N=2), Indochina (IND, N=6), Malay (MA, N=4), Sunda (SU, N=3), a fishing cat, and 2 domestic cats (Miao, 2017) (Figure 10; Table 5).

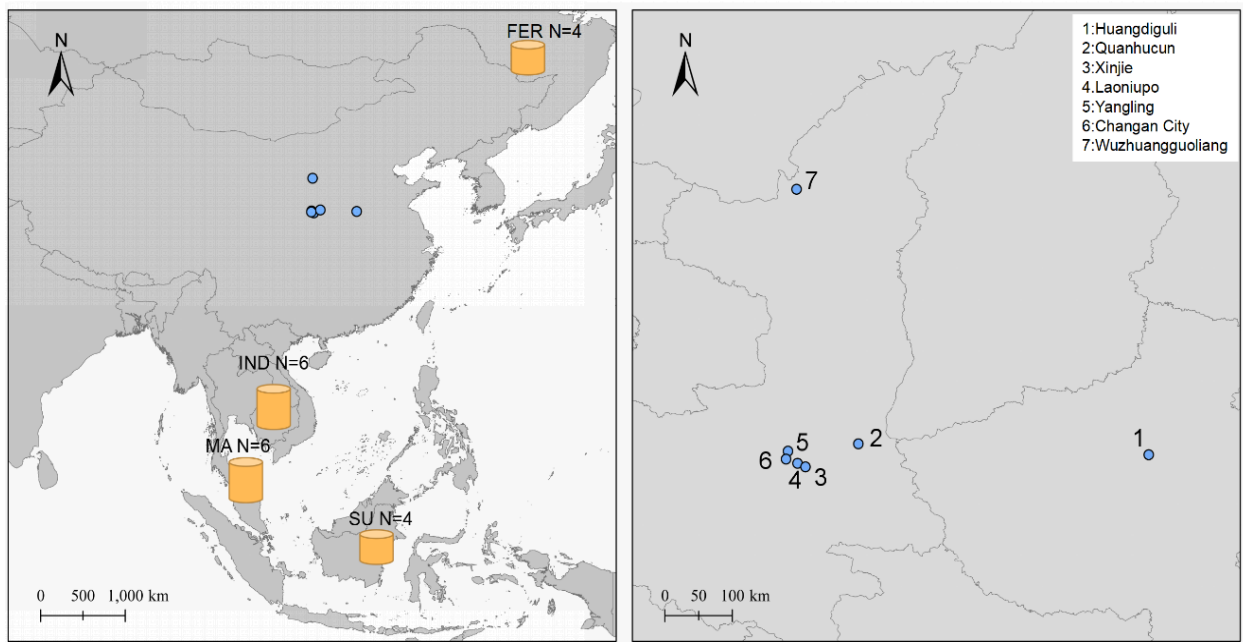


Figure 10 Sampling locations of the leopard cats used in this research. All sampling sites of ancient leopard cats are labeled in blue points. Locations of modern samples are labeled in orange columns. The height of the columns are associated with sampling quantity: Far East Russia (FER, N=4), Indochina (IND, N=6), Malay (MA=6), Sunda (SU=4).

Table 5 Information of ancient leopard cat samples in this research (MYR=the middle reach of the Yellow River Basin)

Sample	Site	Context	Location	Period	Date
FS8	Wuzhuangguoliang	waste pit	MYR	Longshan	4,000BP
FS10	Laoniupo	waste pit	MYR	Shang	3,200BP
FS13	Yanling	accessory burial pit	MYR	Han	2,150BP
FS14	Xinjie	waste pit	MYR	Yangshao	5,000BP
FS15	Quanhucun	waste pit	MYR	Yangshao	5,500BP
FS19	Huangdiguli	tomb lane	MYR	Han	1,800BP
FS24	Changan City	corner of the city wall	MYR	Han	2,000BP

3.3 Methods

3.3.1 Ancient DNA Laboratory Methods

Ancient DNA experiment was conducted in a dedicated ancient genetic laboratory at School of Life Sciences Peking University. Protective clothing (3M) was used to reduce the risk of contamination.

For the methods of sample preparation, Extraction, Library building, qPCR, Library amplification, please see *Methods Chapter 2*.

3.3.1.1 Sample screening

All samples underwent screening sequencing used the NovaSeq 6000 instrument at Novogene, China. We obtained approximately 3Gb of raw data for each sample. Following the screening sequencing, we estimated endogenous DNA content by calculating the ratio of nucleotides that mapped to the leopard cat reference genome (Genome assembly Fcat_Pben_1.1_paternal_pri GCF_016509475.1) or the number of reads mapped to the mitochondrial genome (NC_001700.1). We then determined further sequencing strategies based on these results.

3.3.1.2 Whole-genome deep sequencing

For samples with over 10% endogenous DNA as revealed by screening results, we performed deep sequencing. In total, 3 samples were chosen (FS8, FS15, FS19). These samples correspond to the Late Neolithic Age (5,500 BP), the last phase of the Chinese Neolithic Period

(4,000 BP), and the Late Eastern Han Dynasty (1,800 BP). The required amount of raw sequencing data for each sample was determined based on the endogenous DNA ratio. All samples were sequenced using the NovaSeq 6000 at Novogene, Beijing.

3.3.1.3 Ancient mitochondrial DNA capture sequencing

We performed in-solution targeted capture of the cat mitochondrial genome on 4 samples, following the myBaits (Arbor Biosciences) Hybridization Capture for Targeted Next-Generation Sequencing protocol. We used a hybridization temperature of 55°C and a hybridization time of 48 hours.

The *Felis catus* mitochondrial reference genome (NC_001700.1) was used to synthesize the baits for capture. Due to the degraded nature of ancient DNA, the amount of endogenous DNA per sample is significantly lower than what would be expected in modern samples. This means that the number of probes in a single capture reaction could be significantly overwhelmed for each sample. To achieve cost-effectiveness, we pooled the four samples equimolarly and processed them in a single reaction. We then amplified and sequenced the capture products on the Illumina NovaSeq 6000 instrument at Novogene, China.

3.3.2 Bioinformatics methods

3.3.2.1 Raw Data filtering

See Methods Chapter 2.

3.3.2.2 Mitochondrial DNA analysis

3.3.2.2.1 Mapping and consensus sequence

The cleaned data was then mapped to the leopard cat mitochondrial reference genome (NC_028301.1) using the aln function in BWA v0.1.17 (Li, 2013) with the settings -n 0.01 -o 2 -l 1024. SAMtools v.1.14 (Danecek et al., 2021) were used to sort the bam files and filter out PCR duplicates, unmapped reads, or reads with a mapping quality of less than 30. HTSbox was used to call consensus mitochondrial sequences with the default settings, which were then aligned to each other using MUSCLE v.5 (Edgar, 2021).

3.3.2.2.2 Phylogeny

A Maximum likelihood phylogenetic tree was constructed using IQtree v.2 (Minh et al., 2020; Kalyaanamoorthy et al., 2017 Nguyen et al., 2015) The tree included 7 ancient samples and 20 modern leopard cats, comprising 4 individuals from Far East Russia, 6 individuals from Indochina, 4 individuals from Sunda, and 6 from Malay. In addition to leopard cats, 2 fishing cats and a flat-headed cat were used for comparison. A tiger (NC_010642.1) was used as outgroup. The tree construction followed the TN93+I+G model.

3.3.2.3 Nuclear Genome Analysis

3.3.2.3.1 Mapping

The cleaned data was then mapped to the domestic cat whole nuclear reference genome (Genome assembly Fcat_Pben_1.1_paternal_pri GCF_016509475.1) using the "aln" function in BWA v.0.1.17 (Li et al. 2013) with the settings -n 0.01 -o 2 -l 1024. SAMtools v.1.14 (Danecek et al. 2021) were then used to sort the bam files and filter out PCR duplicates, unmapped reads, or reads with a mapping quality less than 30.

3.3.2.3.2 Call SNPs

The genomic data of the three ancient leopard cats, along with the samples provided by PKU with raw NGS data, were used to generate single nucleotide polymorphisms (SNPs). The SNPs were identified and filtered based on quality using GATK v.4.2 (McKenna et al., 2010), BCFtools v.1.14 (Danecek et al. 2021), and VCFtools v.0.1.16 (Danecek et al., 2011). All transitions were deleted to avoid the influence of deamination (Dabney et al., 2013; Hofreiter et al., 2001). BEDtools v.2.30.0 (Quinlan and Hall, 2010) was used to exclude all coding regions and retain only the neutral regions. PLINK v.1.9 (Purcell et al., 2007) was used to filter out all the SNPs located in the linkage disequilibrium region and the sex chromosomes. To prevent the impact of deamination in ancient DNA, all transitions were deleted. Through all these steps, a total of 20,436,894 SNPs were called and used for downstream analysis.

3.3.2.3.3 Nuclear phylogeny

A ML (maximum likelihood) phylogenetic tree was constructed based on the 20 million SNPs using IQ-TREE v.2 (Minh et al., 2020; Kalyaanamoorthy et al., 2017; Nguyen et al., 2015), following the TN93+I+G model. The bootstrap was set to 1,000 replicates. A total of 18 samples, including 2 Chinese domestic cats and 15 modern leopard cats from Far East Russia (FER, N=2), Indochina (IND, N=6), Malay (MA, N=4), Sunda (SU, N=3), and a fishing cat were included in the analysis. Domestic cat was used as the outgroup.

3.3.2.3.4 Population analysis

We analysed the nucleotide diversity (π) and inbreeding coefficient (F) with VCFtools using the 20 million SNP panel generated during SNP calling. The π was calculated using the command `--window-pi-step` with a sliding window size of 10K. The F was calculated using the command `-het` (Danecek et al., 2011).

Principal component analysis was performed using EIGENSTRAT SmartPCA (Patterson et al., 2006; Price et al., 2006) with a total of 15 samples, including 3 ancient Chinese leopard cats and 15 modern leopard cats from Far East Russia (N=2), Indochina (N=6), Malay (N=4), and Sunda (N=3), along with a fishing cat. Due to the large amount of missing data in ancient samples that could impact PCA results, the PCA was first constructed using high-quality modern data. The ancient leopard cats were then projected onto it by applying the "LsqProject: Yes" function.

ADMIXTURE analysis was performed using ADMIXTURE 1.3.0 (Kenneth, 2011) on over 20 million SNPs derived from a total of 16 samples, which included 3 ancient Chinese leopard cats, 12 modern leopard cats from Far East Russia (N=2), Indochina (N=6), Malay (N=4), Sunda (N=3), and a fishing cat. The population number K was set to range from 2 to 4.

The out-group f3-statistics were informed by the qp3Pop method of EIGENSTRAT AdmixTools v.7.0 (Patterson et al., 2012). Over 20 million SNPs from a total of 18 samples, including 3 ancient Chinese leopard cats, 15 modern leopard cats from FER (N=2), IND (N=6), MA (N=4), and Sunda (N=3), a fishing cat, and two domestic cats, were used. We first conducted the analysis based on individuals. Then, the same analysis was repeated while grouping the individuals according to their species or geographical locations. The ancient leopard cats from the middle reach of the Yellow River Basin (MYR) were treated as an independent population. In the analysis, the domestic cat was used as the outgroup, and the shared drifts of different pairs of individuals and groups were tested.

F4-statistics analysis was conducted using qpDstat method of EIGENSTRAT AdmixTools v.7.0 (Patterson et al. 2012). The same dataset as the outgroup f3-statistics was used. The population combinations were set as (F.catus, P.bengal_X, FER, ancient_MYR). F.catus is used as outgroup. Leopard cats from Indochina, Malay and Sunda are then individually placed in P.bengle_X. Leopard cats from Far East Russia are taken as a population and placed in FER. MYR represents the ancient leopard cats, and they are tested individually in MYR. The target of this analysis is to observe the signals of introgression between the ancient MYR ancient and

IND leopard cats relative to the Far East Russia.

3.3.3 Radiocarbon dating

See Methods Chapter 2.

3.4 Results

3.4.1 Radiocarbon dating results.

Three samples, FS13, FS19, and FS24, were sent to Beta Analytic or the Laboratory of Chronology at Peking University for direct radiocarbon dating. The dating of these samples was crucial, as their association with the contexts in which they were found was uncertain, and small cats could easily stray or fall into human facilities, leaving their carcasses behind. Furthermore, these samples were believed to be from the Han Dynasty, potentially representing the last leopard cats that enjoyed a commensal relationship with humans. Obtaining precise chronological information was essential for determining the time period when leopard cats coexisted with humans. The remaining four samples were either previously dated in published research (Hu et al, 2014) or had a high level of confidence regarding their association with the contexts from which they were excavated (Table 6).

Table 6 Radiocarbon dating results of sample FS13, FS19, and FS24.

Sample	Sites	Context date	Uncalibrated date (BP)	SD	Date cal. BCE/CE (95.4%, overall range IntCal 20)
FS13	Changan City	Han	2095	20	168BC (95.4%) 48BC
FS19	Huangdiguli	Han	1760	30	234 - 380 Cal AD(1716 - 1570 Cal BP)
FS24	Yangling	Han	1995	20	44BC (95.4%) 76AD

The radiocarbon dating results align with expectations based on the archaeological contexts. FS13 and FS24 fall within the range of the Western Han Dynasty, while FS19 is from the end of the Eastern Han Dynasty, consistent with predictions based on the characteristics of the tomb it was buried in. Combined with the dates of the other four samples, we have demonstrated that the commensal relationship between leopard cats could have begun as early as the late Chinese Neolithic Age, around 5,500 years ago, and persisted until after the Eastern Han Dynasty (Table 6).

3.4.2 Genetic analysis

3.4.2.1 Genetic Data Generation

Mitochondrial genomes were effectively retrieved from all seven samples through targeted capture, yielding a final coverage that varied from 12-91X. In order to obtain whole nuclear genome data, three ancient samples – FS8 (4,000 BP), FS15 (5,500 BP), and FS19 (1,750 BP) – underwent deep sequencing. These three whole genomes exhibited a final depth of 3-6X. Following the combined calling of SNPs with modern samples, approximately 20 million SNPs were preserved for further analysis, as outlined in Table 7.

Table 7 Information of nuclear genomes used in this study.

Sample ID	Species	Population	MEAN_DEPTH	SNPs
FCAP0103	<i>Felis catus</i>	Chinese domestic cat	14.1563	19785405
FCAP0137	<i>Felis catus</i>	Chinese domestic cat	14.1563	19785405
FS15	<i>P.bengalensis</i>	Ancient	3.32325	14322059
FS19	<i>P.bengalensis</i>	Ancient	4.70431	15747266
FS8	<i>P.bengalensis</i>	Ancient	5.96269	17194519
PBEP0014	<i>P.bengalensis</i>	Far East Russia	11.8565	20163701
PBEP0023	<i>P.bengalensis</i>	Far East Russia	11.1209	20174913
PVIP0012	<i>P.viverrinus</i>	Fishing cat	13.9554	20065873
PBEP0005	<i>P.bengalensis</i>	Sunda	11.3489	20028118
PBEP0008	<i>P.bengalensis</i>	Sunda	11.7308	19973472
PBEP0009	<i>P.bengalensis</i>	Sunda	11.0208	20048584
PBEP0068	<i>P.bengalensis</i>	Indochina	13.1926	20233140
PBEP0069	<i>P.bengalensis</i>	Indochina	11.9837	19977195
PBEP0066	<i>P.bengalensis</i>	Indochina	11.4505	20137637
PBEP0067	<i>P.bengalensis</i>	Indochina	11.6046	20120124
PBEP0064	<i>P.bengalensis</i>	Indochina	11.3286	20116523
PBEP0065	<i>P.bengalensis</i>	Indochina	11.4206	20141843
PBEP0038	<i>P.bengalensis</i>	Malay	13.0935	20193189
PBEP0039	<i>P.bengalensis</i>	Malay	12.8588	20110897
PBEP0028	<i>P.bengalensis</i>	Malay	12.7609	20102689
PBEP0036	<i>P.bengalensis</i>	Malay	11.7445	20032265

3.4.2.2 Phylogenetic Analysis

3.4.2.2.1 Whole mitochondrial genome phylogeny

The resulting phylogenetic tree separate leopard cats into two distinct groups, representing

mainland and island groups respectively (Figure 11). The mainland group primarily consists of all modern leopard cats from Far East Russia (FER), Indochina (IND), and the seven ancient Chinese samples from the Middle Yellow River Basin (MYR). The island group mainly contains samples from the Malay (MA) and Sunda (SU). Notably, one Malay (MA) individual (PBEP0039) shows a similar mitochondrial genome to the mainland group. Two fishing cats exhibit closer maternal relationship to the mainland (Figure 11).

The mainland further splits into two geographically distinct sub-clades represented by the Far East Russia population and Indochina Population. The ancient Chinese leopard cats used in this study, although all from the same geographical region, clustered across two different sub-clades. FS13, FS14, FS19, FS24 are assigned to clade Far East Russia, and the FS8, FS10, FS15 has integrated into clade Indochina (Figure 11).

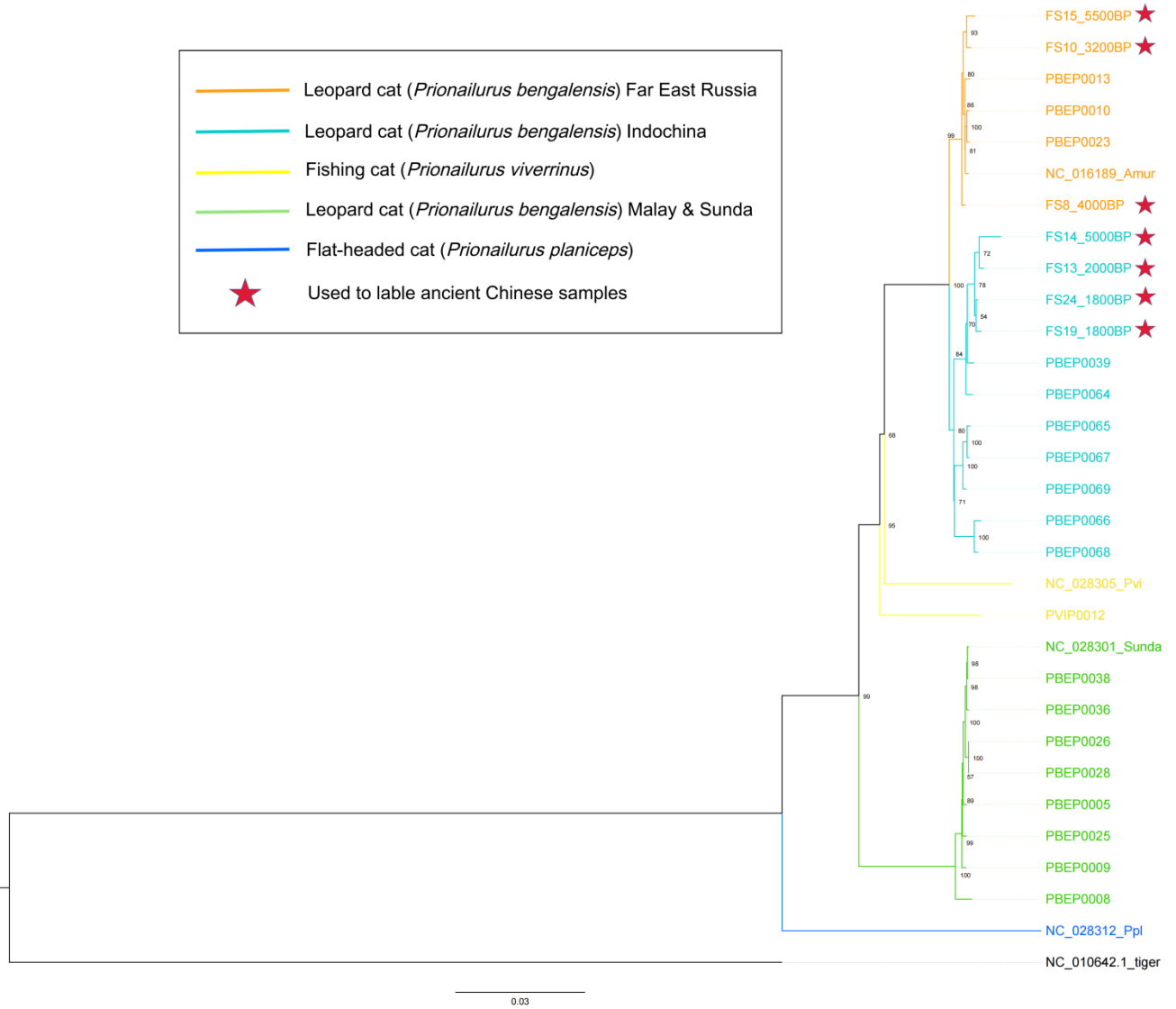


Figure 11 ML tree constructed using the mitochondrial genomes of ancient leopard cat from the middle reach of the Yellow River Basin, and modern leopard cats from Far East Russia, Indochina, Malay and Sunda. Mitochondrial genomes of fishing cat and flat-headed cat are also included for comparison. Tiger is used as outgroup.

3.4.2.2.2 Nuclear genome phylogeny

In this analysis, three ancient leopard cats from the MYR and 15 modern samples from FER, IND, MA, and SU were used, with a fishing cat serving as an outgroup (Miao, 2017). Over two million autosomal SNPs were examined to construct the maximum likelihood (ML) tree. This tree successfully differentiated the four primary populations of leopard cats, namely SU,

MA, IND, and FER, consistent with previous research classifications (Miao, 2017). All branches exhibited high statistical support (bootstrap ≥ 99). The clade formed by the SU population is basal to all other branches.

Interestingly, Malay individuals did not form a unified clade, which may be attributed to the complexity of their evolutionary history, such as the establishment of this population through admixture of SU and IND. As anticipated, the IND and FER populations clustered into the same clade, corresponding to their assignment to the mainland group in previous research. The three ancient samples from MYR—FS8 (4,000 BP), FS15 (5,500 BP), and FS19 (1,800 BP)—formed a monophyletic group that clustered with FER leopard cats (Figure 12). This genetic proximity implies that MYR and FER leopard cats are more closely related than other populations. Notably, FS19 was positioned in the IND clade (Figure 11; Figure 12), which may be explained by introgression from the IND population to the leopard cats in MYR.

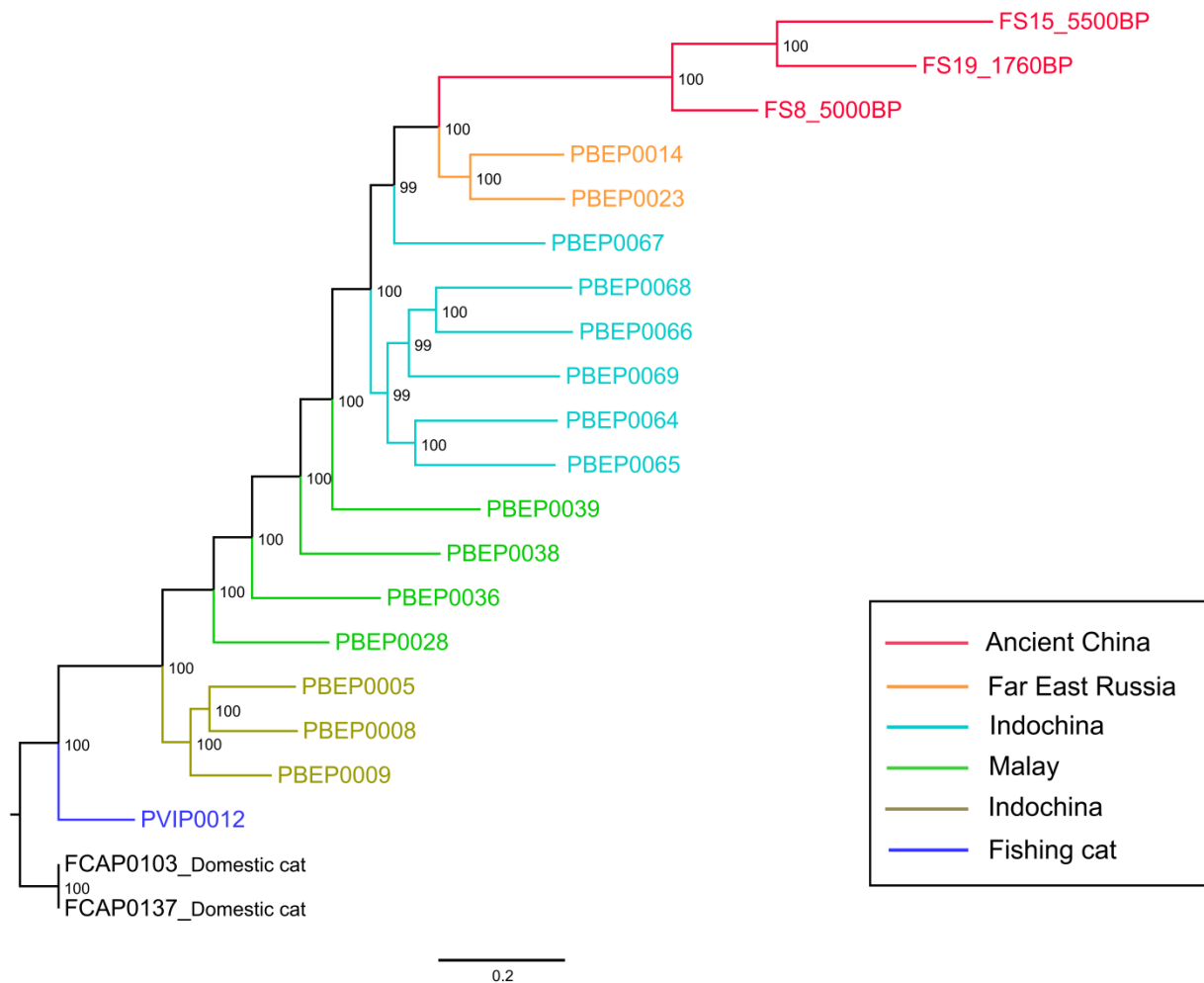


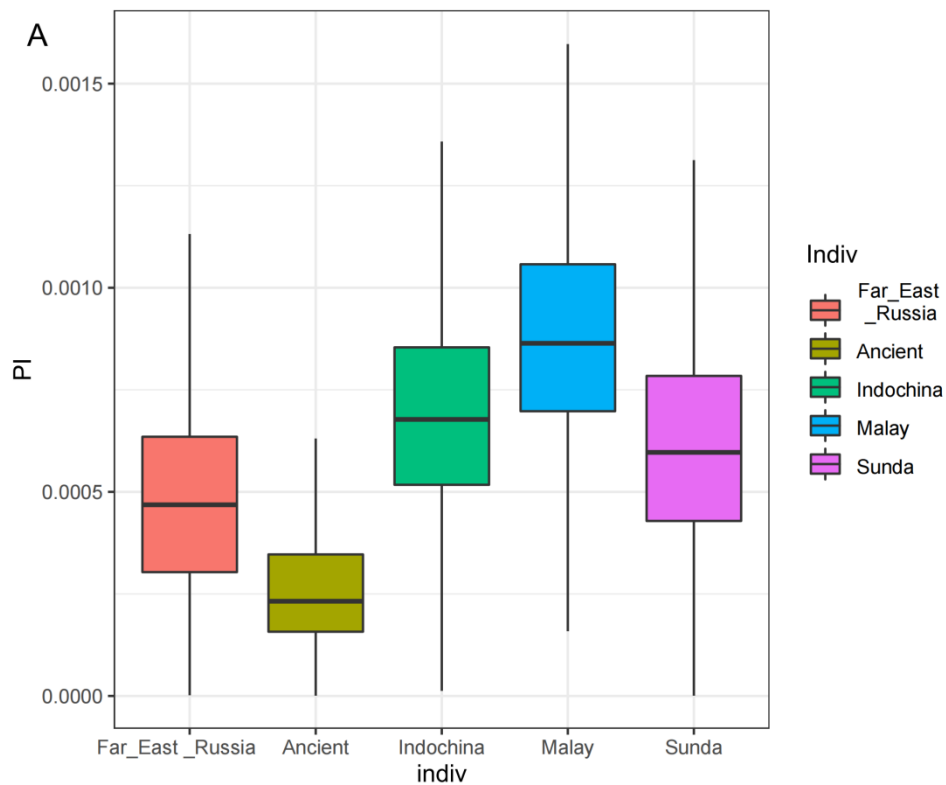
Figure 12 ML phylogeny of leopard cats constructed using 20 million nuclear genomic SNPs of leopard cat. A fishing cat is included in the analysis for comparison. Domestic cat is used as outgroup

3.4.2.3 Genetic Diversity

To examine the genetic profiles of the ancient leopard cats from the MYR, we estimated nucleotide diversity (P_i) (Figure 13A) and inbreeding coefficients (F) (Figure 13B) for different leopard cat populations using VCFtools (Danecek et al., 2011). Among all populations or individuals, the MA leopard cats exhibited the highest P_i and the lowest F , indicating the greatest genetic diversity. This can be attributed to the origin of this population, which was established through the admixture of IND and SU (Miao, 2017). In contrast, the ancient leopard

cats from the MYR displayed the lowest genetic diversity, with the smallest P_i value and the highest F (Figure 13).

The low genetic diversity of the MYR population could be a result of dispersal. Considering the genetic similarity between MYR and FER leopard cats, the establishment of the MYR population might have stemmed from the diffusion of FER leopard cats. During this process, it is perhaps that only a relatively small population successfully colonized the Middle Yellow River Basin.



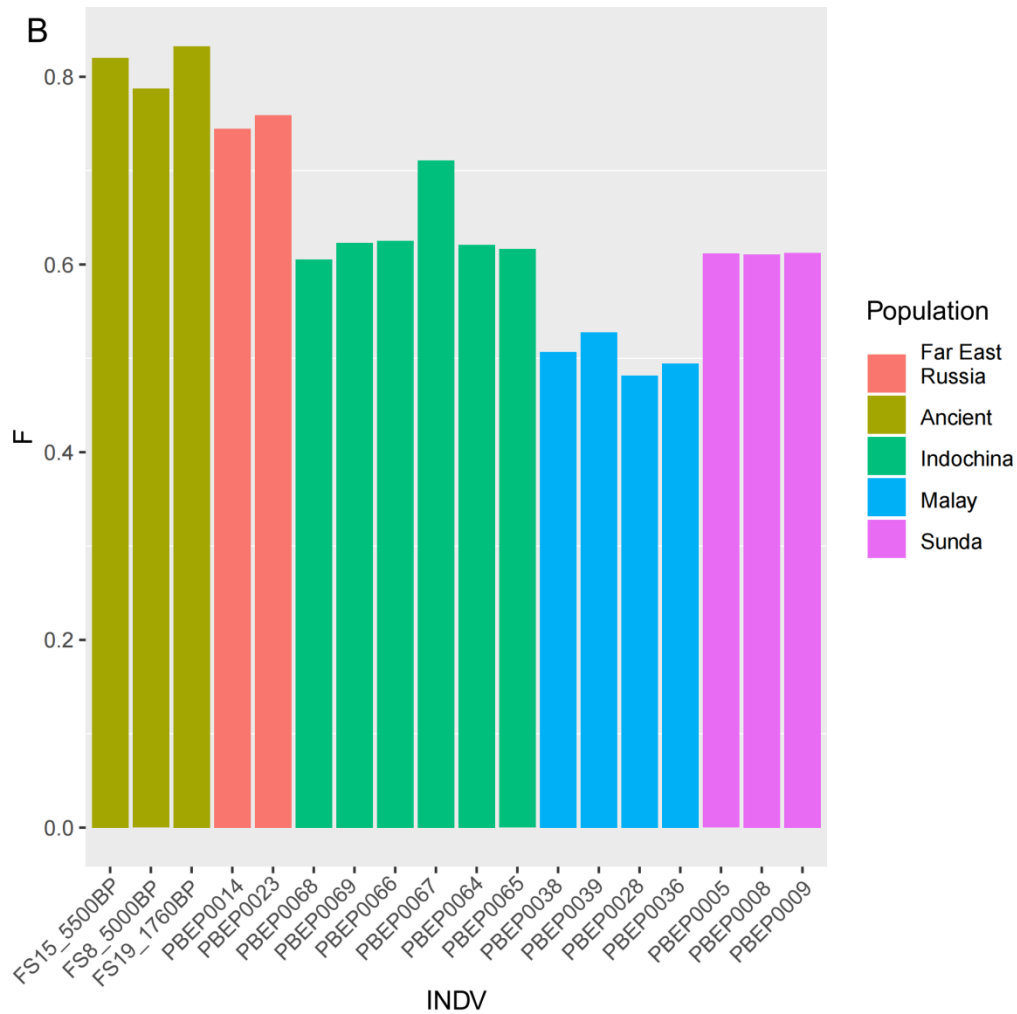


Figure 13 Genetic diversity of different populations of leopard cats. Plot A presents the value of PI. Plot B presents the value of F.

3.4.2.4 PCA

According to the PCA analysis (Figure 14), a clear distinction among geographically diverged populations is observed. Samples from the ancient middle reach of the Yellow River Basin, Far East Russia, Indochina, and Sunda regions formed distinct clades. However, the Malay population did not cluster together. Instead, their distribution formed a continuum between the Indochina and Sunda clades, indicating that some Malay individuals share genetic similarities with the Indochina population, while others are more closely related to the Sunda population.

Populations from the ancient middle reach of the Yellow River Basin, Far East Russia, and Indochina demonstrated similarities along the PC1 axis but were separated by PC2. The PC2 axis reveals that the clade representing ancient leopard cats is situated between the Far East Russia and Indochina populations, indicating a genetic affinity to both groups. This observation may suggest historical gene flow or shared ancestry between these populations and the ancient leopard cats, which warrants further investigation (Figure 14).

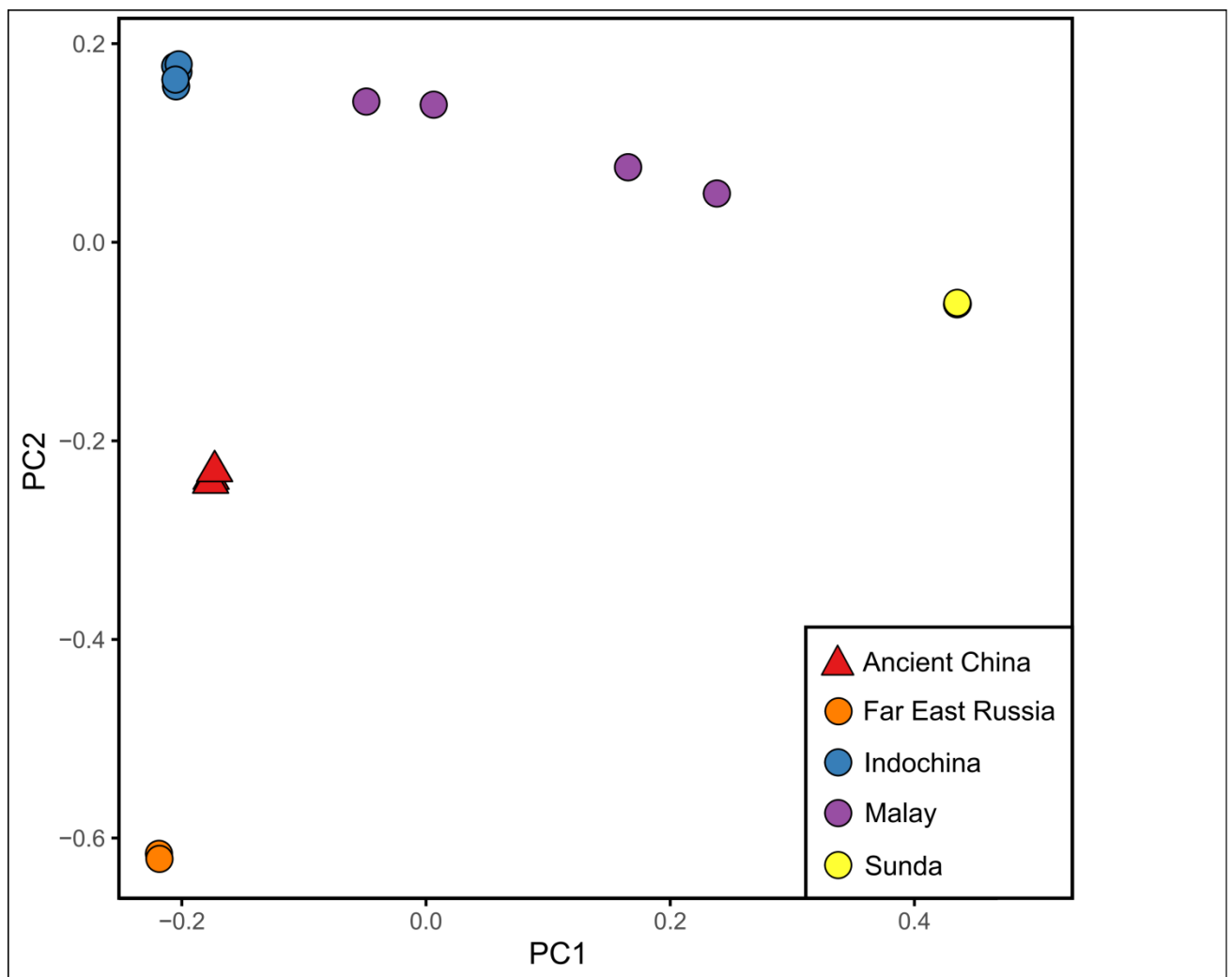


Figure 14 Principal component analysis. A total of 20 million SNPs of 18 samples including 3 ancient Chinese leopard cats, 15 modern leopard cats from Far East Russia (N=2), Indochina (N=6), Malay (N=4), Sunda (N=3), and a fishing cat.

3.4.2.5 Out-group f_3 analysis

To further explore the relationships among different populations, we conducted two outgroup f_3 analyses (Patterson et al., 2012) based on individuals respectively (Figure 15A; Table S6) and groups respectively (Figure 15B; Table S7). Group assignment followed species classification and geographical locations, and the ancient leopard cats from MYR were treated as a single group regardless of date differences. A domestic cat was used as the outgroup in the analysis. The population exhibiting the highest f_3 value with the MYR was the FER, followed by IND, MA, SU, and finally, the fishing cat (Figure 15B; Table S7). Identical results were obtained when analysing populations at the individual level (Figure 15A; Table S6). This indicates that the ancient MYR leopard cats shared more genetic drift or longer evolutionary history with the FER population compared to other populations, reflecting a closer genetic affinity.

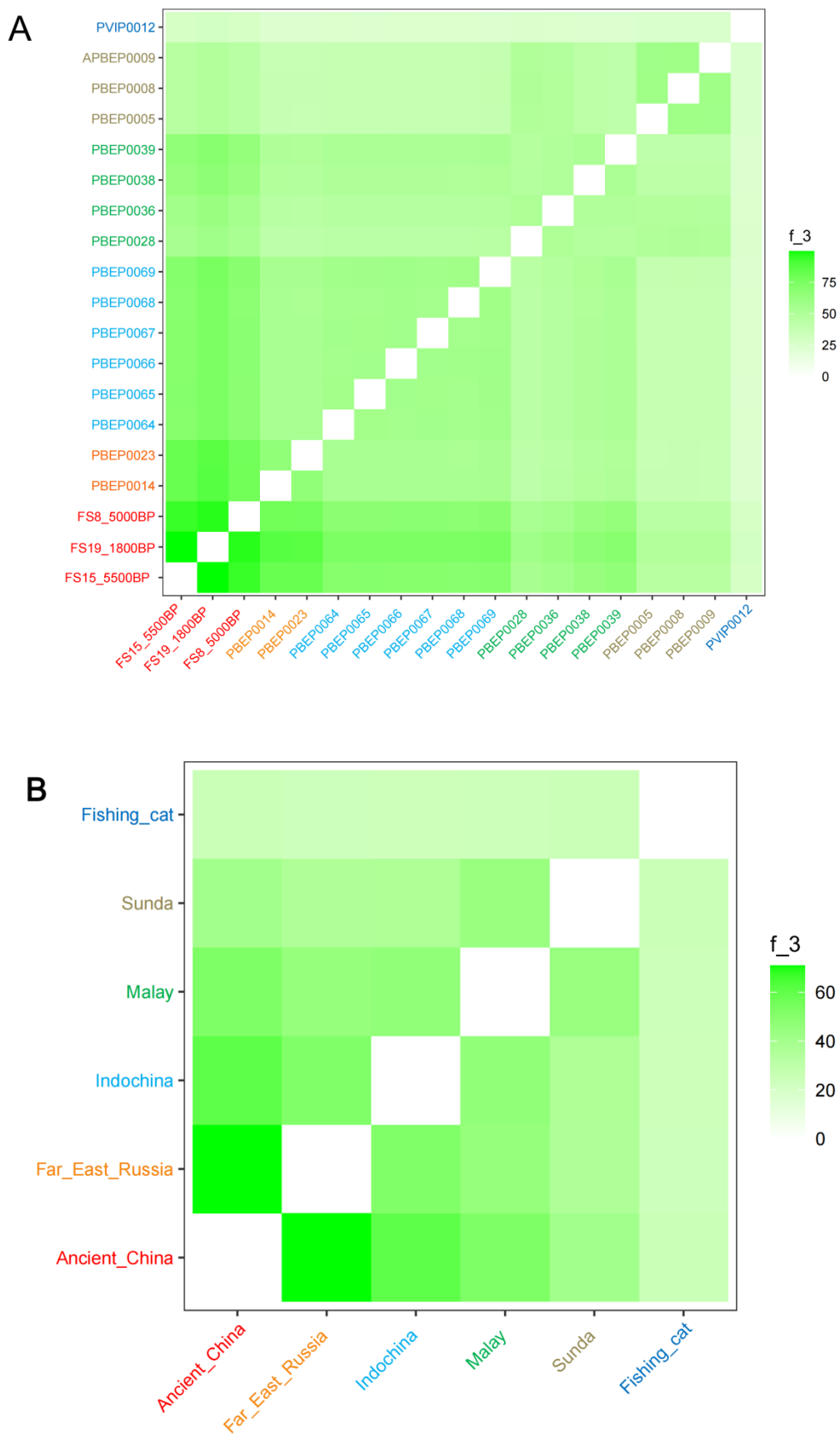


Figure 15 Results of *outgroup* f_3 -statistics. Plot A presents the results of outgroup f_3 analysis

based on individuals. Samples labelled in red font are ancient samples from the middle reach of the Yellow River Basin; samples labelled in orange font are from Far East Russia; samples labelled in light blue font are from Indochina; samples labelled in light green font are from Malay; samples labelled in dark green are from Sunda; the sample labelled in dark blue is a fishing cat. Plot B presents the results of outgroup f3 analysis based on populations.

3.4.2.6 ADMIXTURE

ADMIXTURE analysis was conducted to explore the population structure of leopard cats (Kenneth, 2011). With the ancient samples, 15 modern leopard cats from FER, IDE, MA, SU and two domestic cats and a fishing cat are included. From K=2 to K=5, the MYR ancient leopard cats always present the same ancestry as FER population. They can be viewed as two regional sub-populations of a whole. However, contrary to expectation, no IND ancestries are exhibited in MYR individuals (Figure 16; Figure S4). Mitochondrial genomes similar to IND can be found in several ancient MYR individuals (Figure 11). Based on the PCA and f3 analysis, MYR exhibited affinity with IND to some degree (Figure 14 & Figure 15). We speculated the absence of IND ancestry in MYR population could be caused by the accuracy or sensitivity of the ADMIXTURE analysis.

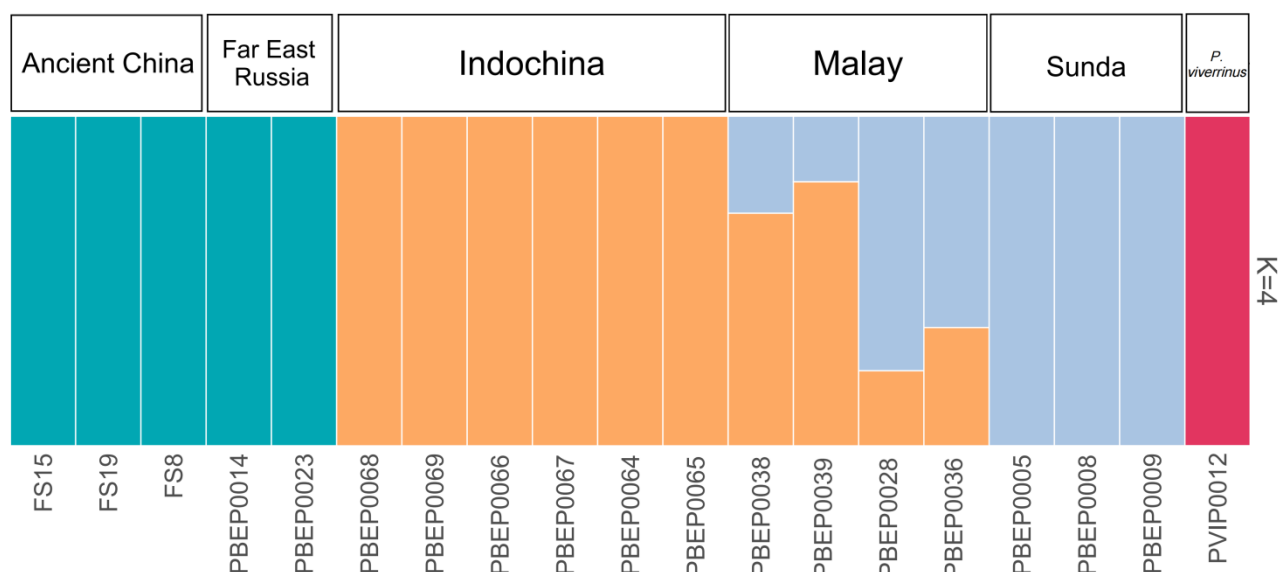


Figure 16 ADMIXTURE analysis using 20 million SNPs and 18 samples including 3 ancient Chinese leopard cats, 15 modern leopard cats from Far East Russia (N=2), Indochina (N=6), Malay (N=4), Sunda (N=3), a fishing cat K=4.

3.4.2.7 F4-Statistics

Although being failed to found IND ancestries from the population of MYR in the ADMIXTURE analysis, we believe that the MYA ancient leopard cats had received introgression from IND population, while the IND ancestries were not resolved in ADMIXTURE analysis. To explore whether the MYR ancient leopard cats were influenced by the IND, we have applied the D-statistics analysis which is more sensitive in revealing minor introgression (Patterson et al. 2012). Taking the combination (F.catus, P.bengal_X, FER, ancient_MYR), the f4 value are always positive while with significant Z value ($Z > 3$). It indicates that there was genetic introgression between ancient leopard cats in MYR and the IND population relative to Far East Russia population(Figure 17; Table S8).

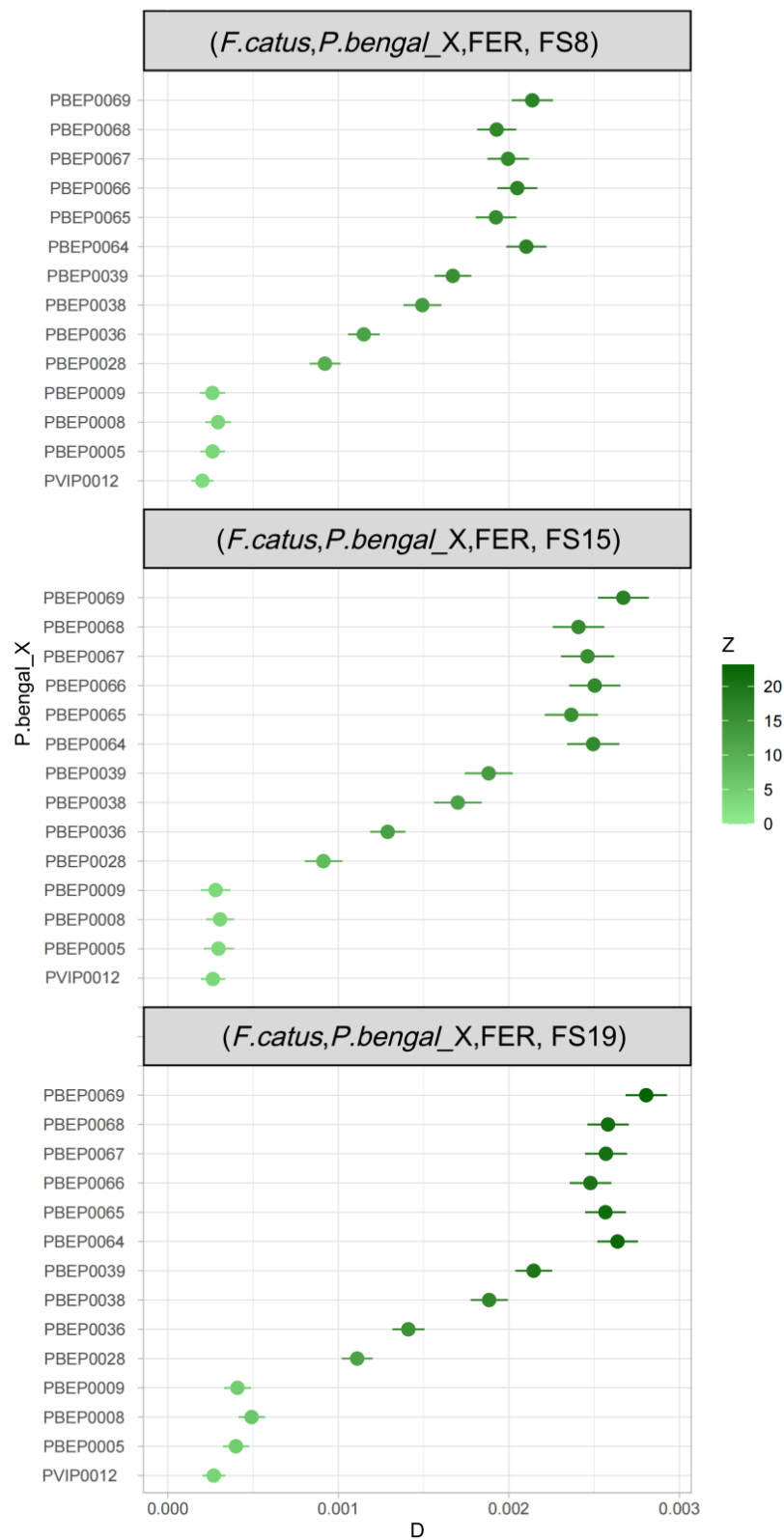


Figure 17 Results of leopard cats f4 statistics.

3.5 Discussion

3.5.1 The population assignment of the ancient Chinese leopard cats from the Middle reach of the Yellow River Basin

Leopard cat are classified into Mainland and Insular groups, as demonstrated. The mainland group consists of the Amur and Indochina populations, while the Insular group includes the Sunda and Malaya populations. However, the genetic origin of leopard cats inhabiting central East Asia is less established due to a scarcity of studies (Miao, 2017; Luo et al, 2014; Koh et al, 2014; Mukherjee et al, 2010; Tamada et al, 2008). Our research utilizes ancient samples from the MYR to bridge the geographical gap.

Our analysis reveal that the MYR and FER leopard cat populations have a close genetic relationship, sharing longer evolutionary history compared to others. Thus, our investigation leads us to assume that MYR leopard cats can be assigned to the Mainland group as a sub-population of the FER leopard cat. Moreover, ancient MYR leopard cats could also receive introgressions from the Indochina population. Because some of the ancient MYR individuals possessed mitochondrial genomes similar to Indochina leopard cats, and *f₄*-statistics exhibited the signals of introgression between the two populations.

The genetic characteristics of ancient MYR leopard cats are linked to their geographic location between FER and IND, which allows them to absorb influences from both the North and South. We hypothesize that the MYR population was formed by the southward spread of FER leopard

cats, followed by bottleneck and founder effects (Barton and Charlesworth, 1984; Nei et al., 1975), which resulted in minimal genetic diversity. The presence of IND mitochondrial genomes and minor ancestries in nuclear genomes suggest possible small-scale admixture between MYR and IND. One reason for the low contribution of IND leopard cats to MYR nuclear genomes could be their adaptation to the milder environment of the Indochina Peninsula and Southern China, limiting their ability to colonize Northern China (Domrös and Peng, 2012; Zhang et al., 2005).

In summary, our analysis of ancient samples places MYR leopard cats in the same population as FER, with influence from IND introgression. The formation of the MYR population may have resulted from the dispersal of FER leopard cats and admixture with the IND population. However, the limited adaptation to colder climates in Northern China (Domrös and Peng, 2012; Zhang et al., 2005) might contribute to the low level of IND population influence on MYR genomes.

3.5.2 A commensal relationship between leopard cats and human

The concept of commensalism implies that one side benefits from the relationship without directly profiting or causing harm to the other. As small-sized felids, leopard cats pose no threat to humans, we think that there is strong evidence supporting a commensal relationship between leopard cats and humans. (Lenski Gerhard, 1976).

We believe that leopard cats and humans have formed a commensal relationship for the

following reasons: First, the occurrence of leopard cats in association with human populations coincided with the prosperity of ancient Chinese agriculture (Jia et al., 2013; Li et al., 2020; Pechenkina et al., 2005; Yang et al., 2022). Leopard cat remains were first discovered in the Middle Reach of the Yellow River Basin during the late phase of the Chinese Neolithic. This period marked the maturity of regional agriculture, with an abundance of millet, a predominance of domestic animals, and food production as the primary subsistence strategy (Pechenkina et al., 2005; Yang et al., 2022). Thriving agriculture could have created an environment conducive to rodent populations, attracting leopard cats to agricultural villages. Secondly, leopard cat remains were not exclusive to the Quanhucun Site but were found in at least seven sites spanning 3,500 years. The frequency and time span of coexistence between leopard cats and humans further demonstrate their affinity. Thirdly, leopard cats indeed hunted rats and mice associated with human settlements, benefiting from this food resource. Studies have shown that leopard cats in Quanhucun had a diet including a significant amount of C4 food, similar to domestic animals feeding on millet products (Zhu et al., 2022; Vigne et al., 2016; Hu et al., 2014). As carnivores, leopard cats likely acquired this C4 contribution by consuming rodents attracted to the settlement.

In addition to these points, ancient literature from before and during the Han Dynasty recorded leopard cats' behaviour of chasing and eating rats and mice (Wang, 2010). This fact suggests that the presence and activities of leopard cats in human environments were widespread and frequent, further supporting the commensal relationship between leopard cats and humans in ancient China. As circumstantial evidence of this relationship, leopard cats were integrated into

ancient Chinese culture, with their images used to decorate artifacts. In the Mawangdui Han Tomb, several service plates featured abstract yet recognizable depictions of leopard cats (Yang, 2016; Hunan Museum and CASS, 1973). The use of leopard cats in decoration indicates that people were likely familiar with the species and might have considered them as guardians of food, reflecting their commensal relationship.

However, this commensal relationship has not persisted until today. According to prior research and radiocarbon dating results from this study, the commensal relationship between humans and leopard cats lasted for over 3,500 years but came to an end around 1,800 years ago.

We associate the termination of this affinity with the collapse of the Eastern Han Dynasty, which resulted in a drastic decline in ancient Chinese society and agricultural economy. Furthermore, the commensal relationship between leopard cats and humans never re-emerged, even after the reunification of mainland East Asia and the resurgence of agriculture during the Tang Dynasty. This can be attributed to the introduction of domestic cats during that time. Sharing a similar ecological niche, domestic cats also preyed on rats and mice and had a considerable degree of habitat overlap with leopard cats. As a result, they occupied the ecological niche formerly held by leopard cats, preventing the re-establishment of the commensal relationship between leopard cats and humans.

3.5.3 Were the Chinese leopard cats ever domesticated?

Commensalism has been identified as a crucial pathway leading to domestication (Zeder, 2012a,

2012b). This process was instrumental in the domestication of African wild cats, the ancestors of modern domestic cats, which established commensal relationships with humans during the Neolithic transition (Zeder, 2012a; Driscoll et al., 2007). Similarly, ancient China witnessed the domestication of commensal animals, such as pigs and dogs (Jing and Flad, 2002; Zeder, 2012a; Zhang et al., 2020), raising the question of whether leopard cats underwent domestication in ancient China.

However, our analysis reveals no substantial evidence supporting the domestication of leopard cats in ancient China. Domestication typically encompasses processes such as captivity, artificial dispersal, feeding, and selective breeding, which result in specific phenotypic or genetic traits (Bökönyi, 2014; Ducos and Matthews, 2014; Zeder, 2015, 2012b). Although ancient leopard cats in MYR displayed lower genetic diversity compared to other groups (Figure 4), this observation could be attributed to bottleneck or founder effects rather than domestication (Allaby et al., 2019).

Moreover, leopard cats exhibit a limited cultural connection with humans. While occasionally found in tombs (Guo et al., 1992; Team of the Dabaotai Han Tomb, 1986), their burial sites are situated far from the main chamber, indicating no strong affinity with the deceased. Furthermore, the representation of leopard cats in Chinese literature and art is scarce compared to domestic cats (Yang, 2016; Hunan Museum and Chinese Academy of Social Sciences, 1973), and they disappeared from archaeological sites after the Han Dynasty, being replaced by domestic cats (see Chapter 2).

In conclusion, despite the potential for domestication in ancient China, we have not identified robust genetic evidence or cultural connections supporting leopard cat domestication. As a result, we characterize the relationship between humans and leopard cats as commensalism, rather than domestication.

3.6 Conclusion

In conclusion, through multidisciplinary research, which incorporated genetics, radiocarbon dating, archaeology, and history, we discovered that: 1) leopard cats from the Middle Reach of the Yellow River Basin and Far East Russia can be assigned to the same population, although the former was also genetically influenced by the Indochina population; 2) leopard cats and humans have interacted in some form of relationship since the late Neolithic Age, 5,500 years ago. This commensal relationship disappeared or nearly disappeared following the breakdown of the Han Dynasty and was never resumed. We attribute the disappearance of the commensal relationship between humans and leopard cats to the recession of the agricultural economy and the social chaos that lasted over 500 years after the collapse of the Han Dynasty. The role of leopard cats in human-associated environments was eventually replaced by domestic cats, introduced around the 7th century CE; 3) although leopard cats had close ties with humans, we found no significant evidence suggesting that they were domesticated in this region or during this period.

Humans have had significant impacts on animals since the Holocene, affecting not only

domesticated species but also wildlife. The leopard cat in ancient China serves as an exemplary case to enhance our understanding of these effects. The commensal relationship between leopard cats and humans demonstrates that anthropogenic landscape modifications, such as agriculture, can profoundly alter animal behaviour and subsistence strategies. In this context, the habitat and dietary preferences of leopard cats may have been closely synchronized with the rise and fall of human societies.

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Supplementary Information

Description of Archaeological contexts

Quanhucun Site

It is a late Neolithic complex comprised of settlement and cemetery (6,000 to 5,000 BP) located in the Middle reach of the Yellow Basin. The main body of the sites belongs to the Banpo type, Yangshao culture. Agriculture was the major subsistence of the site. There were at least three individuals unearthed from the pits of the settlement (Peking University, 2003). These cat remains were initially thought to be domestic cats and the stable isotopic analysis indicated that they had consumed certain amount of C4 food which was close to the domestic animals. However, later the research using GM method suggested that they actually belonged to leopard cats, and the result has been further confirmed by ancient DNA. A mandible has been sampled and analyzed in this research (Hu et al., 2014b; Vigne et al., 2016; Zhu et al., 2022).

Huangdiguli Site

It is an archaeological complex belonging to the Xinzheng City Site. The Xinzheng City Site is in the middle reach of the Yellow River Basin. During the Warring States Period (475 BC-221 BC), the city was made the capital of the State of Zheng and then the State of Han in succession. After the establishment of the Qin Dynasty (221-207 BC), the city was continued to be used while its importance gradually declined. In the site, a leopard cat skeleton was found in a tomb. Based on the archaeological contexts, the tomb was thought to be built during the late Eastern Han Dynasty (25 AD-220 AD) which has later been confirmed by the radiocarbon dating results. Considering the standard of artifacts buried and the scale of the tomb, the dead might be an official or nobility. The cat skeleton belonged to a single individual and lie on the side of the tomb's lane.

Wuzhuangguoliang Site

It is a settlement located in the upper part of the Middle reach of the Yellow River Basin, Western China. The site was used as a settlement from the Late Neolithic (5,000 BP) to the Warring State Period (475 BC-221BC). The Neolithic materials of the site are with typical

features of the Majiayao Culture. Agriculture and animal farming was the major subsistence of the site. One piece of bones of leopard cat (FS8) was excavated from a pit of the Longshan Period (4,000 BP) .

Han Changan City Site

It was the Capital of the Western Han Dynasty (202 BC -8 AD) located in the Guanzhong Plain, Middle Reach of the Yellow River Basin. The city was constructed on the basis of the renovation of the Changle Palace of the Qin Dynasty (221-207 BC), and the project started since 200 BC. One ulna of leopard cat (FS24) was unearthed from the Southwest corner of the city wall. Through direct radiocarbon dating on the bone, we confirmed that it indeed belongs to the Western Han Dynasty (Hu et al., 2006).

Yangling mausoleum

It is the burial of the Emperor Yang of the Western Han Dynasty. The tomb is located on the Xianyang tableland, the Middle Reach of the Yellow River Basin. The construction of the Yangling cemetery started in 153 AD lasted for 28 years. The cemetery was on a large scale, except main tombs of the emperor and his empress there are also multiple subsidiary facilities build surround them, such as the ancestral temple and some subordinate tombs, etc. A tibia belonging to leopard cat (FS13) was unearthed from a funerary pit attached to the main tomb. It is not clear whether a leopard cat had been intentionally buried, or just break into the pit occasionally. However, our radiocarbon dating results directly on this piece of bone indicated that it was of the same age with the tomb.

Laoniupo Site

The site is located in the Middle Reach of the Yellow River Basin and contains the remains from the late Neolithic Age (6,000 BP) to the Shang Dynasty (3,600 - 3,046 BP). The major subsistence of the site was agriculture. A right tibia of leopard cats (FS10) was found in the sediment of the Shang Dynasty .

Lantianxinjie Site

The site is located in the Middle Reach of the Yellow River Basin. It was used as a settlement during the late Neolithic Age. The major subsistence of the site was agriculture. A left humerus (FS14) unearthed from a pit belonging to the late phase of Yangshao culture was sampled.

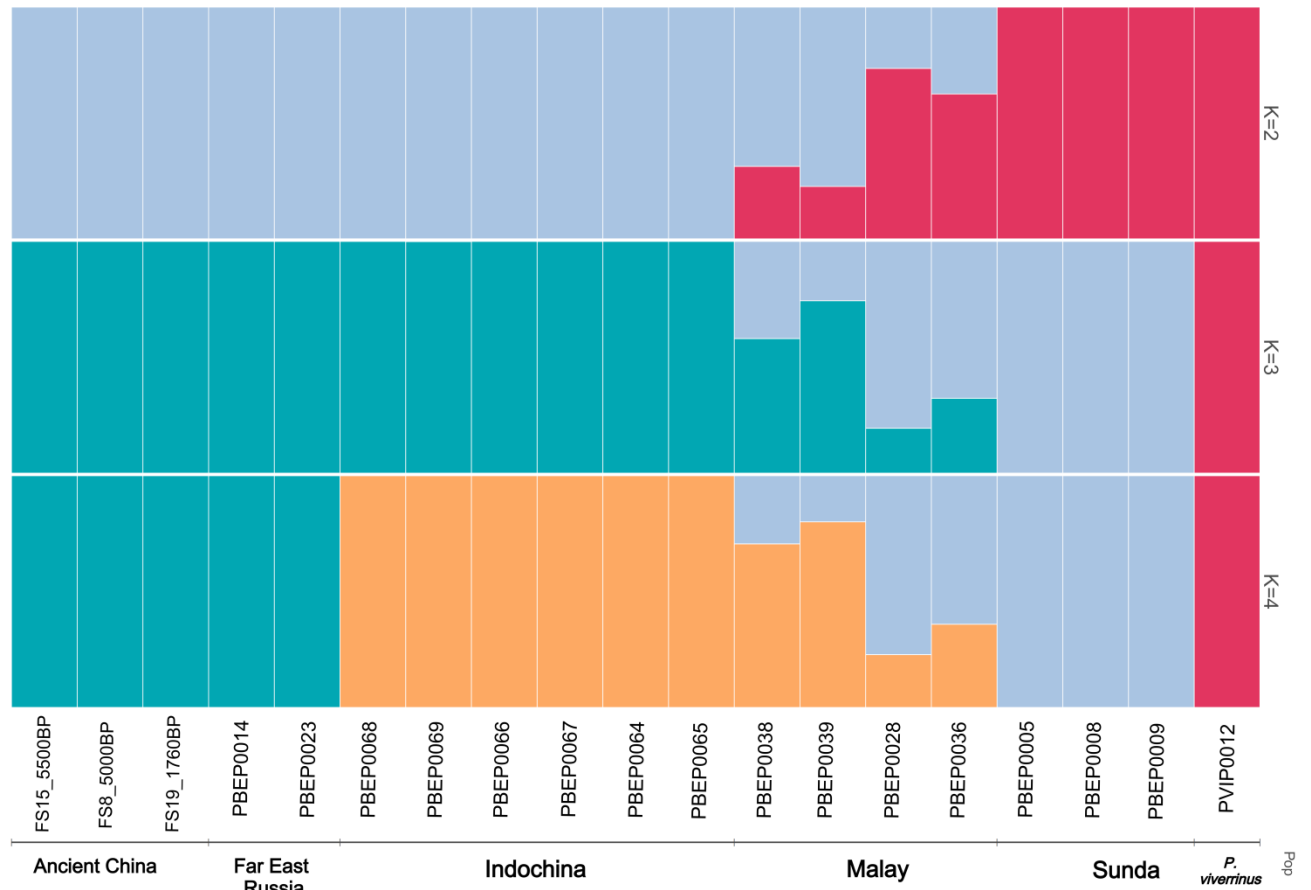


Figure S4 Results of leopard cat ADMIXTURE analysis. K=2 to 4.

Table S6 Results of outgroup f3 analysis of leopard cats conducted on Individuals.

A	B	C	f ₃	std.err	Z	SNPs
FS15	FS15	Domestic	0	-1	0	-1
FS15	FS8	Domestic	94.479252	3.161095	29.888	703660
FS15	FS19	Domestic	99.536262	3.275273	30.39	642087
FS15	PBEP0014	Domestic	82.320955	2.499533	32.935	785997
FS15	PBEP0023	Domestic	82.094914	2.511656	32.686	780733
FS15	PVP0012	Domestic	28.737047	0.917571	31.319	700068
FS15	PBEP0005	Domestic	46.123892	1.4261	32.343	858528
FS15	PBEP0008	Domestic	46.164718	1.428339	32.321	857682
FS15	PBEP0009	Domestic	45.917531	1.403135	32.725	858237
FS15	PBEP0068	Domestic	69.950411	2.10229	33.273	894240

FS15	PBEP0069	Domestic	71.417436	2.211924	32.287	882128
FS15	PBEP0066	Domestic	70.576964	2.153776	32.769	884207
FS15	PBEP0067	Domestic	70.503829	2.15352	32.739	851996
FS15	PBEP0064	Domestic	70.628207	2.143967	32.943	885700
FS15	PBEP0065	Domestic	70.682337	2.159481	32.731	887683
FS15	PBEP0038	Domestic	62.92979	1.919277	32.788	915250
FS15	PBEP0039	Domestic	65.392363	1.992234	32.824	907600
FS15	PBEP0028	Domestic	54.652416	1.671718	32.692	911959
FS15	PBEP0036	Domestic	57.967153	1.790102	32.382	907314
FS8	FS8	Domestic	0	-1	0	-1
FS8	FS15	Domestic	94.479252	3.161095	29.888	703660
FS8	FS19	Domestic	96.856843	3.252197	29.782	760198
FS8	PBEP0014	Domestic	79.309033	2.459222	32.25	974613
FS8	PBEP0023	Domestic	78.955336	2.462822	32.059	969790
FS8	PVIP0012	Domestic	28.183928	0.906908	31.077	888823
FS8	PBEP0005	Domestic	45.603351	1.436178	31.753	1074427
FS8	PBEP0008	Domestic	45.538826	1.439044	31.645	1072055
FS8	PBEP0009	Domestic	45.358966	1.405665	32.269	1073212
FS8	PBEP0068	Domestic	68.206189	2.100244	32.475	1107559
FS8	PBEP0069	Domestic	69.39284	2.205899	31.458	1090504
FS8	PBEP0066	Domestic	68.65733	2.146608	31.984	1094816
FS8	PBEP0067	Domestic	68.672333	2.137885	32.122	1055827
FS8	PBEP0064	Domestic	68.828178	2.138988	32.178	1095339
FS8	PBEP0065	Domestic	68.826358	2.144858	32.089	1098540
FS8	PBEP0038	Domestic	61.557468	1.923598	32.001	1135282
FS8	PBEP0039	Domestic	64.069036	1.998685	32.056	1123996
FS8	PBEP0028	Domestic	53.800577	1.676275	32.095	1134106
FS8	PBEP0036	Domestic	56.795691	1.798561	31.578	1126856
FS19	FS19	Domestic	0	-1	0	-1
FS19	FS15	Domestic	99.536262	3.275273	30.39	642087
FS19	FS8	Domestic	96.856843	3.252197	29.782	760198
FS19	PBEP0014	Domestic	87.648307	2.650553	33.068	852906
FS19	PBEP0023	Domestic	87.220844	2.666313	32.712	847048
FS19	PVIP0012	Domestic	30.388756	0.97351	31.216	766663
FS19	PBEP0005	Domestic	49.332079	1.51644	32.532	940866
FS19	PBEP0008	Domestic	49.320579	1.528168	32.274	939748
FS19	PBEP0009	Domestic	49.121241	1.508188	32.57	940176
FS19	PBEP0068	Domestic	75.043667	2.265701	33.122	974334
FS19	PBEP0069	Domestic	75.997938	2.354484	32.278	962071
FS19	PBEP0066	Domestic	75.1633	2.299679	32.684	964114
FS19	PBEP0067	Domestic	75.071234	2.287896	32.812	928363
FS19	PBEP0064	Domestic	75.280656	2.28575	32.935	965174
FS19	PBEP0065	Domestic	75.449196	2.293242	32.901	966873

FS19	PBEP0038	Domestic	67.478661	2.06905	32.613	998387
FS19	PBEP0039	Domestic	69.956229	2.136028	32.751	990065
FS19	PBEP0028	Domestic	58.522595	1.79248	32.649	997491
FS19	PBEP0036	Domestic	61.711387	1.896681	32.537	992332
PBEP0014	PBEP0014	Domestic	0	-1	0	-1
PBEP0014	FS15	Domestic	82.320955	2.499533	32.935	785997
PBEP0014	FS8	Domestic	79.309033	2.459222	32.25	974613
PBEP0014	FS19	Domestic	87.648307	2.650553	33.068	852906
PBEP0014	PBEP0023	Domestic	66.197117	2.082669	31.785	1190087
PBEP0014	PVIP0012	Domestic	23.948674	0.758141	31.589	1186896
PBEP0014	PBEP0005	Domestic	37.477295	1.185413	31.615	1377780
PBEP0014	PBEP0008	Domestic	37.671307	1.204158	31.284	1371085
PBEP0014	PBEP0009	Domestic	37.416043	1.177416	31.778	1376009
PBEP0014	PBEP0068	Domestic	53.490127	1.666208	32.103	1419107
PBEP0014	PBEP0069	Domestic	54.881495	1.770938	30.99	1379219
PBEP0014	PBEP0066	Domestic	54.002324	1.700729	31.752	1394879
PBEP0014	PBEP0067	Domestic	53.889496	1.691444	31.86	1349399
PBEP0014	PBEP0064	Domestic	54.118805	1.708585	31.675	1393811
PBEP0014	PBEP0065	Domestic	54.12236	1.701883	31.801	1399547
PBEP0014	PBEP0038	Domestic	48.951724	1.532748	31.937	1449191
PBEP0014	PBEP0039	Domestic	50.68284	1.589837	31.879	1429287
PBEP0014	PBEP0028	Domestic	43.426948	1.377289	31.531	1445233
PBEP0014	PBEP0036	Domestic	45.649228	1.458796	31.292	1430275
PBEP0023	PBEP0023	Domestic	0	-1	0	-1
PBEP0023	FS15	Domestic	82.094914	2.511656	32.686	780733
PBEP0023	FS8	Domestic	78.955336	2.462822	32.059	969790
PBEP0023	FS19	Domestic	87.220844	2.666313	32.712	847048
PBEP0023	PBEP0014	Domestic	66.197117	2.082669	31.785	1190087
PBEP0023	PVIP0012	Domestic	23.837789	0.760999	31.324	1174225
PBEP0023	PBEP0005	Domestic	37.35184	1.195286	31.249	1366269
PBEP0023	PBEP0008	Domestic	37.504124	1.215381	30.858	1359709
PBEP0023	PBEP0009	Domestic	37.248091	1.187525	31.366	1364656
PBEP0023	PBEP0068	Domestic	53.251322	1.677011	31.754	1410661
PBEP0023	PBEP0069	Domestic	54.652481	1.789079	30.548	1370795
PBEP0023	PBEP0066	Domestic	53.74023	1.716968	31.299	1386675
PBEP0023	PBEP0067	Domestic	53.715082	1.708844	31.434	1340270
PBEP0023	PBEP0064	Domestic	53.891182	1.714463	31.433	1385607
PBEP0023	PBEP0065	Domestic	53.891158	1.713174	31.457	1391553
PBEP0023	PBEP0038	Domestic	48.685729	1.54992	31.412	1441364
PBEP0023	PBEP0039	Domestic	50.451412	1.603709	31.459	1420513
PBEP0023	PBEP0028	Domestic	43.196526	1.382828	31.238	1435808
PBEP0023	PBEP0036	Domestic	45.449093	1.469906	30.92	1420822
PVIP0012	PVIP0012	Domestic	0	-1	0	-1

PVIP0012	FS15	Domestic	28.737047	0.917571	31.319	700068
PVIP0012	FS8	Domestic	28.183928	0.906908	31.077	888823
PVIP0012	FS19	Domestic	30.388756	0.97351	31.216	766663
PVIP0012	PBEP0014	Domestic	23.948674	0.758141	31.589	1186896
PVIP0012	PBEP0023	Domestic	23.837789	0.760999	31.324	1174225
PVIP0012	PBEP0005	Domestic	25.634429	0.832234	30.802	1039423
PVIP0012	PBEP0008	Domestic	25.673187	0.844428	30.403	1032625
PVIP0012	PBEP0009	Domestic	25.638723	0.825598	31.055	1037937
PVIP0012	PBEP0068	Domestic	24.329028	0.767674	31.692	1298418
PVIP0012	PBEP0069	Domestic	24.554984	0.814497	30.147	1244679
PVIP0012	PBEP0066	Domestic	24.290088	0.777031	31.26	1265061
PVIP0012	PBEP0067	Domestic	24.293093	0.780768	31.114	1200500
PVIP0012	PBEP0064	Domestic	24.218761	0.776431	31.192	1265322
PVIP0012	PBEP0065	Domestic	24.356293	0.783999	31.067	1272804
PVIP0012	PBEP0038	Domestic	24.560739	0.782955	31.369	1278932
PVIP0012	PBEP0039	Domestic	24.501967	0.786403	31.157	1269500
PVIP0012	PBEP0028	Domestic	25.061814	0.803433	31.193	1202629
PVIP0012	PBEP0036	Domestic	24.969234	0.808118	30.898	1209863
PBEP0005	PBEP0005	Domestic	0	-1	0	-1
PBEP0005	FS15	Domestic	46.123892	1.4261	32.343	858528
PBEP0005	FS8	Domestic	45.603351	1.436178	31.753	1074427
PBEP0005	FS19	Domestic	49.332079	1.51644	32.532	940866
PBEP0005	PBEP0014	Domestic	37.477295	1.185413	31.615	1377780
PBEP0005	PBEP0023	Domestic	37.35184	1.195286	31.249	1366269
PBEP0005	PVIP0012	Domestic	25.634429	0.832234	30.802	1039423
PBEP0005	PBEP0008	Domestic	59.109367	1.954609	30.241	1046564
PBEP0005	PBEP0009	Domestic	58.574828	1.900872	30.815	1052285
PBEP0005	PBEP0068	Domestic	38.187278	1.224464	31.187	1466928
PBEP0005	PBEP0069	Domestic	38.972885	1.292857	30.145	1424331
PBEP0005	PBEP0066	Domestic	38.377598	1.235497	31.062	1441564
PBEP0005	PBEP0067	Domestic	38.422704	1.239383	31.001	1384954
PBEP0005	PBEP0064	Domestic	38.320087	1.237798	30.958	1442591
PBEP0005	PBEP0065	Domestic	38.46402	1.24436	30.911	1448224
PBEP0005	PBEP0038	Domestic	43.72363	1.403629	31.15	1392550
PBEP0005	PBEP0039	Domestic	42.41455	1.37545	30.837	1402302
PBEP0005	PBEP0028	Domestic	50.648952	1.643772	30.813	1261340
PBEP0005	PBEP0036	Domestic	48.824514	1.58665	30.772	1290000
PBEP0008	PBEP0008	Domestic	0	-1	0	-1
PBEP0008	FS15	Domestic	46.164718	1.428339	32.321	857682
PBEP0008	FS8	Domestic	45.538826	1.439044	31.645	1072055
PBEP0008	FS19	Domestic	49.320579	1.528168	32.274	939748
PBEP0008	PBEP0014	Domestic	37.671307	1.204158	31.284	1371085
PBEP0008	PBEP0023	Domestic	37.504124	1.215381	30.858	1359709

PBEP0008	PVIP0012	Domestic	25.673187	0.844428	30.403	1032625
PBEP0008	PBEP0005	Domestic	59.109367	1.954609	30.241	1046564
PBEP0008	PBEP0009	Domestic	58.850098	1.936179	30.395	1046302
PBEP0008	PBEP0068	Domestic	38.376315	1.24739	30.765	1458998
PBEP0008	PBEP0069	Domestic	39.171144	1.314889	29.79	1419140
PBEP0008	PBEP0066	Domestic	38.564098	1.259783	30.612	1434541
PBEP0008	PBEP0067	Domestic	38.617735	1.261538	30.612	1378483
PBEP0008	PBEP0064	Domestic	38.535139	1.25923	30.602	1436094
PBEP0008	PBEP0065	Domestic	38.647117	1.265993	30.527	1441722
PBEP0008	PBEP0038	Domestic	43.885647	1.430986	30.668	1385733
PBEP0008	PBEP0039	Domestic	42.62025	1.396354	30.523	1396048
PBEP0008	PBEP0028	Domestic	50.883481	1.662097	30.614	1255154
PBEP0008	PBEP0036	Domestic	49.032855	1.617148	30.321	1285300
PBEP0009	PBEP0009	Domestic	0	-1	0	-1
PBEP0009	FS15	Domestic	45.917531	1.403135	32.725	858237
PBEP0009	FS8	Domestic	45.358966	1.405665	32.269	1073212
PBEP0009	FS19	Domestic	49.121241	1.508188	32.57	940176
PBEP0009	PBEP0014	Domestic	37.416043	1.177416	31.778	1376009
PBEP0009	PBEP0023	Domestic	37.248091	1.187525	31.366	1364656
PBEP0009	PVIP0012	Domestic	25.638723	0.825598	31.055	1037937
PBEP0009	PBEP0005	Domestic	58.574828	1.900872	30.815	1052285
PBEP0009	PBEP0008	Domestic	58.850098	1.936179	30.395	1046302
PBEP0009	PBEP0068	Domestic	38.118426	1.216623	31.331	1465594
PBEP0009	PBEP0069	Domestic	38.861658	1.284087	30.264	1422781
PBEP0009	PBEP0066	Domestic	38.251629	1.225227	31.22	1440249
PBEP0009	PBEP0067	Domestic	38.330881	1.235669	31.02	1383051
PBEP0009	PBEP0064	Domestic	38.248548	1.229581	31.107	1440539
PBEP0009	PBEP0065	Domestic	38.352801	1.237209	30.999	1446463
PBEP0009	PBEP0038	Domestic	43.641591	1.394671	31.292	1391361
PBEP0009	PBEP0039	Domestic	42.336655	1.367342	30.963	1400471
PBEP0009	PBEP0028	Domestic	50.585553	1.634403	30.95	1259817
PBEP0009	PBEP0036	Domestic	48.71554	1.581173	30.81	1288682
PBEP0068	PBEP0068	Domestic	0	-1	0	-1
PBEP0068	FS15	Domestic	69.950411	2.10229	33.273	894240
PBEP0068	FS8	Domestic	68.206189	2.100244	32.475	1107559
PBEP0068	FS19	Domestic	75.043667	2.265701	33.122	974334
PBEP0068	PBEP0014	Domestic	53.490127	1.666208	32.103	1419107
PBEP0068	PBEP0023	Domestic	53.251322	1.677011	31.754	1410661
PBEP0068	PVIP0012	Domestic	24.329028	0.767674	31.692	1298418
PBEP0068	PBEP0005	Domestic	38.187278	1.224464	31.187	1466928
PBEP0068	PBEP0008	Domestic	38.376315	1.24739	30.765	1458998
PBEP0068	PBEP0009	Domestic	38.118426	1.216623	31.331	1465594
PBEP0068	PBEP0069	Domestic	58.441624	1.913531	30.541	1411327

PBEP0068	PBEP0066	Domestic	57.58135	1.824227	31.565	1426744
PBEP0068	PBEP0067	Domestic	56.680701	1.803141	31.434	1398764
PBEP0068	PBEP0064	Domestic	56.393183	1.796036	31.399	1443298
PBEP0068	PBEP0065	Domestic	56.737073	1.812383	31.305	1446165
PBEP0068	PBEP0038	Domestic	50.571	1.608067	31.448	1511299
PBEP0068	PBEP0039	Domestic	52.322734	1.654707	31.621	1487696
PBEP0068	PBEP0028	Domestic	44.533386	1.423341	31.288	1518078
PBEP0068	PBEP0036	Domestic	46.901036	1.520153	30.853	1498384
PBEP0069	PBEP0069	Domestic	0	-1	0	-1
PBEP0069	FS15	Domestic	71.417436	2.211924	32.287	882128
PBEP0069	FS8	Domestic	69.39284	2.205899	31.458	1090504
PBEP0069	FS19	Domestic	75.997938	2.354484	32.278	962071
PBEP0069	PBEP0014	Domestic	54.881495	1.770938	30.99	1379219
PBEP0069	PBEP0023	Domestic	54.652481	1.789079	30.548	1370795
PBEP0069	PVIP0012	Domestic	24.554984	0.814497	30.147	1244679
PBEP0069	PBEP0005	Domestic	38.972885	1.292857	30.145	1424331
PBEP0069	PBEP0008	Domestic	39.171144	1.314889	29.79	1419140
PBEP0069	PBEP0009	Domestic	38.861658	1.284087	30.264	1422781
PBEP0069	PBEP0068	Domestic	58.441624	1.913531	30.541	1411327
PBEP0069	PBEP0066	Domestic	58.552373	1.922908	30.45	1395854
PBEP0069	PBEP0067	Domestic	57.836758	1.902992	30.393	1363949
PBEP0069	PBEP0064	Domestic	57.746294	1.89774	30.429	1407299
PBEP0069	PBEP0065	Domestic	58.039258	1.919653	30.234	1410521
PBEP0069	PBEP0038	Domestic	51.890591	1.71557	30.247	1468425
PBEP0069	PBEP0039	Domestic	53.71418	1.762043	30.484	1450464
PBEP0069	PBEP0028	Domestic	45.55532	1.507132	30.226	1477220
PBEP0069	PBEP0036	Domestic	47.900895	1.604823	29.848	1461155
PBEP0066	PBEP0066	Domestic	0	-1	0	-1
PBEP0066	FS15	Domestic	70.576964	2.153776	32.769	884207
PBEP0066	FS8	Domestic	68.65733	2.146608	31.984	1094816
PBEP0066	FS19	Domestic	75.1633	2.299679	32.684	964114
PBEP0066	PBEP0014	Domestic	54.002324	1.700729	31.752	1394879
PBEP0066	PBEP0023	Domestic	53.74023	1.716968	31.299	1386675
PBEP0066	PVIP0012	Domestic	24.290088	0.777031	31.26	1265061
PBEP0066	PBEP0005	Domestic	38.377598	1.235497	31.062	1441564
PBEP0066	PBEP0008	Domestic	38.564098	1.259783	30.612	1434541
PBEP0066	PBEP0009	Domestic	38.251629	1.225227	31.22	1440249
PBEP0066	PBEP0068	Domestic	57.58135	1.824227	31.565	1426744
PBEP0066	PBEP0069	Domestic	58.552373	1.922908	30.45	1395854
PBEP0066	PBEP0067	Domestic	57.043378	1.830176	31.168	1377887
PBEP0066	PBEP0064	Domestic	56.830802	1.8231	31.173	1421893
PBEP0066	PBEP0065	Domestic	57.080744	1.832016	31.157	1425738
PBEP0066	PBEP0038	Domestic	50.912261	1.634442	31.15	1487144

PBEP0066	PBEP0039	Domestic	52.786739	1.686925	31.292	1465598
PBEP0066	PBEP0028	Domestic	44.785824	1.435889	31.19	1493950
PBEP0066	PBEP0036	Domestic	47.165809	1.535822	30.71	1476621
PBEP0067	PBEP0067	Domestic	0	-1	0	-1
PBEP0067	FS15	Domestic	70.503829	2.15352	32.739	851996
PBEP0067	FS8	Domestic	68.672333	2.137885	32.122	1055827
PBEP0067	FS19	Domestic	75.071234	2.287896	32.812	928363
PBEP0067	PBEP0014	Domestic	53.889496	1.691444	31.86	1349399
PBEP0067	PBEP0023	Domestic	53.715082	1.708844	31.434	1340270
PBEP0067	PVIP0012	Domestic	24.293093	0.780768	31.114	1200500
PBEP0067	PBEP0005	Domestic	38.422704	1.239383	31.001	1384954
PBEP0067	PBEP0008	Domestic	38.617735	1.261538	30.612	1378483
PBEP0067	PBEP0009	Domestic	38.330881	1.235669	31.02	1383051
PBEP0067	PBEP0068	Domestic	56.680701	1.803141	31.434	1398764
PBEP0067	PBEP0069	Domestic	57.836758	1.902992	30.393	1363949
PBEP0067	PBEP0066	Domestic	57.043378	1.830176	31.168	1377887
PBEP0067	PBEP0064	Domestic	57.033927	1.826729	31.222	1378841
PBEP0067	PBEP0065	Domestic	57.425261	1.845202	31.121	1380255
PBEP0067	PBEP0038	Domestic	50.865001	1.628588	31.233	1444001
PBEP0067	PBEP0039	Domestic	52.606408	1.68338	31.25	1424429
PBEP0067	PBEP0028	Domestic	44.835111	1.441555	31.102	1445197
PBEP0067	PBEP0036	Domestic	47.149441	1.536355	30.689	1429407
PBEP0064	PBEP0064	Domestic	0	-1	0	-1
PBEP0064	FS15	Domestic	70.628207	2.143967	32.943	885700
PBEP0064	FS8	Domestic	68.828178	2.138988	32.178	1095339
PBEP0064	FS19	Domestic	75.280656	2.28575	32.935	965174
PBEP0064	PBEP0014	Domestic	54.118805	1.708585	31.675	1393811
PBEP0064	PBEP0023	Domestic	53.891182	1.714463	31.433	1385607
PBEP0064	PVIP0012	Domestic	24.218761	0.776431	31.192	1265322
PBEP0064	PBEP0005	Domestic	38.320087	1.237798	30.958	1442591
PBEP0064	PBEP0008	Domestic	38.535139	1.25923	30.602	1436094
PBEP0064	PBEP0009	Domestic	38.248548	1.229581	31.107	1440539
PBEP0064	PBEP0068	Domestic	56.393183	1.796036	31.399	1443298
PBEP0064	PBEP0069	Domestic	57.746294	1.89774	30.429	1407299
PBEP0064	PBEP0066	Domestic	56.830802	1.8231	31.173	1421893
PBEP0064	PBEP0067	Domestic	57.033927	1.826729	31.222	1378841
PBEP0064	PBEP0065	Domestic	57.47477	1.844152	31.166	1420493
PBEP0064	PBEP0038	Domestic	50.949881	1.634895	31.164	1487328
PBEP0064	PBEP0039	Domestic	52.791184	1.687717	31.28	1466847
PBEP0064	PBEP0028	Domestic	44.772867	1.436449	31.169	1494998
PBEP0064	PBEP0036	Domestic	47.143791	1.536757	30.677	1477374
PBEP0065	PBEP0065	Domestic	0	-1	0	-1
PBEP0065	FS15	Domestic	70.682337	2.159481	32.731	887683

PBEP0065	FS8	Domestic	68.826358	2.144858	32.089	1098540
PBEP0065	FS19	Domestic	75.449196	2.293242	32.901	966873
PBEP0065	PBEP0014	Domestic	54.12236	1.701883	31.801	1399547
PBEP0065	PBEP0023	Domestic	53.891158	1.713174	31.457	1391553
PBEP0065	PVIP0012	Domestic	24.356293	0.783999	31.067	1272804
PBEP0065	PBEP0005	Domestic	38.46402	1.24436	30.911	1448224
PBEP0065	PBEP0008	Domestic	38.647117	1.265993	30.527	1441722
PBEP0065	PBEP0009	Domestic	38.352801	1.237209	30.999	1446463
PBEP0065	PBEP0068	Domestic	56.737073	1.812383	31.305	1446165
PBEP0065	PBEP0069	Domestic	58.039258	1.919653	30.234	1410521
PBEP0065	PBEP0066	Domestic	57.080744	1.832016	31.157	1425738
PBEP0065	PBEP0067	Domestic	57.425261	1.845202	31.121	1380255
PBEP0065	PBEP0064	Domestic	57.47477	1.844152	31.166	1420493
PBEP0065	PBEP0038	Domestic	51.049324	1.641499	31.099	1493130
PBEP0065	PBEP0039	Domestic	52.893594	1.693741	31.229	1471647
PBEP0065	PBEP0028	Domestic	44.908315	1.455199	30.861	1499890
PBEP0065	PBEP0036	Domestic	47.319543	1.547917	30.57	1482257
PBEP0038	PBEP0038	Domestic	0	-1	0	-1
PBEP0038	FS15	Domestic	62.92979	1.919277	32.788	915250
PBEP0038	FS8	Domestic	61.557468	1.923598	32.001	1135282
PBEP0038	FS19	Domestic	67.478661	2.06905	32.613	998387
PBEP0038	PBEP0014	Domestic	48.951724	1.532748	31.937	1449191
PBEP0038	PBEP0023	Domestic	48.685729	1.54992	31.412	1441364
PBEP0038	PVIP0012	Domestic	24.560739	0.782955	31.369	1278932
PBEP0038	PBEP0005	Domestic	43.72363	1.403629	31.15	1392550
PBEP0038	PBEP0008	Domestic	43.885647	1.430986	30.668	1385733
PBEP0038	PBEP0009	Domestic	43.641591	1.394671	31.292	1391361
PBEP0038	PBEP0068	Domestic	50.571	1.608067	31.448	1511299
PBEP0038	PBEP0069	Domestic	51.890591	1.71557	30.247	1468425
PBEP0038	PBEP0066	Domestic	50.912261	1.634442	31.15	1487144
PBEP0038	PBEP0067	Domestic	50.865001	1.628588	31.233	1444001
PBEP0038	PBEP0064	Domestic	50.949881	1.634895	31.164	1487328
PBEP0038	PBEP0065	Domestic	51.049324	1.641499	31.099	1493130
PBEP0038	PBEP0039	Domestic	52.325796	1.674354	31.251	1462386
PBEP0038	PBEP0028	Domestic	48.251508	1.536723	31.399	1453227
PBEP0038	PBEP0036	Domestic	49.911117	1.611623	30.969	1441111
PBEP0039	PBEP0039	Domestic	0	-1	0	-1
PBEP0039	FS15	Domestic	65.392363	1.992234	32.824	907600
PBEP0039	FS8	Domestic	64.069036	1.998685	32.056	1123996
PBEP0039	FS19	Domestic	69.956229	2.136028	32.751	990065
PBEP0039	PBEP0014	Domestic	50.68284	1.589837	31.879	1429287
PBEP0039	PBEP0023	Domestic	50.451412	1.603709	31.459	1420513
PBEP0039	PVIP0012	Domestic	24.501967	0.786403	31.157	1269500

PBEP0039	PBEP0005	Domestic	42.41455	1.37545	30.837	1402302
PBEP0039	PBEP0008	Domestic	42.62025	1.396354	30.523	1396048
PBEP0039	PBEP0009	Domestic	42.336655	1.367342	30.963	1400471
PBEP0039	PBEP0068	Domestic	52.322734	1.654707	31.621	1487696
PBEP0039	PBEP0069	Domestic	53.71418	1.762043	30.484	1450464
PBEP0039	PBEP0066	Domestic	52.786739	1.686925	31.292	1465598
PBEP0039	PBEP0067	Domestic	52.606408	1.68338	31.25	1424429
PBEP0039	PBEP0064	Domestic	52.791184	1.687717	31.28	1466847
PBEP0039	PBEP0065	Domestic	52.893594	1.693741	31.229	1471647
PBEP0039	PBEP0038	Domestic	52.325796	1.674354	31.251	1462386
PBEP0039	PBEP0028	Domestic	47.650117	1.532367	31.096	1459517
PBEP0039	PBEP0036	Domestic	49.743939	1.61294	30.841	1444072
PBEP0028	PBEP0028	Domestic	0	-1	0	-1
PBEP0028	FS15	Domestic	54.652416	1.671718	32.692	911959
PBEP0028	FS8	Domestic	53.800577	1.676275	32.095	1134106
PBEP0028	FS19	Domestic	58.522595	1.79248	32.649	997491
PBEP0028	PBEP0014	Domestic	43.426948	1.377289	31.531	1445233
PBEP0028	PBEP0023	Domestic	43.196526	1.382828	31.238	1435808
PBEP0028	PVIP0012	Domestic	25.061814	0.803433	31.193	1202629
PBEP0028	PBEP0005	Domestic	50.648952	1.643772	30.813	1261340
PBEP0028	PBEP0008	Domestic	50.883481	1.662097	30.614	1255154
PBEP0028	PBEP0009	Domestic	50.585553	1.634403	30.95	1259817
PBEP0028	PBEP0068	Domestic	44.533386	1.423341	31.288	1518078
PBEP0028	PBEP0069	Domestic	45.55532	1.507132	30.226	1477220
PBEP0028	PBEP0066	Domestic	44.785824	1.435889	31.19	1493950
PBEP0028	PBEP0067	Domestic	44.835111	1.441555	31.102	1445197
PBEP0028	PBEP0064	Domestic	44.772867	1.436449	31.169	1494998
PBEP0028	PBEP0065	Domestic	44.908315	1.455199	30.861	1499890
PBEP0028	PBEP0038	Domestic	48.251508	1.536723	31.399	1453227
PBEP0028	PBEP0039	Domestic	47.650117	1.532367	31.096	1459517
PBEP0028	PBEP0036	Domestic	51.988861	1.676199	31.016	1360165
PBEP0036	PBEP0036	Domestic	0	-1	0	-1
PBEP0036	FS15	Domestic	57.967153	1.790102	32.382	907314
PBEP0036	FS8	Domestic	56.795691	1.798561	31.578	1126856
PBEP0036	FS19	Domestic	61.711387	1.896681	32.537	992332
PBEP0036	PBEP0014	Domestic	45.649228	1.458796	31.292	1430275
PBEP0036	PBEP0023	Domestic	45.449093	1.469906	30.92	1420822
PBEP0036	PVIP0012	Domestic	24.969234	0.808118	30.898	1209863
PBEP0036	PBEP0005	Domestic	48.824514	1.58665	30.772	1290000
PBEP0036	PBEP0008	Domestic	49.032855	1.617148	30.321	1285300
PBEP0036	PBEP0009	Domestic	48.71554	1.581173	30.81	1288682
PBEP0036	PBEP0068	Domestic	46.901036	1.520153	30.853	1498384
PBEP0036	PBEP0069	Domestic	47.900895	1.604823	29.848	1461155

PBEP0036	PBEP0066	Domestic	47.165809	1.535822	30.71	1476621
PBEP0036	PBEP0067	Domestic	47.149441	1.536355	30.689	1429407
PBEP0036	PBEP0064	Domestic	47.143791	1.536757	30.677	1477374
PBEP0036	PBEP0065	Domestic	47.319543	1.547917	30.57	1482257
PBEP0036	PBEP0038	Domestic	49.911117	1.611623	30.969	1441111
PBEP0036	PBEP0039	Domestic	49.743939	1.61294	30.841	1444072
PBEP0036	PBEP0028	Domestic	51.988861	1.676199	31.016	1360165

Table S7 Results of outgroup f3 analysis of leopard cats conducted on populations.

Source1	Source2	Target	f_3	std.err	Z	SNPs
Ancient	North	Domestic	70.93032	2.012327	35.248	1426324
Ancient	Fishing_cat	Domestic	25.78662	0.765319	33.694	1293957
Ancient	Sunda	Domestic	40.638839	1.147033	35.43	1755910
Ancient	Indochina	Domestic	60.78772	1.715523	35.434	2131220
Ancient	Malay	Domestic	52.481229	1.489341	35.238	2056985
North	Ancient	Domestic	70.93032	2.012327	35.248	1426324
North	Fishing_cat	Domestic	23.719445	0.74542	31.82	1408412
North	Sunda	Domestic	36.201983	1.109549	32.628	1892885
North	Indochina	Domestic	52.033974	1.596423	32.594	2310369
North	Malay	Domestic	45.528832	1.399902	32.523	2224408
Fishing_cat	Ancient	Domestic	25.78662	0.765319	33.694	1293957
Fishing_cat	North	Domestic	23.719445	0.74542	31.82	1408412
Fishing_cat	Sunda	Domestic	25.317031	0.798271	31.715	1403772
Fishing_cat	Indochina	Domestic	24.095854	0.753765	31.967	2240202
Fishing_cat	Malay	Domestic	24.530297	0.767601	31.957	2043424
Sunda	Ancient	Domestic	40.638839	1.147033	35.43	1755910
Sunda	North	Domestic	36.201983	1.109549	32.628	1892885
Sunda	Fishing_cat	Domestic	25.317031	0.798271	31.715	1403772
Sunda	Indochina	Domestic	36.968113	1.133961	32.601	2603046
Sunda	Malay	Domestic	44.559514	1.369964	32.526	2246719
Indochina	Ancient	Domestic	60.78772	1.715523	35.434	2131220
Indochina	North	Domestic	52.033974	1.596423	32.594	2310369
Indochina	Fishing_cat	Domestic	24.095854	0.753765	31.967	2240202
Indochina	Sunda	Domestic	36.968113	1.133961	32.601	2603046
Indochina	Malay	Domestic	47.036042	1.442084	32.617	2682885
Malay	Ancient	Domestic	52.481229	1.489341	35.238	2056985
Malay	North	Domestic	45.528832	1.399902	32.523	2224408
Malay	Fishing_cat	Domestic	24.530297	0.767601	31.957	2043424
Malay	Sunda	Domestic	44.559514	1.369964	32.526	2246719
Malay	Indochina	Domestic	47.036042	1.442084	32.617	2682885

Table S8 F4-statistics results of leopard cats.

O	C	pop	B	A	D	stdr	Z
Domestic	PBEP0068	PBEP0068	Far_East_Russia	FS8	0.001928	0.000114	16.92
Domestic	PBEP0069	PBEP0069	Far_East_Russia	FS8	0.002137	0.00012	17.775
Domestic	PBEP0066	PBEP0066	Far_East_Russia	FS8	0.002049	0.000116	17.679
Domestic	PBEP0067	PBEP0067	Far_East_Russia	FS8	0.001995	0.00012	16.601
Domestic	PBEP0064	PBEP0064	Far_East_Russia	FS8	0.002102	0.000117	17.903
Domestic	PBEP0065	PBEP0065	Far_East_Russia	FS8	0.001924	0.000118	16.301
Domestic	PBEP0038	PBEP0038	Far_East_Russia	FS8	0.001492	0.00011	13.516
Domestic	PBEP0039	PBEP0039	Far_East_Russia	FS8	0.001671	0.000107	15.668
Domestic	PBEP0028	PBEP0028	Far_East_Russia	FS8	0.000921	0.000089	10.387
Domestic	PBEP0036	PBEP0036	Far_East_Russia	FS8	0.001149	0.000092	12.492
Domestic	PBEP0005	PBEP0005	Far_East_Russia	FS8	0.000262	0.000071	3.703
Domestic	PBEP0008	PBEP0008	Far_East_Russia	FS8	0.000294	0.000074	3.954
Domestic	PBEP0009	PBEP0009	Far_East_Russia	FS8	0.000261	0.000073	3.573
Domestic	PVIP0012	PVP0012	Far_East_Russia	FS8	0.000202	0.000064	3.173
Domestic	PBEP0068	PBEP0068	Far_East_Russia	FS15	0.002408	0.00015	16.032
Domestic	PBEP0069	PBEP0069	Far_East_Russia	FS15	0.002671	0.000148	18.103
Domestic	PBEP0066	PBEP0066	Far_East_Russia	FS15	0.002503	0.000149	16.786
Domestic	PBEP0067	PBEP0067	Far_East_Russia	FS15	0.002461	0.000155	15.885
Domestic	PBEP0064	PBEP0064	Far_East_Russia	FS15	0.002494	0.000152	16.439
Domestic	PBEP0065	PBEP0065	Far_East_Russia	FS15	0.002366	0.000155	15.312
Domestic	PBEP0038	PBEP0038	Far_East_Russia	FS15	0.0017	0.000138	12.338
Domestic	PBEP0039	PBEP0039	Far_East_Russia	FS15	0.001881	0.000139	13.565
Domestic	PBEP0028	PBEP0028	Far_East_Russia	FS15	0.000912	0.000109	8.337
Domestic	PBEP0036	PBEP0036	Far_East_Russia	FS15	0.001289	0.000102	12.622
Domestic	PBEP0005	PBEP0005	Far_East_Russia	FS15	0.000297	0.000087	3.432
Domestic	PBEP0008	PBEP0008	Far_East_Russia	FS15	0.000306	0.000083	3.709
Domestic	PBEP0009	PBEP0009	Far_East_Russia	FS15	0.00028	0.000086	3.264
Domestic	PVIP0012	PVP0012	Far_East_Russia	FS15	0.000264	0.000071	3.728
Domestic	PBEP0068	PBEP0068	Far_East_Russia	FS19	0.002581	0.00012	21.513
Domestic	PBEP0069	PBEP0069	Far_East_Russia	FS19	0.002805	0.000121	23.159
Domestic	PBEP0066	PBEP0066	Far_East_Russia	FS19	0.002478	0.000121	20.545
Domestic	PBEP0067	PBEP0067	Far_East_Russia	FS19	0.002569	0.000122	21.055
Domestic	PBEP0064	PBEP0064	Far_East_Russia	FS19	0.002637	0.000118	22.31
Domestic	PBEP0065	PBEP0065	Far_East_Russia	FS19	0.002566	0.000118	21.719
Domestic	PBEP0038	PBEP0038	Far_East_Russia	FS19	0.001884	0.000108	17.379
Domestic	PBEP0039	PBEP0039	Far_East_Russia	FS19	0.002145	0.000107	20.029
Domestic	PBEP0028	PBEP0028	Far_East_Russia	FS19	0.00111	0.00009	12.295
Domestic	PBEP0036	PBEP0036	Far_East_Russia	FS19	0.00141	0.000093	15.156
Domestic	PBEP0005	PBEP0005	Far_East_Russia	FS19	0.000399	0.000075	5.324
Domestic	PBEP0008	PBEP0008	Far_East_Russia	FS19	0.000491	0.000076	6.437
Domestic	PBEP0009	PBEP0009	Far_East_Russia	FS19	0.000408	0.000078	5.198
Domestic	PVIP0012	PVP0012	Far_East_Russia	FS19	0.000269	0.000066	4.061

Permissions from co-authors

I hereby give permission to Yu Han to use our joint work "Early commensalism between leopard cat (*Prionailurus bengalensis*) and human in ancient China" as a contribution towards his DPhil thesis, to be submitted for examination at the University of Oxford.

I confirm that to the best of my knowledge, the author contribution statement below is accurate, that Yu Han's overall contribution was equal or greater to that of the other co-first authors.

Author contributions:

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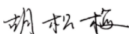
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4 Human-mediated translocation of fallow deer (*Dama dama*) in Western Eurasia from an ancient DNA perspective

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Abstract

The Holocene human-mediated transfer of animals has had a substantial impact on global biogeography. The fallow deer (*Dama dama*), encompassing the Persian fallow deer (*Dama dama mesopotamica*) and the European fallow deer (*Dama dama dama*), exemplifies a species whose post-glacial distribution was largely facilitated by human activity. To investigate the mechanisms underlying fallow deer distribution and their evolutionary history, we generated and analysed 29 partial or complete mitochondrial genomes of both species and subspecies. Utilizing ancient genetic techniques, we determined that the two species or subspecies of fallow deer diverged approximately 0.57 million years ago, with evidence suggesting potential interbreeding in Southwestern Anatolia. Through historical maritime trading routes, the Persian fallow deer from the Levant were transported to Cyprus and subsequently westward to the Mediterranean region. Concurrently, the European fallow deer originating from Anatolia were dispersed throughout continental Europe.

Key words: Fallow deer; human-mediated dispersal; ancient DNA.

4.1 Introduction

Since the Holocene, one of the most profound and far-reaching events in natural history has been the anthropogenic modification of faunal biogeography (Banks et al., 2015; Capinha et al., 2015). This period saw the translocation of animals, especially those with biological or social bonds to humans, from their original habitats to colonize new ecosystems (Dobney et al., 2008; Gippet et al., 2019; Xiang et al., 2022). The fallow deer (*Dama dama*) serves as a prime example of this phenomenon, with its post-glacial distribution being largely mediated by human activity (Chapman and Chapman, 1980).

Belonging to the Cervidae family, the fallow deer comprises two species or subspecies: the Persian fallow deer (*Dama dama mesopotamica*) and the European fallow deer (*Dama dama dama*). These two species or subspecies are hypothesized to have diverged between 0.4 to 4 million years ago (Masseti et al., 2008; Pitra et al., 2004). However, their classification as distinct species or subspecies remains a topic of debate due to differences in their natural habitats and morphology (Chapman and Chapman, 1975, 1980). Historically, the fallow deer had an extensive range. The distribution of the Persian fallow deer is thought to have extended from the western Iranian plateau to the Mediterranean Sea and from southern Anatolia to Palestine (Uerpmann, 1987: 61-sq). The European fallow deer, on the other hand, likely had a distribution that encompassed various parts of Europe and Anatolia (Baker et al., 2017; Uerpmann, 1987). It is possible that the native ranges of these two species or subspecies once overlapped in southeastern Anatolia (Uerpmann 1987).

Fallow deer has played a significant role in human societies throughout history. Since the Neolithic period, it has been intensively exploited and used for various purposes, such as a food source (Vigne et al., 2016), medicine, and cultural objects including decorations, ritual artifacts, and religious deities (Miller and Sykes, 2016). Additionally, fallow deer have been highly valued as one of the most popular game animals (De Marinis and Masseti, 2021; Zenke et al., 2022) and as ornaments in deer parks (Fletcher, 2011; Ward, 2017). This deep-rooted association with humans further highlights the anthropogenic influence on the biogeography and conservation of the fallow deer, demonstrating the interconnectedness of human and natural history.

The genetic diversity of the two fallow deer species or subspecies is currently extremely low (Baker et al., 2017; Fernández-García, 2012). Persian fallow deer, thought to be extinct in the Mediterranean regions since the 16th and 17th centuries, was rediscovered and described by the British Vice Consul in 1875 AD. Believed to have disappeared worldwide in the 1940s, a well-established wild population was found in southwestern Iran during the 1950s, leading to the establishment of a wildlife reserve (Haltenorth, 1961; Jantschke, 1990).

The European fallow deer seemingly became extinct in most European regions at least 10,000 years ago (Chapman & Chapman, 1975, 1997; Masseti et al., 1996). Today, Anatolia serves as the centre of the European fallow deer's most secure native range (Baker et al., 2017). By 1934, they were restricted to the western Taurus foothills (Chapman & Chapman, 1975, 1997), and after the 1960s, only a small wild population has survived in the Duzlercami Wildlife Reserve

(Termessos National Park, Antalya) in Anatolia, established in the mid-1960s (De Marinis and Masseti, 2009; Sayan and Atik, 2011; Ünal et al., 2022). The fallow deer residing in these two reserves represent the only two naturally established wild populations worldwide.

Past climate changes have had a significant impact on the population dynamics of both fallow deer species. The Persian fallow deer experienced an expansion in the early Holocene when they began to appear in a series of sites in the Near East. Some research suggests that increased aridity in their home range around Mount Carmel from 14,000 BP onwards could have driven this expansion. The first Holocene massive extinction of ungulates in southern Levant during the Iron Age (1,200-586 BC), a major habitat for Persian fallow deer, may have played a crucial role in their population decline (Belmaker, 2017; Tsahar et al., 2009).

The endangered state of the European fallow deer is attributed to the last glacial period. The ice age dramatically transformed the bio-landscape of Western Eurasia, significantly reducing the fallow deer's habitat and variability. Current knowledge suggests that the European fallow deer could have most likely survived in southern Anatolia during the last Glacial Period (Masetti et al., 2008, 1996; Masetti and Rustioni, 1988). The survival of only small populations in a few restricted regions may have led to a genetic bottleneck, causing the loss of a substantial proportion of the species' genetic variation. This reduction in genetic diversity could have diminished the European fallow deer's ability to adapt to new natural environments outside of their refuges, consequently constraining their potential to naturally recolonize Europe following glaciation. (Sykes, 2004a; Nei et al., 1975).

Both the Persian and European fallow deer experienced rapid diffusion during the Holocene epoch. This widespread distribution was significantly influenced by human activities, which facilitated their translocation across various regions (Chapman & Chapman, 1975, 1980).

One of the most salient examples of human-mediated translocation of Persian fallow deer is their presence on Mediterranean islands. Although absent from the Late Glacial mammalian faunal remains in Cyprus, the Persian fallow deer first appeared on the island around the eighth millennium BC (Daujat, 2013; Vigne et al., 2016). Subsequently, Cyprus became one of the few, if not the only, locations where fallow deer contributed substantially to human subsistence during the Holocene, from the Early Neolithic to the Bronze Age (Croft, 2002; Flourentzos, 2002; Vigne et al., 2016). Given the considerable distance between Cyprus and the mainland of the Near East, it is evident that the fallow deer must have been transported to the island via boat. Despite this understanding, the precise origins of the Cypriot Persian fallow deer remain uncertain. Comparisons with samples from the middle Euphrates River Valley, dating between 8,000 and 6,000 BC, reveal a significantly smaller body size in the Persian fallow deer, suggesting that they did not originate from this region (Vigne et al., 2016). Consequently, the deer must have originated from another location within the Levant.

Another intriguing example of fallow deer translocation is the population found in Mallorca. Situated in the Western Mediterranean, far from the natural range of Persian fallow deer, the island hosted a mysterious population during the Roman Period. It is believed that the fallow deer were first introduced to the island in the 2nd century AD and proliferated in the 4th century AD (Pinya & Lassnig, 2018; Valenzuela et al., 2016). Mitochondrial DNA analysis confirms

their classification as Persian fallow deer, forming a monophyletic clade; however, their origins remain enigmatic (Sykes et al., 2011). Intriguingly, morphological analysis suggests that the Mallorca fallow deer may have descended from European fallow deer. This discrepancy between genetic and morphological classifications has led some researchers to hypothesize that the Mallorca population could be hybrids (Valenzuela et al., 2016).

The translocation of European fallow deer was more frequent and extensive. Following human footsteps, they have recolonized the Aegean region from Anatolia (Baker et al., 2017). It is believed that fallow deer was introduced to mainland Greece and Aegean Island such as Makri, Crete and Rhode Rhodian since Neolithic (De Marinis and Masseti, 2021; Hubbard, 1995; Sykes et al., 2013). Same age of death frequencies from Late Neolithic sites throughout Greece and Anatolia indicated the two places might have been sharing the same herd management or exploitation pattern such as ranching or parking (Hubbard, 1995). The similarity in *D. dama dama* faunal assemblages between Anatolia and Greece is unlikely to be a coincidence. The extensive trade routes existed between Asia Minor, mainland and island Greece facilitating the dispersal and introduction of *D. dama dama* across the Aegean (Michailidou and Dogan, 2008; Sykes et al., 2013).

In addition to the Aegean region, fallow deer expanded northward to Western Europe. Facilitated by human intervention, they reached Western Europe (Lepetz & Jean-Herve, 2002), the Apennine Peninsula (Bökönyi, 1986; Madgwick et al., 2013), Iberia (Davis & MacKinnon, 2009), and Britain (Sykes, 2004b) from the Roman Period to the Medieval Ages.

Regarding the British fallow deer (Beglane et al., 2018; Sykes, 2004b; Sykes et al., 2011), although it is evident that humans introduced the species, the specific group responsible for the introduction, the timing of the establishment, and the origin of the imported deer remain ambiguous. Four groups, the Phoenicians, Romans, Saxons, and Normans, have been hypothesized as the potential agents of introduction (Sykes, 2004). The Phoenicians may have been responsible for the first occurrence of fallow deer in Britain; however, this theory is supported by minimal evidence, such as a single shed antler. This antler could also come to the region through trade of dismembered body parts from dead fallow deer rather than a locally established population.

During the Roman Period, fallow deer remains were discovered at various sites (Sykes et al., 2011). The genetics, diet, body size, and gender composition of the remains from sites such as Monkon and Thanet suggest a local population origin, although some bones may have been derived from traded carcasses (Miller et al., 2016; Sykes et al., 2006). It is believed that Roman British fallow deer were not directly imported from Anatolia or Greece, as the ancient individuals did not genetically cluster with any modern haplotypes from those regions. Furthermore, this population did not survive the Roman withdrawal from the British Isles. Subsequently, the Anglo-Saxons are thought to have reintroduced the species, after which the fallow deer population began to flourish (Sykes, 2004, 2010; Jones, 1963).

The historical dispersal of fallow deer represents one of the most substantial and widespread migrations of animals facilitated by human intervention. Although numerous global fallow deer

populations are evidently the product of anthropogenic translocations, their source populations remain uncertain (Baker et al., 2017; Vigne et al., 2016). This ambiguity is primarily attributed to the limited availability of reference samples, a consequence of the reduced states of both Persian and European fallow deer and the disappearance of many populations. Consequently, ancient samples can serve a crucial role in addressing this knowledge gap.

In this study, we have generated 29 partial or near-complete ancient mitochondrial genomes of Persian and European fallow deer from 13 sites spanning a period of at least 8,000 years. This was achieved through targeted capture and Next Generation Sequencing (NGS) techniques. By analysing these samples, we aim to identify the origins of populations throughout Western Eurasia and gain valuable insights into the processes underlying the anthropogenic movement of animals.

4.2 Materials

To investigate the post-glacial dispersal of fallow deer, we assembled a collection of 125 samples from 20 sites situated along the eastern and northern coasts of the Mediterranean Sea as well as England, covering a time span of approximately 8,000 years. This sample set encompasses both Persian and European fallow deer (Figure 18 & Table S9).



Figure 18 Sampling locations of fallow deer. There are 125 samples from 20 sites across the east and north coast of Mediterranean Sea and England spanning 8,000 years. The sample set contains both Persian and European fallow deer. The blue line separates the natural range of Persian and European fallow deer.

4.3 Methods

4.3.1 Ancient DNA laboratory methods

4.3.1.1 Sample preparation

All samples were processed in a dedicated ancient DNA laboratory at the School of Archaeology, University of Oxford. To minimize the risk of contamination from experimenters, protective clothing (3M) was worn throughout the procedure. Bone samples, ranging between 100-250 mg, were prepared using a Dremel multi-tool drill. The bone surface was carefully

removed with a circular cutting disk to eliminate any exogenous DNA present on the exterior. To minimize the possibility of cross-contamination, the drill was cleaned with 10% bleach after each sample, and both the spindle and the disposable disk were replaced after cutting each sample. The benchtop was also sanitized with 10% bleach and 70% ethanol between each sample, and gloves were changed frequently. Negative controls for each batch of tested samples at every stage of the laboratory procedures, from extraction to capture. Bones were subsequently powdered using a micro-abrader. All equipment and surfaces that had been in contact with the samples were meticulously cleaned between each sample using 10% bleach and 70% ethanol.

4.3.1.2 Extraction

DNA extraction was performed using a protocol based on Dabney et al. (2013a), incorporating a pre-digestion step according to Damgaard et al. (2015). The bone powder was pre-digested in 1 ml of extraction buffer, containing water, 10 mg/ml Proteinase K, TWEEN 20, and 0.5 M EDTA at pH 8.0, for 30 minutes to further release and wash off exogenous DNA from the surface of the bone particles. Following this, the samples were centrifuged for one minute, and the supernatants were discarded. The bone powder was then incubated overnight in 1 ml of fresh extraction buffer to ensure thorough dissolution of the powder. Subsequently, the samples were centrifuged to pellet the bone powder, and the supernatant was collected and combined with sodium acetate and binding buffer, which contained guanidine hydrochloride, isopropanol, TWEEN 20, and water. The DNA was then collected by spinning the samples mixed with sodium acetate and binding buffer through MinElute spin columns. The columns were washed

with PE buffer (QIAGEN) and finally eluted with 42 µl of TET (0.5 M EDTA at pH 8.0, Tris HCl at pH 8.0, TWEEN 20, and water) into a fresh Lo-bind collection tube.

4.3.1.3 Library Building

See Methods Chapter 2

4.3.1.4 Screening

All samples subsequently underwent screening sequencing on a single lane of an Illumina HiSeq 4000 at Microgene, Sacramento, USA. The sequencing data were then mapped to the *Dama dama* isolate CYTO mitochondrion complete genome (NCBI: NC_020700.1). For each site, samples with the highest mapping efficiencies for mitochondrial genome capture were selected. Ultimately, a total of 55 samples, ranging from 2 to 5 per site, were chosen for further analysis.

4.3.1.5 Ancient mitochondrial DNA capture

In-solution targeted capture of the fallow deer mitochondrial genome was performed on 55 samples using the myBaits (Arbor Biosciences) Hybridization Capture for Targeted Next-Generation Sequencing (NGS) protocol. The following conditions were applied: a hybridization temperature of 60 °C and a hybridization time of 48 hours. The *Dama dama* isolate CYTO mitochondrion complete genome (NCBI:NC_020700.1) was utilized to synthesize the baits for capture. Due to the degraded nature of ancient DNA, the amount of endogenous DNA per sample is significantly lower than in modern samples. As a result, 5 to 7

samples were pooled equimolarly per reaction. The pools were determined based on the percentage of endogenous DNA with a mapping quality above 30. The capture products of the 55 samples, divided into 9 capture reactions (Table S10), were subsequently sequenced on an Illumina HiSeq 4000 at Microgene, Sacramento, USA.

4.3.2 Bioinformatics methods

4.3.2.1 Raw Data filtering

See Methods Chapter 2.

4.3.2.2 Mapping

The cleaned data was then mapped to the *Dama dama* isolate CYTO mitochondrion, complete genome (NC_020700.1) using the aln function in BWA v0.1.17 (Li, 2013) with the settings -n 0.01 -o 2 -l 1024 . SAMtools v.1.14 (Danecek et al., 2021) were then used to sort the bam files, filter out the PCR duplicates, unmapped read, or the reads with the mapping quality that were less than 30. HTSbox was used to call consensus mitochondrial sequences with the default settings. The consensus sequences were then aligned to each other with MUSCLE v.5 (Edgar, 2021).

4.3.2.3 Phylogeny

A maximum likelihood (ML) tree was constructed using IQ-TREE v.2 (Minh et al., 2020; Nguyen et al., 2015), following the TN93+I+G model with 1,000 replicates. In total, 55 fallow

deer were captured and sequenced. Only samples with coverage over 30% were retained. After filtering, 29 samples from 13 sites were utilized in the phylogenetic analysis. A red deer (*Cervus elaphus*, NCBI: NC_007704.2) was selected as the outgroup. The tree included 29 ancient samples, as well as a modern Persian fallow deer (NCBI: JN632630.1) and a modern European fallow deer (NCBI: NC_020700.1) as reference points.

4.4 Results and discussions

4.4.1 Data generation

Fallow deer from 22 sites across Western Eurasia were captured and sequenced, with only samples having coverage over 30% retained for further analysis (Table 8). After filtering, 29 samples from 13 sites, spanning from the early Holocene to the Medieval Age, were included in downstream analyses. The sequencing depths ranged from 0.5X to 38X (Table 8). Out of the initial sample size of 125 bone or teeth specimens, only 23.2% were utilized in the final analysis. The low success rate is primarily attributed to the poor preservation conditions of the faunal remains (Table 8). Excluding the five samples from England, 96% of the samples were excavated from regions or islands surrounding the Mediterranean, where relatively high temperatures accelerated DNA degradation (Karni et al., 2013).

Table 8 Context information and sequencing results of samples used in downstream bioinformatics analysis.

ID	Reads_mapped(Q30)	coverage(%)	depth(X)	country	Site	Time
YH006	184	73.2	1.87	Lebanon	Sidon	Neolithic-Medieval
YH008	323	87.1	2.71	Lebanon	Fadous	3000BC

YH04 5	1577	81.4552	5.3	Turkey	Yeni Kapi	400BC-1000AD
YH06 0	324	71.7273	1.48	Spain	Sa Mesquida (Mallorca)	5th to 6th century AD
YH06 8	2715	98.8872	12.3	Spain	Pollentia (Mallorca)	5th to 6th century AD
YH07 3	219	80.269	2.07	Lebano n	Tell Arqa	1350BC
YH07 4	481	73.421	1.59	Lebano n	Tell Arqa	1350BC
YH07 6	212	88.4	2.56	Lebano n	Kroum el Jabal Cave	18000-8000BC
YH10 4	48	35.26	0.49	Cyprus	Politiko-Troullia	2100-1850 BC
YH10 7	269	73.84	2.57	Turkey	Sirkeli Höyük	1290-1240BC
YH10 8	150	75.8	1.59	Turkey	Sirkeli Höyük	1290-1240BC
YH10 9	3389	97.6338	11.3	Turkey	Lidar Höyük	3000BC-1300AD
YH11 0	1885	84.8976	6.73	Greece	Makri	Neolithic
YH11 1	2759	86.9887	9.06	Greece	Makri	Neolithic
YH11 2	1663	82.4396	4.93	Greece	Makri	Neolithic
YH11 3	1039	77.8294	3.54	Greece	Makri	Neolithic
YH11 4	374	58.0801	1.27	Greece	Makri	Neolithic
YH11 5	4687	90.2171	18.06	England	Dudley Castle	Medieval
YH11 6	2349	84.7631	8.83	England	Dudley Castle	Medieval
YH11 7	865	73.4026	3.14	England	Dudley Castle	Medieval
YH11 8	7421	99.6026	37.74	Spain	Sa Mesquida (Mallorca)	5th to 6th century AD
YH12 0	9032	99.9022	37.67	Spain	Sa Mesquida (Mallorca)	5th to 6th century AD
YH12 2	1141	98.3186	7.22	Spain	Pollentia (Mallorca)	5th to 6th century AD

YH12 4	595	90.9691	3.27	Spain	Sa Mesquita (Mallorca)	5th to 6th century AD
YH12 5	595	66.3895	2.69	England	Faccombe Netherton	Medieval
YH13 4	124	62	1.07	Turkey	Tell Atchana	2000-1200BC
YH13 6	119	64	1.18	Turkey	Tell Atchana	2000-1200BC
YH13 7	88	41	0.8	Turkey	Tell Atchana	2000-1200BC
YH13 8	227	68	1.48	Turkey	Tell Atchana	2000-1200BC

4.4.2 Phylogeny

In the tree (Figure 19), Persian and European fallow deer are separated and have diverged into two primary clades. The Persian clade (orange) contains 18 ancient samples (62.07%) from Anatolia (N=6), Cyprus (N=1), Levant (N=5), and Mallorca (N=6). The European clade (Green) contains 11 ancient samples (37.93%) from Anatolia (N=2), Makri, Greece (N=5) and England (N=4).

The Cyprus samples (YH104) from Politiko Troullia (2100-1850 BC) are closely related to samples from Turkey (YH136, YH108, YH137, YH134, and YH107) and Lebanon (YH074, YH073, YH008, YH006, and YH076) within the tree. This close genetic relationship suggests shared material ancestries among fallow deer populations from Cyprus, Turkey, and Lebanon, indicating shared ancestry or migration events among these regions.

The Mallorca samples (YH060, YH124, YH122, YH118, YH120, and YH068) represent the Persian fallow deer and form a distinct clade within the phylogenetic tree. These samples

include specimens from Sa Mesquida (YH124, YH118, and YH120) and Pollentia (YH122 and YH068), both from Mallorca, Spain. The separate lineage of the Persian fallow deer suggests a unique evolutionary history compared to other fallow deer populations.

The Makri samples (YH112, YH111, YH110, and YH113) from Greece (Neolithic period), and English samples (YH125, YH117, YH115, and YH116) are found within the European fallow deer clade, indicating a shared ancestry or gene flow with other European fallow deer populations. The English samples from Faccombe Netherton and Dudley Castle (Medieval period) formed a monophyletic group. This clustering demonstrates a close genetic relationship among English fallow deer populations from the Medieval period.

And notably, both the Persian and European mitochondrial genomes are found in the Tell Atchana individuals (Figure 19). Like what has been presented in the tree, samples from the same site, Tell Atchana, Turkey including YH134, YH136, and YH137 fell into the Persian clade, while YH138 (Tell Atchana, Turkey) clustered with the European group. It demonstrates that the Persian and European fallow deer were co-existing in the same region.

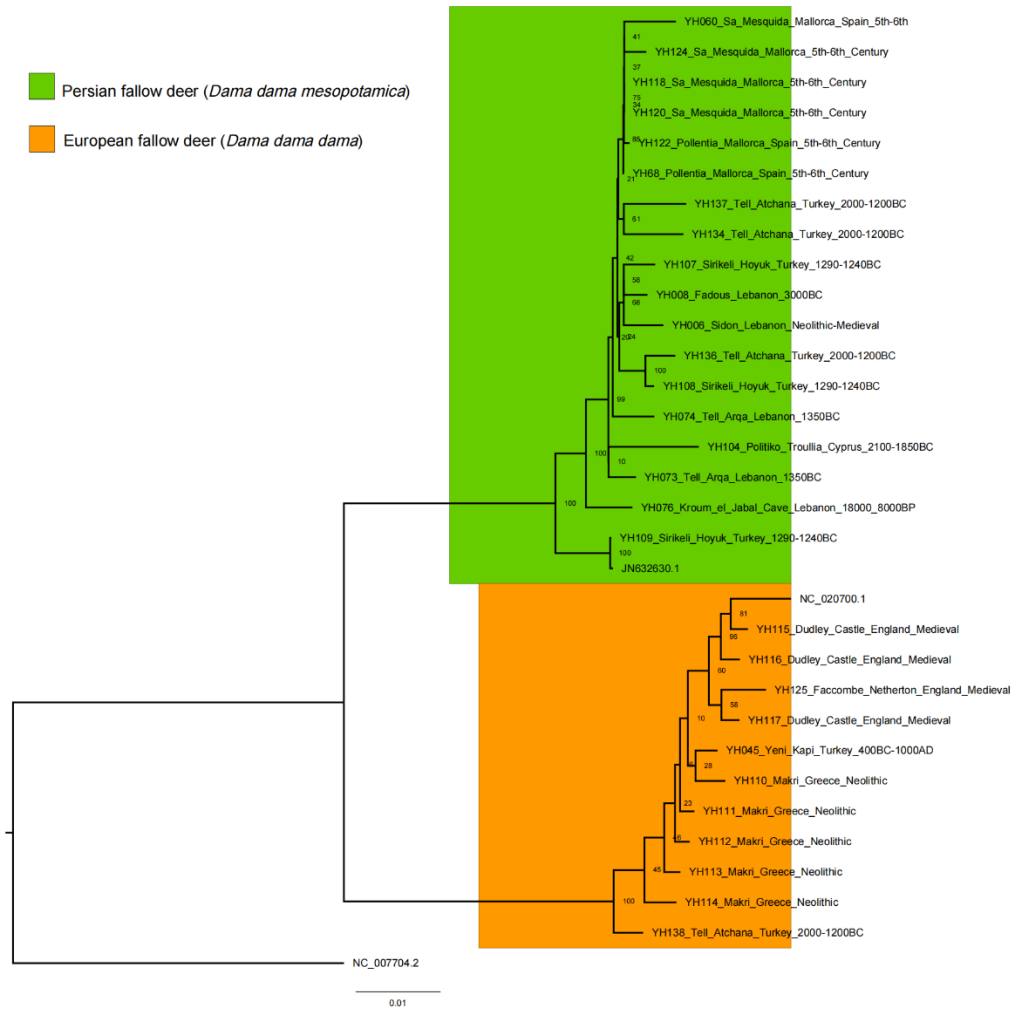


Figure 19 ML tree constructed using 29 ancient samples with red deer as outgroup.

4.4.3 The origins of Cypriot fallow deer

Cypriot fallow deer has played a significant role in both subsistence and cultural practices on the island. They first appeared in Cyprus around 8,000 BC, with their dominance extending until the Bronze Age (Vigne et al., 2016).

We generated a partial mitochondrial genome of a Cypriot fallow deer (YH104 2100-1850 BC) from the Politiko Troulli site. As illustrated in the Maximum Likelihood (ML) tree (Figure 19), the YH104 sample was classified as a Persian fallow deer and clustered with sample YH073

from Tell Arqa, Levant (Figure 18). This result supports the previous hypothesis that Cypriot fallow deer were derived from the Levant (Vigne et al., 2016).

Cultural interconnections between the Levant and Cyprus have been well-established, with evidence of exchanges in various domains such as fauna, pottery, copper, and architecture (Bar-Yosef Mayer et al., 2015; Charaf, 2013; Crewe, 2012; Gourichon and Kolska Horwitz, 2021; Hein, 2014; Peltenburg, 2008; Peters et al., 1999). Tell Arqa, an ancient coastal city in the Levant, played a pivotal role in these interactions, serving as an important stopover with convenient access to both the hinterland of the Near East and the islands of the Mediterranean (Thalmann, 2006). Given its abundant natural resources, it is plausible that Tell Arqa or a nearby region functioned as a critical intermediary point for the transportation of Persian fallow deer from the Levant to Cyprus.

Nonetheless, it is essential to recognize the limitations of our study concerning the genetic diversity of fallow deer in Cyprus. As we have only obtained a single mitochondrial genome from the island, our findings may not fully represent the extent of the species' genetic diversity. Consequently, we cannot rule out the possibility that Cypriot fallow deer have multiple origins. Therefore, future research is warranted to further investigate this issue and enhance our understanding of the complex interconnections between the mainland and Cyprus.

4.4.4 The origins of the Mallorca fallow deer

According to Pinya and Lassnig (2018), the first occurrence of fallow occurred in Mallorca

was during the second century AD and they experienced a population boom in the fourth century AD. Mallorca fallow deer are unique comparing to the other populations, as evidenced by both phylogenetic and morphological analysis (Pinya and Lassnig, 2018; Valenzuela et al., 2016).

In our study, we analysed samples from six individuals obtained from two sites in Mallorca. All these individuals formed a monophyletic group. Interestingly, two Persian fallow deer samples (YH134, YH137) unearthed at Tell Atchana, a site located in Southwest Anatolia near the Levant, formed a sister group to the Mallorca samples, as supported by phylogenetic analysis (Figure 19). Given the considerable geographic distance between Mallorca and the native range of Persian fallow deer, it is highly likely that the Mallorca population was introduced from the Levant or nearby regions, rather than being indigenous to the island.

By synthesizing with historical and archaeological contexts, we deduce that the spread of Persian fallow deer was likely associated with a Mediterranean trading network, connecting the Levant and Cyprus to Southern Iberia, established prior to the 8th century BC (Bar-Yosef Mayer et al., 2015; Williams-Thorpe and Thorpe, 1993). Most importantly, Mallorca emerged as one of the most significant nodes (Sherratt S and Sherratt A 1993) of this trading network.

In the island, the site of Pollentia, where two of our samples were discovered, functioned as a major Roman-era port city (Giaime et al., 2017). As a result of these established trade routes, people from the eastern Mediterranean began settling in Mallorca as early as the seventh

century BC. The island bears traces of Levantine glass (Van Strydonck et al., 2018) and ceramic technologies from the eastern Mediterranean (Arthur, 2008), further substantiating the connection between these regions.

In summary, the arrival of Persian fallow deer in the Mallorca possibly coincided with the establishment of east-west trade routes. It is likely that the Persian fallow deer constituted one of the items in the cultural package imported from the Levant, illustrating the interconnectedness of the Mediterranean world during this time.

4.4.5 The origins of the Makri fallow deer

The history of fallow deer in Makri remains relatively unexplored, with limited information currently available. Makri is situated at the convergence of the Balkan and Anatolian regions, and as a Neolithic settlement, its fauna predominantly consisted of domestic animals. Fallow deer represented only a minor fraction of the local fauna (Curci & Tagliacozzo, 2003; Efstratiou & Fumanal García, 1998; Halstead & Isaakidou, 2016).

Our phylogenetic analysis demonstrates that the Makri fallow deer population is closely associated with the European clade (Figure 19). Given that Anatolia has been identified as the most plausible refugium for European fallow deer during the last glacial period (Baker et al., 2017; Masseti et al., 2008, 1996; Masseti and Rustioni, 1988), it is highly probable that the Makri fallow deer population originated from Anatolia.

Since the Neolithic period, the Balkan and Anatolian regions have engaged in frequent and enduring cultural exchanges. Archaeological findings at Makri reveal the presence of glume wheat, which has its origins in Anatolia, as well as faunal remains such as cattle, pigs, sheep, and goats (Curci & Tagliacozzo, 2003). These discoveries, in conjunction with other evidence indicative of Near Eastern influences, strongly support the notion of a cultural linkage between Makri and the Near East. This connection further reinforces the hypothesis of a shared ancestry and interregional exchange between these populations, and it is reasonable to hypothesize that the importation of European fallow deer might have occurred concurrently with the communication and exchange between these two regions.

4.4.6 The origin of the England fallow deer of the Medieval Age

The origin of fallow deer in Britain is complex, as historical records suggest that they were introduced by four distinct groups of people, and the medieval population was likely re-introduced following the Norman conquest of England in the eleventh century (Sykes, 2004).

In this study, we successfully extracted mitochondrial genomes from four individuals at two sites (Figure 18; Table 8), and these individuals clustered with European fallow deer to form a monophyletic clade (Figure 19), indicating a close genetic relationship among English fallow deer populations. These findings suggest that the fallow deer population in Medieval England might originate from a relatively single source.

Considering that Anatolia could be the only refugium region for European fallow deer after the

last glacial (Baker et al., 2017; Masseti et al., 2008, 1996; Masseti and Rustioni, 1988), the origin of fallow deer in England must be traced back to Anatolia. Thinking the geographical and temporal gaps involved, there must have been intermediate points between Anatolia and England in the spread of fallow deer. However, it is currently difficult to map its precise migration route due to the lack of nuclear genome data and reference datasets from mainland Europe. Further studies using nuclear genome data and reference datasets from mainland Europe could provide additional insight into the evolutionary history and origin of fallow deer in Britain.

4.4.7 Transportation routes of fallow deer

Based on the results presented in this study, it is clear that the human-mediated dispersal of fallow deer followed two distinct routes. The Persian fallow deer, as evidenced by the mitochondrial genome analysis of the Cypriot, Mallorca, and Levantine samples, dispersed to the Mediterranean region. On the other hand, European fallow deer, as demonstrated by the Makri, Mallorca, and English fallow deer mitochondrial genomes, spread to Europe from their likely refugium in Anatolia.

Two factors are likely to have contributed to the separate dispersal of fallow deer. Firstly, the adaptive ability of the Persian and European fallow deer is a significant factor. European fallow deer can tolerate and adapt to lower temperatures, making them more suited to cooler climates. On the other hand, the Persian fallow deer, which are originally distributed in the land of the two rivers, have thrived only in high temperatures, making them more suitable for warmer

regions (Uerpmann, 1987: 61-sq; Chapman and Chapman, 1975, 1980). As a result, the Mediterranean region, with its warm climate, is more suitable to Persian fallow deer.

This difference in adaptation could have played a significant role in the separation of these species' dispersal. It is likely that the two species dispersed to different regions based on their tolerance for temperature variations, with the Persian fallow deer migrating towards the warmer Mediterranean region, while the European fallow deer dispersed towards the cooler regions of Europe.

Secondly, the establishment of trade routes since 800 BC, linking the Levant and western Mediterranean region Bar-Yosef Mayer et al., 2015; Williams-Thorpe and Thorpe, 1993, likely associated with the dispersal of fallow deer. The Levant is the natural range of the Persian fallow deer, and frequent and long-lasting cultural interactions between Anatolia and Europe likely led to the dispersion of European fallow deer to Europe from Anatolia.

4.4.8 Past hybridization of the Persian and European fallow deer

Persian and European fallow deer have been demonstrated to interbreed, producing. First-generation hybrids exhibit a 30% likelihood of conception when back-crossed with Persian fallow deer and a 50% chance when back-crossed with European fallow deer (Asher et al., 1993; Mylrea et al., 1991; Nugent and Yockney, 2004). However, these examples of hybridization primarily resulted from artificial insemination aimed at improving meat quality and the efficiency of deer farming. The ability of these two species or subspecies to naturally

interbreed remains uncertain (Asher et al., 1993; Mylrea et al., 1991; Nugent and Yockney, 2004).

Research has begun exploring the potential existence of hybrid fallow deer within the archaeological record (Fernández-García, 2012; Valenzuela et al., 2016). Owing to challenges in sample preservation and a scarcity of reference nuclear genomes, these investigations have yet to provide definitive conclusions regarding the presence of ancient hybrids. Nevertheless, our findings indicate a potential admixture between Persian and European fallow deer, as evidenced by the coexistence of their mitochondrial genomes at Tell Atchana. This site or its surrounding areas may have served as an admixture zone for these two species or subspecies. However, the current lack of nuclear DNA data precludes the direct determination of whether admixed individuals were indeed present. Despite this limitation, the coexistence of maternal lineages from both species or subspecies in the same region suggests an increased likelihood of admixture. Further research, particularly focusing on nuclear DNA analysis, is necessary to provide a more conclusive understanding of the potential admixture between Persian and European fallow deer.

The Mallorca population has been posited to have originated from admixtures of Persian and European fallow deer (Fernández-García, 2012; Valenzuela et al., 2016). Genetic analysis using mitochondrial DNA reveals that all Mallorca individuals analysed possess Persian fallow deer lineages, while zooarchaeologists generally classify them as European fallow deer based on morphological characteristics. This apparent discrepancy between genetics and morphology

has led some researchers to suggest that hybridization between European and Persian fallow deer could be the underlying cause (Fernández-García, 2012; Valenzuela et al., 2016).

In our study, all six mitochondrial genomes of Mallorca fallow deer analysed belong to the Persian clade (Figure 19), at least from a maternal perspective, providing no direct evidence of admixture. However, given that mitochondrial DNA is exclusively maternally inherited, the possibility of European traits being present in the nuclear genomes of Mallorca fallow deer cannot be ignored. As such, a definitive conclusion regarding the admixture of Persian and European fallow deer cannot be drawn until a comprehensive analysis of the nuclear genome is conducted.

5 Conclusion

In conclusion, this study sheds light on the origins and dispersal patterns of fallow deer populations in different regions. The adaptive ability of the two main species or subspecies of fallow deer could have played a significant role in their separate dispersal towards warmer and cooler regions, respectively. Additionally, the establishment of trade routes between the Levant and western Mediterranean region likely contributed to the dispersal of fallow deer. Our findings also suggest the possibility of admixture between the Persian and European subspecies, which warrants further investigation using nuclear genome data. Understanding the evolutionary history and genetic diversity of fallow deer populations can provide insights into human-animal interactions, the spread of animal domestication, and the cultural and ecological dynamics of the regions they inhabit.

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Supplementary

The information of the key sites associated with this research

Makri

Prehistoric Makri is a coastal site, located on top of a steep cliff edge, 50 m high, overlooking the Thracian Sea, ten km W of the modern town of Alexandroupolis. The Neolithic deposit has been damaged by the modern military facilities. The systematic excavations at Makri were carried out by the Ephorate of Prehistoric and Classical Antiquities of Komotini between 1988 and 1996 under the direction of Professor N. Efstratiou of the University of Thessaloniki. The extensive excavations revealed an important Neolithic settlement dated between the middle of the 6th and the beginning of the 5th millennium BC, with deep undisturbed deposits, well-preserved architectural features and abundant archaeological remains.

Tell Atchana

Tell Atchana (Alalakh), located in the Amuq Valley in modern day Turkey, is one among many urban sites in the Middle and Late Bronze Age (MBA and LBA, respectively; ca. 2000–1200 BC) Levant that functioned as the capital of a local kingdom named Mukish in the LBA, characterized by complex diplomatic and interregional relations and frequently shifting loyalties to bigger entities of the ancient Near East.

Tell Arqa

The site of Tell Arqa is located in the south of the Akkar plain, northern Lebanon, on the northern Levantine coast. It lies 22 km north of the modern city of Tripoli, 12 km south of the Nahr el-Kebir, the current Syrian Lebanese border, and 6 km east from the coast. The site is located in the northwestern foothills of Mount Lebanon, on the north bank of the Nahr Arqa, a small watercourse flowing from Mount Lebanon to the Mediterranean Sea. The tell is around 30 m high and 4.5 ha in extent at the top. It is one of three major sites on the Akkar plain, along with Tell Kazel and Tell Jamous. Tell Arqa should, therefore, be considered an important Bronze Age site in the central Levant, and one sitting in a favourable geographical location, at the junction of major routes.

Sa Mesquida

The site of Sa Mesquida is located in Santa Ponça bay in the area of Calvia in the western part of the island of Mallorca in the Balearics. Archaeological excavations uncovered the remains of a rural settlement or villa. It has been demonstrated that the villa was founded perhaps in the Augustan period (approx. 27 BC- AD 14) or slightly earlier at the very end of the Late Republican period. Subsequent transformations led to structural changes in some floors and rooms of the main building that was destroyed by a fire by the end of the 2nd or early 3rd century.

Pollentia

The site of Pollentia is located in the modern town of Alcúdia in the island of Mallorca, in Spain. It is the best preserved Roman city of the island and it has become an important reference for the study of the introduction and spread of the Roman culture in the Balearic Islands. It extends about 300 m from East to West and 600 m from North to South for a total area of about 180,000 m². The city was a colony founded after the invasion of the Balearic Islands by Quintus Caecilius Metellus in 123 BC, but the first Roman constructions in the area of the forum did not take place before approximately 70 BC. Pollentia has a designed plan with a regular orthogonal layout. The city was modified after the Republican foundation by a refurbishment under Augustus and by a series of deep transformations at the end of the second century and the beginning of the third century AD. The forum of the city was partially destroyed in the late third century AD by a large fire. The decline of Pollentia would be dated around to the 270–280 AD, but the site was occupied also in Late Antiquity and the Middle Ages.

Dudley Castle

Dudley Castle is located in the West Midlands, England, 15 km north-west of Birmingham (Fig. 1). Excavations were carried out within the keep and the confines of the inner bailey between 1983 and 1993 and Ten phases of activity were identified during the excavations stretching from the eleventh to the mid-eighteenth century this phasing was established

following archaeological confirmation of historically-attested periods of building activity and was verified by ceramic spot-dating.

Facombe Netherton

Facombe Netherton is a manorial site in north west Hampshire. Facombe was owned by Wynflaed apparently the grandmother of Kings Edwy and Edgar in the 10th century and later by wealthy Norman families. In the mid-14th century part of the estate was sold, the manorial site was later abandoned and the original village of Facombe declined in favour of a newer settlement in the center of the manor. The manor is well documented, particularly from the 14th century. There was some slight evidence of occupation in prehistoric and Roman times, but the first settled occupation found of excavation was in the 9th century in an area of early fields on the west side of the valley. A series of buildings interpreted as halls, private apartments and kitchens developed from the 9th century, through to the mid-14th century.

Location	Total (site)	Total (region)	HQ (site)	HQ (region)	HQ (site)	HQ (region)	HQ (site)	HQ (region)	LQ (site)	LQ (region)	LQ (site)	LQ (region)
Tell Arqa (Lebanon)	9		0		0		1		5		3	
Fadous (Lebanon)	1	19	0	0	0	0	0	1	1	11	0	7
Kroum el Jabal Cave	3		0		0		0		2		1	
Sidon (Lebanon)	6		0		0		0		3		3	
Marki-Alonia (Cyprus)	17		0		1	1	1	2	8		7	
Politiko-Kokkinorotsos	18	49	0	0	0	1	0	2	10	25	8	21
Politiko-Troullia (Cyprus)	14		0		0		1		7		6	
Kinet Höyük (Turkey)	1		0		0		0		1		0	
Yeni Kapi (Marmaray-Turkey)	2		0		0		0		1		1	
Sirkeli Höyük (Turkey)	2		0		0		0		1		1	
Lidar Höyük (Turkey)	1	13	0	1	1	2	0	3	0	5	0	2
Bademagaci Höyük (Turkey)	1		0		0		0		1		0	
Uluçak (Turkey)	1		0		0		1		0		0	
Tell Atchana (Turkey)	5		1		1		2		1		0	
Makri (Greece)	6	6	0	0	2	2	1	1	3	3	0	0
Morgantina (Sicily)	1	1	0	0	0	0	0	0	1	1	0	0
Pollentia (Mallorca)	11	29	4	7	1	2	2	3	2	8	2	9
Sa Mesquida (Mallorca)	18		3		1		1		6		7	
Dudley Castle (England)	7	8	0	0	2	3	2	2	3	3	0	0
Facombe Netherton	1		0		1		0		0		0	
Total		125		8		10		12		56		39

high-quality (HQ)samples: the samples that contain endogenous DNA over 3%.
medium-quality (MQ)samples: the samples that contain endogenous DNA from 1-3%.
regular-quality (RQ)samples: the samples that contain endogenous DNA from 0.5-1%.
low-quality (LQ)samples: the samples that contain endogenous DNA 0.1-0.5%.
failed (F)samples: the samples that contain endogenous DNA lower than 0.1%.

Table S9 Background information and preservation of the fallow deer samples collected in this study.

Table S10 Pooling strategy for fallow deer samples.

Lab Code	Endogenous %	Pools
YH068_L1A1	11.9	1
YH031_L1A2	8.2	
YH037_L1A2	6.5	
YH033_L1A2	5.06	
YH042_L1A1	4	
YH120_L1A1	2.1	2
YH118_L1A1	2	
YH137_L1A2	1.5	
YH109_L1A2	1.5	
YH115_L1A1	1.3	
YH113_L1A1	0.93	
YH122_L1A1	0.92	3
YH125_L1A1	0.87	
YH087_L1A1	0.68	
YH112_L1A1	0.61	
YH130_L1A1	0.57	
YH116_L1A1	0.51	
YH074_L1A1	0.45	
YH038_L1A1	0.34	4
YH132_L1A2	0.32	
YH136_L1A1	0.32	
YH138_L1A1	0.31	
YH126_L1A2	0.23	
YH117_L1A1	0.22	
YH124_L1A1	0.21	5
YH134_L1A1	0.19	
YH003_L1A2	0.18	

YH110_L1A1	0.15	
YH019_L1A2	0.13	
YH111_L1A1	0.12	
YH045_L1A1	0.11	6
YH076_L1A1	0.11	
YH060_L1A1	0.1	
YH135_L1A2	0.09	
YH006_L1A1	0.07	
YH073_L1A1	0.07	
YH077_L1A1	0.07	7
YH008_L1A1	0.06	
YH114_L1A1	0.06	
YH131_L1A1	0.06	
YH002_L1A1	0.05	
YH009_L1A1	0.05	
YH010_L1A1	0.05	8
YH024_L1A1	0.05	
YH047_L1A2	0.05	
YH097_L1A1	0.05	
YH107_L1A1	0.05	
YH025_L1A1	0.04	
YH058_L1A2	0.03	9
YH080_L1A1	0.03	
YH108_L1A1	0.03	
YH121_L1A1	0.03	
YH013_L1A1	0.02	
YH079_L1A1	0.02	
YH104_L1A1	0.01	

Permission from co-authors

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I confirm that to the best of my knowledge. The author contribution statement below is accurate, that Yu Han's overall contribution was equal or greater to that of the other co-first authors.

Author contributions:

Greger Larson, Naomi Sykes, Julie Daujat, Karis Baker and Yu Han conceived of the project and designed the research;
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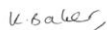
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5 Ancient genomic and zooarchaeological investigation into the domestic pigs (*Sus scrofa*) in the Middle Reach of the Yellow River Basin from 5,500 to 2,500 BP

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Abstract

Domestic pig (*Sus scrofa*) is a crucial livestock species in China, particularly within the Middle Reach of the Yellow River Basin (MYR), which is considered a key region for its origin. However, the extent to which MYR pig populations have contributed to contemporary breeds remains inadequately understood, and further investigation into the evolution of pig management in this area is warranted. In this study, we employed ancient genomic analysis to generate five nuclear and nine mitochondrial genomes of ancient domestic pigs from two distinct MYR sites, spanning a period of over 3,000 years. Furthermore, we analysed more than 1,400 faunal remains, including pigs from the Zheng-Han City site. Our findings indicate that the ancient domestic pig populations from the MYR played a significant role in shaping modern Chinese breeds, despite these populations exhibiting genetic differentiation. Through the application of zooarchaeological methods, we observed a decline in the prominence of pigs within archaeological sites and potential intensification of pig management in the MYR, from the late Neolithic to the Iron Age. These results provide valuable insights into the evolutionary

history of pig domestication and management in the MYR region and elucidate the contributions of MYR pig populations to the genetic makeup of contemporary breeds.

Key words: MYR; ancient domestic pigs; ancient genomics; zooarchaeology

5.1 Introduction

The domestication of pigs

Domestic pig (*Sus scrofa*) is one of the most widely distributed mammalian livestock globally and serves as a crucial source of animal-based protein for humans (Rischkowsky and Pilling, 2007). It is widely accepted that the common ancestor of domestic pigs is the wild boar (*Sus scrofa*) (Frantz et al., 2016, 2013; Larson et al., 2005; Zeder, 2012). *Sus scrofa* is one of the 17 species in the Suidae family that diverged from Suiodae during the Eocene or early Oligocene period (34.50–39.69 Ma) based on molecular estimates. The species originated in the islands of Southeast Asia around 4 million years ago and subsequently spread throughout continental Eurasia. Despite originating in tropical areas, *Sus scrofa* quickly adapted to various environments across Eurasia. Around 1.2 million years ago, the species further diverged into European and Asian clades, with glacial events leading to geographical isolation that restricted genetic communication (Frantz et al., 2016, 2013; Giuffra et al., 2000; Groenen et al., 2012). With the Neolithic transition, pigs were independently domesticated in both western and eastern Eurasia (Larson et al., 2005; Yu et al., 2013).

The domestication of pigs in western Eurasia has been extensively studied and is believed to have originated in the Near East around 9,500 BP (Ervynck et al., 2001). Near Eastern pig populations entered Europe around 8,000 BP with the migration of local farmers (Larson et al., 2007; Lazaridis et al., 2016; Ottoni et al., 2013). However, over the subsequent two to three thousand years, the Near Eastern population was replaced by native European wild boar stocks. Today, Near Eastern ancestry is almost completely absent from European domestic pig populations, except for Southern Europe, where some minor Near Eastern traits remain (Frantz et al., 2019, 2015; Larson et al., 2007; Ottoni et al., 2013). Historical and genetic research (Bosse et al., 2014; White, 2011) indicates that Asian domestic pigs were introduced to Europe after the industrial revolution to improve commercial breeds. Today, Asian traits can be detected in mitochondrial genomes (Clop et al., 2004; Frantz et al., 2019; Traspov et al., 2016), as well as in several functional genes associated with fertility or disease resistance of some European commercial breeds (Bosse et al., 2015, 2014; Wilkinson et al., 2013).

Pig domestication was a significant milestone in the establishment and advancement of Chinese agricultural societies (Jing, 2008; Yuan et al., 2008). Today, China boasts over 100 pig breeds that make up one third of the world's pig population, making it the largest population worldwide (Fang et al., 2005; Huang et al., 2020). Pork is the dominant source of protein for the Chinese population, with more than half of global pork consumption occurring in China (Larsen, 2012; Shimokawa, 2015; Whitnall and Pitts, 2019). Despite being a crucial part of China's biodiversity resources and society, the origin and evolutionary history of Chinese domestic pigs remain poorly understood.

Origin of Chinese domestic pigs from a zooarchaeological perspective

Zooarchaeological studies suggest that the domestication of pigs in China occurred separately in several geographical regions or cultural backgrounds (Jing, 2008; Liu et al., 2017). The earliest domestic pigs in Northern China were considered to have occurred at the Cishan Site (8,000 BP) (Jing and Flad, 2002; Rodwell, 1984). In the Northeast, the Xinglongwa Site (10,000 to 7,000 BP) is believed to have domesticated the region's earliest pigs (Jing and Flad, 2002; Womack, 2020). Along the Yellow River Basin, wild boars in the Dadiwan Site in the upper reach (Bettinger et al., 2010; Yang et al., 2022), the Jiahu Site in the middle reach (Cucchi et al., 2011; Juzhong and Qilong, 2013), and the Houli Site in the lower reach (Fen, 2013; Hou et al., 2022) may have been domesticated independently. In the Yangtze River Basin, pigs were domesticated in the middle and lower reach represented by the Daxi Site (Dong and Yuan, 2020; Jing and Flad, 2002; Peregrine, 2001) and Kuahuqiao Site, respectively (Innes et al., 2009; Jing and Flad, 2002; Leping, 2013). In Southern China, the Zengpiyan Site (Jing and Flad, 2002; Zhou et al., 2022) is also believed to have had its own locally domesticated pigs.

In addition to addressing the origins of Chinese domestic pigs, zooarchaeology has also proved instrumental in informing the management and exploitation of pig populations, particularly those in the Middle Reach of the Yellow River Basin. Using zooarchaeological techniques, it has demonstrated that the frequency of pig remains in archaeological sites along the Yellow River Basin exhibited a declining trend, dropping from 90% in 6,000 BP to 30%-60% in 2,000 BP. However, the management of pig herds intensified following the advancement of agriculture, dating back to 7,000 BP (Luo, 2012; Dong and Yuan, 2020; Zhang, 2021).

zooarchaeology mainly consults archaeological contexts, age of death and the morphology of pig bones or teeth (Rowley-Conwy et al., 2012). While such information can be important to answer a wide range of questions, it is challenging to determine the interactions or relationships of pigs living in different spatial and temporal contexts. To explore the population dynamics of domestic pigs, the conduction of genetic research is more than necessary.

Origin of Chinese domestic pigs from a genetic perspective

Genetics has emerged as a powerful tool for understanding the evolution of organisms. However, past research on Chinese domestic pigs was limited by its reliance on short mitochondrial DNA sequences, which constrained the exploration of their evolutionary history (Larson et al., 2010; Wang et al., 2022; Xiang et al., 2017a). Advances in next-generation sequencing (NGS) and ancient DNA technology have enabled more comprehensive studies using ancient and modern genomic materials, thereby enhancing our understanding of Chinese pig population history at a higher resolution (Carøe et al., 2018; Dabney et al., 2013a; Goodman et al., 2016).

Traditionally, Chinese pigs were categorized into six types based on geographic distribution and phenotype, reflecting adaptations to the varying conditions in Northern and Southern China (Ai et al., 2015). However, recent research has shifted towards classifying Chinese domestic pigs into three groups according to their genetic characteristics. Demographic models use an

approximation of Bayesian calculation indicated that local Chinese pigs are descended from an extinct *Sus scrofa* population that existed 22,000 years ago. The three groups then separated from their forebears 16,000, 11,000, and 8,700 years ago, respectively. (Peng et al., 2022). Zhang et al. (2021) analysed 42 mitochondrial genomes, revealing only minor discrepancies in maternal haplotypes among East Asian pigs, with most branches sharing a common ancestor dating to less than 20,000 years ago. East Asian domestic pigs may have undergone two population expansions approximately 7,000 and 4,000 years ago, corresponding to climate changes and the spread of agriculture.

In a study by Wen et al. (2022), the analysis of six low-coverage ancient nuclear genomes from the Shimao Site in the Yellow River Basin and modern samples from across Eurasia revealed that European domestic pig traits were introduced into the Chinese population, a process likely commencing less than 3,900 years ago. Despite the identification of a domestication centre in the lower reach of the Yangtze River Basin, modern pig breeds in the region display a greater ancestry connection with Shimao domestic pigs, indicating cultural exchanges between the Yellow and Yangtze River basins since the final phase of the Neolithic Age.

Pig domestication in the middle reach of the Yellow River Basin

The integration of ancient and modern genomic studies has significantly enhanced our understanding of the evolutionary history of Chinese domestic pigs. However, research on ancient domestic pigs from the middle reach of the Yellow River Basin remains limited, primarily due to the lack of high-quality ancient genomic data from the eastern part of the MYR,

also known as the Central Plain. While previous studies have investigated the western part of the MYR, the eastern portion played a crucial role in the formation of Chinese civilization and, notably, may have been one of the first regions to establish agriculture and manage pig herds across Eurasia (Jing and Flad, 2002; Xinwei, 2013; Yanping, 2013). Given the potential significance of the MYR in the origin and evolution of domestic pigs, further investigation into the ancient pigs of this region is warranted.

In this study, we have collected ancient domestic pig samples from the Miaodigou (MIA) site (Han, 2013), dated to 6,000-5,000 BP (n=10), and the Zheng and Han City (Z&H) site (Relics and Archaeology, 2001), dated to approximately 2,500 BP (n=4) in the Central Plain. By employing ancient genomic technology in conjunction with zooarchaeological analysis, we aimed to 1) elucidate the genetic background of ancient Chinese domestic pigs in the middle reach of the Yellow River Basin, 2) assess the genetic relationships between ancient domestic pigs in the Central Plain and those from other locations in the MYR, and 3) determine the ancestral contributions of the pigs from Miaodigou and Zheng and Han City to modern breeds worldwide.

5.2 Materials



Figure 20 Sampling locations of ancient Chinese pigs .In this study, five samples were unearthed from the Miaodigou Site (6,000-5,000 BP) and 4 were unearthed from the Zheng and Han City Site (2,770 -2,230 BP).

Eight ancient pigs were collected from two sites in the Middle Reach of the Yellow River Basin (MYR), spanning over 3,000 years and 250 km. Five of these pigs were excavated from the Miaodigou Site (6,000-5,000 BP) (Han, 2013)The remaining four were discovered at the Zheng and Han City Site (2,770-2,230 BP) (Relics and Archaeology, 2001) (Figure 20 and Table 9).

The Miaodigou archaeological site is located on the Loess Plateau between the Qinglongjian River and Canglongjian River, which flow into the Yellow River, covering an area of

approximately 240,000 square meters. The early phase of the site belongs to the Miaodigou Type Culture of the Yangshao Culture, dating from around 6000 BC to 5000 BC. The upper phase of the site is associated with the early Miaodigou Phase II Culture of the Central Plains Longshan Culture, dating from around 4900 BC to 4800 BC. The primary subsistence at this site were agriculture and animal husbandry (Han 2013).

The Zheng-Han Ancient City was one of the capital cities during the Eastern Zhou Dynasty. The city was initially established by the State of Zheng during the Spring and Autumn Period (770-476 BC). The State of Han conquered Zheng, and moved its capital to this location, with both Zheng and Han successively building their capitals here for a total of 539 years. The layout of the Zheng-Han Ancient City is an irregular triangle, with a north-south dividing wall in the middle that separates the city into eastern and western sections. The western section served as the political centre, with the palace districts and noble residences of both Zheng and Han states concentrated here. The eastern section functioned as the economic centre, featuring areas for handicraft workshops, commoner residential zones, religious and ceremonial worship areas, and the tombs of Zheng nobles ((Relics and Archaeology, 2001).

In addition to these ancient samples, 152 ancient and modern mitochondrial genomes from Zhang et al., 2021 and Frantz et al., 2019, as well as 150 nuclear genomes from Frantz et al. 2019, were utilized in the study.

Table 9 Information of pig samples collected from the Miaodigou Site, and the Zheng and Han City Sites.

ID	Species	Sites	Region	Date (BP)	Bones
SS1	<i>Sus scrofa domesticus</i>	Miaodigou	MYR	6,000-5,000	petrosal bone
SS2	<i>Sus scrofa domesticus</i>	Miaodigou	MYR	6,000-5,000	petrosal bone
SS3	<i>Sus scrofa domesticus</i>	Miaodigou	MYR	6,000-5,000	petrosal bone
SS4	<i>Sus scrofa domesticus</i>	Miaodigou	MYR	6,000-5,000	petrosal bone
SS10	<i>Sus scrofa domesticus</i>	Miaodigou	MYR	6,000-5,000	petrosal bone
SS11	<i>Sus scrofa domesticus</i>	Zheng/Han	MYR	2,770 -2,230 BP	petrosal bone
SS13	<i>Sus scrofa domesticus</i>	Zheng/Han	MYR	2,770 -2,230 BP	petrosal bone
SS14	<i>Sus scrofa domesticus</i>	Zheng/Han	MYR	2,770 -2,230 BP	petrosal bone
Total	8				

In conjunction with the genetic research, we collected and analysed 1,436 animal bone fragments excavated from two locations within the Zheng and Han City Site. To examine the changes in pig management and utilization over time in the Middle Reach of the Yellow River Basin, we also integrated published zooarchaeological findings from the Miaodigou (Liu et al. 2021) and Yinxu (Li, 2011) sites into our research for comparative purposes.

5.3 Methods

5.3.1 Ancient DNA methods

5.3.1.1 Ancient DNA laboratory methods

For the methods of sample preparation, Extraction, Library building, qPCR, Library amplification, please see *Methods Chapter 2*.

5.3.1.1.1 Sample screening

All samples underwent screening sequencing using the NovaSeq 6000 instrument at Novogene, China. Approximately 3G of raw data was obtained for each sample. To estimate endogenous DNA content, we calculated the ratio of nucleotides mapped to the domestic pig reference genome (GCF_000003025.5_Sscrofa10.2) or the number of reads mapped to the mitochondrial genome (NC_000845.1). Based on these results, we determined our subsequent sequencing strategies. Samples with a higher endogenous DNA content (>15%) were chosen for whole-genome deep sequencing.

5.3.1.1.2 Whole-genome deep sequencing

A total of five samples were selected (SS2, SS3, SS10, SS13, SS14) (Table S7). Among these, SS2, SS3, and SS10 were excavated from the Miaodigou Site, while SS13 and SS14 were unearthed from the Zheng-Han City Site. The required amount of raw sequencing data for each sample was determined based on the percentage of endogenous DNA. All samples were sequenced using the NovaSeq 6000 platform at Novogene, Beijing.

5.3.1.2 Bioinformatics methods

5.3.1.2.1 Raw Data filtering

See Methods Chapter 2..

5.3.1.2.2 Mitochondrial DNA analysis

5.3.1.2.2.1 Mapping and consensus sequence

Cleaned data was then mapped to the domestic pig mitochondrial reference genome (NC_000845.1) using the `aln` function in BWA v0.1.17 (Li, 2013) with the default settings. SAMtools v.1.14 (Danecek et al., 2021) were then used to sort the bam files and filter out the PCR duplicates, unmapped reads, or the reads with the mapping quality of less than 30. HTSbox was used to call consensus mitochondrial sequences with the default settings. Then they were aligned to each other with MUSCLE v.5 (Edgar, 2021).

5.3.1.2.2.2 Phylogeny

We employed BEAST v2.6.7 (Bouckaert et al., 2019) to construct a timetree, utilizing the dates of the tips to calibrate divergence times. We implemented the Tamura-Nei 93 (TN93) nucleotide substitution model, incorporating both a gamma distribution (G) and a proportion of invariant sites (I). A strict molecular clock and constant population size were assumed, with uniform priors applied. Our dataset comprised 159 pigs, including three ancient mitochondrial genomes from the Zheng and Han City sites and five from the Miaodigou site. The BEAST analysis was run for 100 million generations, with parameters and trees sampled every 10,000 generations.

5.3.1.2.3 Nuclear Genome Analysis

5.3.1.2.3.1 Mapping

The cleaned data was subsequently mapped to the domestic pig whole nuclear reference genomes (GCF_000003025.5_Sscrofa10.2) using the "aln" function in BWA v.0.1.17 (Li, 2013) with settings -n 0.01 -o 2 -l 1024. Following this, SAMtools v.1.14 (Danecek et al., 2021) were employed to sort the BAM files and filter out PCR duplicates, unmapped reads, or reads with mapping quality below 30.

5.3.1.2.3.2 Call SNPs

SNPs for 150 samples from Frantz et al. 2019 were initially identified and filtered based on quality using GATK v.4.2 (Van der Auwera and O'Connor, 2020), BCFtools v.1.14 (Danecek et al. 2021), and VCFtools v.0.1.16 (Danecek et al. 2011). All transitions were removed to mitigate the impact of deamination (Dabney et al., 2013b; Hofreiter et al., 2001). BEDtools v.2.30.0 (Quinlan and Hall, 2010) was employed to exclude all non-neutral loci. PLINK v.1.9 (Purcell et al., 2007) was utilized to filter out SNPs within linkage disequilibrium regions and sex chromosomes. A panel of 2,958,161 SNPs was obtained, which was then used as a reference to identify SNPs from the five ancient Chinese samples with ANGSD (Korneliussen et al., 2014). Finally, the VCF files of the 150 samples and our five ancient samples were merged using the "merge" function in VCFtools (Danecek et al., 2011).

5.3.1.2.3.3 Principal component analysis

Principal Component Analysis (PCA) was conducted using EIGENSTRAT SmartPCA (Price et al., 2006) on a dataset comprising 2,958,161 SNPs and 155 samples, which included five ancient samples from the MYR region and 150 modern and ancient pigs from across Eurasia, as published in Frantz et al., 2019. Due to the high level of missing data in ancient samples, the PCA was initially constructed using high-quality modern data. Subsequently, the ancient samples were projected onto the PCA by applying the "LsqProject: Yes" function.

5.3.1.2.3.4 ADMIXTURE analysis

Population structure analysis was conducted using ADMIXTURE 1.3.0 (Kenneth, 2011) with a dataset comprising 2,958,161 SNPs and 155 samples. These samples included five ancient specimens from MYR and 150 modern and ancient pigs from across Eurasia, as published in Frantz et al., 2019. The population number K was set to values ranging from 2 to 6.

5.3.1.2.3.5 Outgroup f3 statistics

The out-group f_3 statistics were determined using the qp3Pop method of EIGENSTRAT AdmixTools v.7.0 (Patterson et al., 2012). The analysis involved a dataset of 2,958,161 SNPs and 59 samples, which included five ancient samples from MIA and Z&H sites, 22 modern pigs from seven Chinese native breeds, 9 modern East Asian wild boars, and 23 modern European wild boars. In this assay, the European wild boar served as the out-group, while the ancient Chinese pigs, each modern breed, and East Asian wild boars were compared in pairs to assess their affinity.

5.3.1.2.3.6 f4-statistics analysis

We conducted an f4-statistics analysis using the qpDstat method of EIGENSTRAT AdmixTools v.7.0 (Patterson et al., 2012). The dataset comprised 2,958,161 SNPs and 155 samples, including five ancient samples from MYR and 30 modern Chinese breeds and wild boars as published in Frantz et al., 2019. In the assay, modern sample were divided into populations based on breeds, while ancient samples were separated according to their excavation sites. The introgressions of MIA and Z&H to all modern populations were examined separately, employing two population combinations: (Warthog, MIA, East Asia wild boar, Modern X) and (Warthog, Z&H, East Asia wild boar, Modern X).

5.3.2 Zooarchaeological method

5.3.2.1 Species identification and measurement

I identified the species of faunal remains excavated at the Z&H site using the Animal Skeleton Atlas (Adams and Crabtree, 2009) and standard specimens collected at the research station of the Zheng and Han City Site. Following identification, the ratio of the Number of Identifiable Specimens (NISP) (Flannery, 1967; Grayson, 1979, 1973) was employed to infer the faunal composition at the site. The Minimum Number of Individuals (MNI) (Flannery, 1967; Grayson, 1979, 1973) was not calculated in this research due to the relatively small quantity of identifiable bones or teeth at the Zheng and Han site. Many of these samples were not suitable for determining skeletal regions. As a result, the ratio of MNI among different species could lead to significant deviation from the actual value.

Measurements of animal bones or teeth were conducted according to Von den Driesch (1976). Only bones with intact measuring points and suitable for species identification were measured. The weights of animal bones were also recorded. To understand the body mass changes of pigs from Miaodigou to Zheng and Han City, we selected the humerus and third lower molars of pigs for comparison, as these two parts were most frequently found at both sites. For the humerus, the breadth of the distal end, trochlea, and the depth of the distal end were used. Regarding the third mandibular molars, their length and width were adopted (Von den Driesch, 1976).

Typically, only one side (either right or left) of the targeted bones or teeth is included in measurements or analysis to prevent redundant data collection from the same individual. However, to maximize data and limit bias caused by a small sample size, we measured and analysed both the left and right humerus and lower molars. We carefully observed the morphological features, weathering degree, and wear stage of the bones or teeth to ensure that all specimens were derived from different individuals, thereby avoiding repeated sampling.

5.3.3.2 Age of death

The determination of the death age of pigs was based on the eruption (Silver, 1969) and wear stage of the whole dentition (Grant, 1982; Hillson, 2005). Grant (1982) defined seven stages, from I to VII, representing ages ranging from 0 to over 37 months.

A procedure similar to the measurement was employed to avoid repeated sampling while

maximizing data quantity. In addition to the Z&H pigs, aging data from the Miaodigou (Liu et al., 2021) and Yinxu (Li, 2011) sites were also incorporated into this analysis. The death age determination of pigs at these two sites was also based on Grant (1982), making the results comparable to our research.

5.3.3.3 Diet contribution

The meat contributions of animals to human diets was estimated based on the weight of bones belonging to different species. This evaluation can also be done through the calculation of the Minimum Number of Individuals (MNI) and presumed volume of meat (Becker, 2001). However, we did not use the MNI-based method to calculate the relative contribution to diet because the number of identifiable bones and teeth among the Zheng and Han samples was relatively small, and a large proportion of them were not suitable for skeletal region determination. Calculating meat contribution based on MNI between various species would result in significant deviation from the actual value.

5.4 Results

5.4.1 Genetic results

5.4.1.1 Data generation

Eight ancient pigs were collected from two sites in the Middle Reach of the Yellow River Basin, spanning approximately 3,000 years. Five samples (SS1, SS2, SS3, SS4, SS10) were unearthed from the Miaodigou Site (MIA) dating back to 5,500 BP, while the remaining three (SS11,

SS13, SS14) were discovered at the Zhang and Han City Site (Z&H). In total, 8 mitochondrial genomes were generated with an average mean coverage ranging between 2-15X, and five whole genomes with coverages varying from 0.8-5.5X (Table 10). A single nucleotide polymorphisms (SNPs) panel, based on 150 samples from Frantz et al. (2019), was used to call 2,958,161 nuclear genome SNPs for downstream analysis.

Table 10 Sequencing results of ancient Chinese domestic pigs.

ID	Species	Sites	Date (BP)	Endogenous %	Reads_mapped (Nuc)	Depth (Nuc) X	Num_SNPs	Reads_mapped (MT)	Depth (MT) X
SS1	<i>Sus scrofa domestica</i>	Miaodigou	6,000 - 5,000	5.85	—	—	—	486	1.92
SS2	<i>Sus scrofa domestica</i>	Miaodigou	6,000 - 5,000	64.48	165479769	5.49	729656	1399	6.47
SS3	<i>Sus scrofa domestica</i>	Miaodigou	6,000 - 5,000	21.57	25098712	0.76	467864	486	1.92
SS4	<i>Sus scrofa domestica</i>	Miaodigou	6,000 - 5,000	21.27	—	—	—	2312	11.13
SS10	<i>Sus scrofa domestica</i>	Miaodigou	6,000 - 5,000	25.29	132710349	3.9	720500	430	1.68
SS11	<i>Sus scrofa domestica</i>	Zheng/Han	2,770 - 2,230 BP	25.08	—	—	—	481	1.72
SS13	<i>Sus scrofa domestica</i>	Zheng/Han	2,770 - 2,230 BP	59.55	149140001	4.5	724863	2229	9.95
SS14	<i>Sus scrofa domestica</i>	Zheng/Han	2,770 - 2,230 BP	16.51	95539511	2.69	683271	1396	6.14

5.4.1.2 Mitochondrial phylogeny

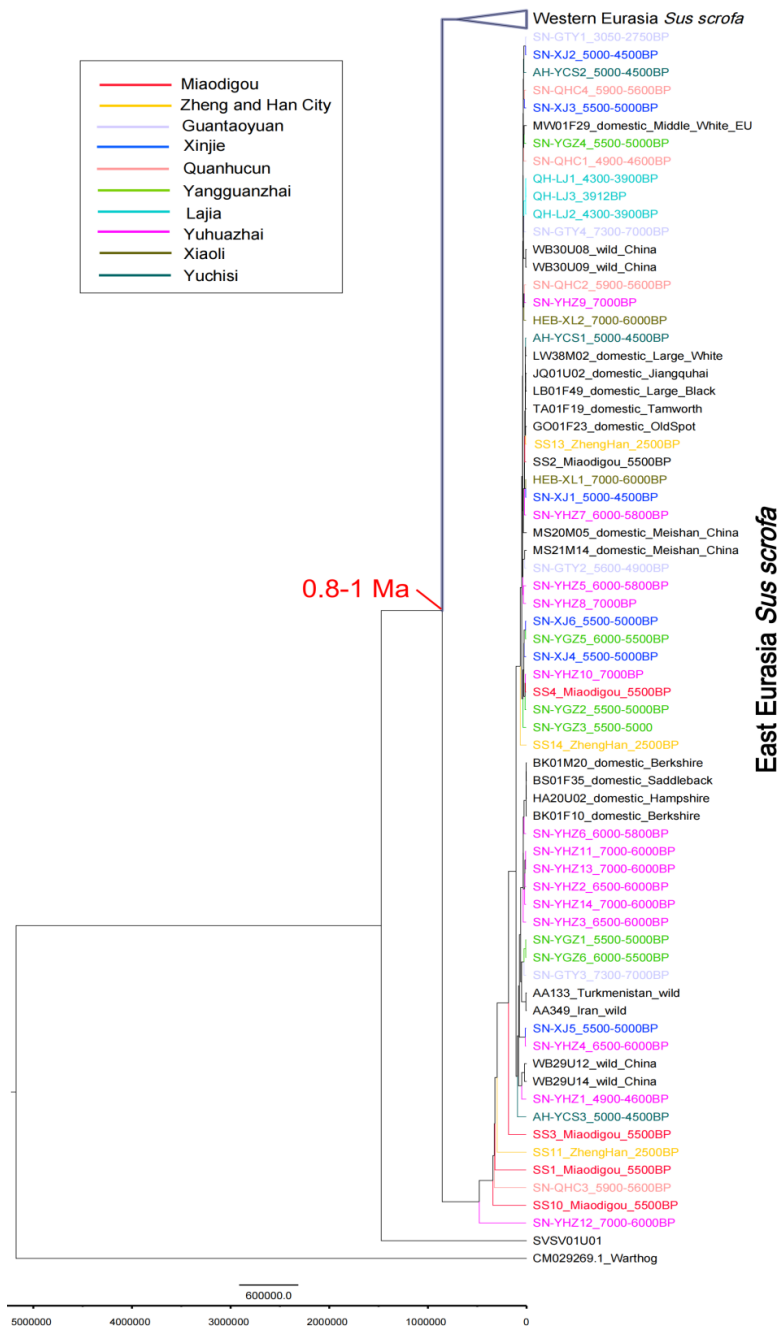


Figure 21 MCC tree inferring the maternal genetic relationship of Eurasian *Sus scrofa*. *Sus scrofa* from Western Eurasia were collapsed in the plot. For the tree with more details please see Figure S 5.

Analysed samples are distinctly separated into two deeply diverged clades, representing *Sus scrofa* from Western and Eastern Eurasia, respectively. Our analysis indicates that these two clades diverged approximately 0.8 to 1 million years ago (Ma). Ancient samples from

Miaodigou, Zheng, and Han City all cluster within the Eastern clade. Interestingly, several modern individuals sampled in the Near East or Europe also group with the domestic pigs of East Asia. These individuals predominantly belong to European commercial breeds, including Large White, Large Black, Middle White, Tamworth, and Gloucestershire Old Spot.(Figure 21).

The phylogenetic analysis does not exhibit any discernible temporal or spatial patterns. Rather, samples from various geographic locations and time periods appear intermixed, instead of clustering into groups associated with specific archaeological sites. Moreover, pigs from the Miaodigou, Zheng, and Han City sites generally demonstrate a relatively deep divergence time. Our analysis reveals that the deepest branch (SS10) shares a common ancestor with other Miaodigou (MIA) or Zheng and Han City (Z&H) pigs, dating back approximately 0.27 to 0.43 million years ago (Ma). Furthermore, the most recent divergence within MIA and Z&H individuals occurred around 0.04 to 0.1 Ma (Figure 21). These dates are likely significantly prior to their domestication, suggesting that the domestication of pigs in the middle reaches of the Yellow River Basin may have involved substantial genetic diversity or multiple independent domestication events.

5.4.1.3 Principal component analysis

To elucidate the genetic relationships among domestic pigs or wild boars across Eurasia, we conducted a principal component analysis (PCA) (Price et al., 2006). We included 2,958,161 SNPs derived from five ancient genomes originating from MIA and Z&H, as well as 150 ancient or modern pigs or wild boars from the Frantz et al., 2019 study in the analysis. Given

that ancient samples may contain significant amounts of missing data, which could impact the results, we initially constructed the PCA using only modern samples. Subsequently, the ancient samples were projected onto the established PCA (Figure 22).

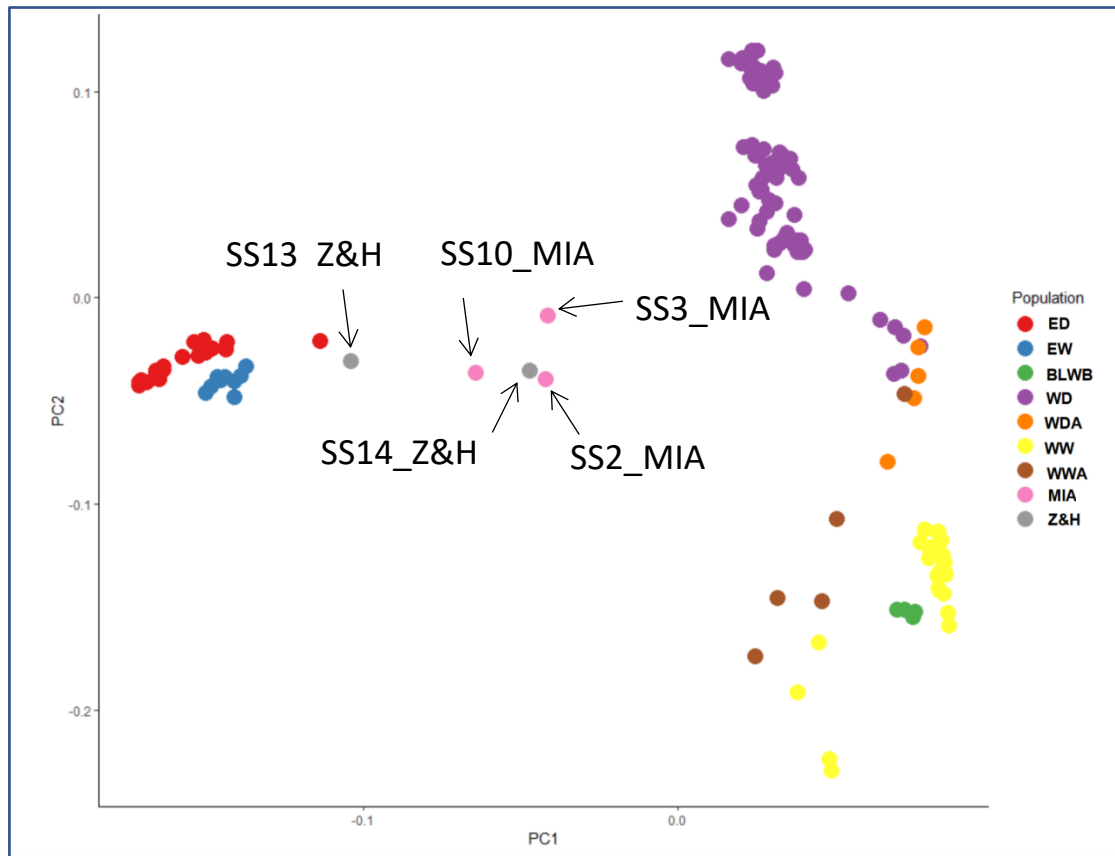


Figure 22 Results of principal component analysis of *sus scrofa* from Eurasia.

PC1 effectively separated pigs from Eastern and Western Eurasia, while PC2 distinguished between domestic pigs and wild boars in East Asia, as well as different populations in Western Eurasia. Intriguingly, the ancient Chinese pigs did not cluster tightly with the modern East Asian domestic pigs or wild boars. Instead, the five samples were distributed across a broader range. Although generally closer to the East Asia group, they exhibited a trend towards the Western Eurasia populations (Figure 22). The distribution of the ancient Chinese samples could be influenced by the fact that the modern Chinese domestic pigs used in this analysis were

primarily collected from Southern China, while our ancient samples originated from the North.

Additionally, it is important to consider that the characteristics of ancient genomes, such as substantial missing data, could potentially contribute to the observed phenomenon.

5.4.1.4 Out-group f_3 analysis

We conducted an out-group f_3 analysis (Patterson et al., 2012) to examine the relationship between ancient domestic pigs from MIA and Z&H and modern native breeds or wild boars in China, using the European wild boar as out-group (Figure 23).

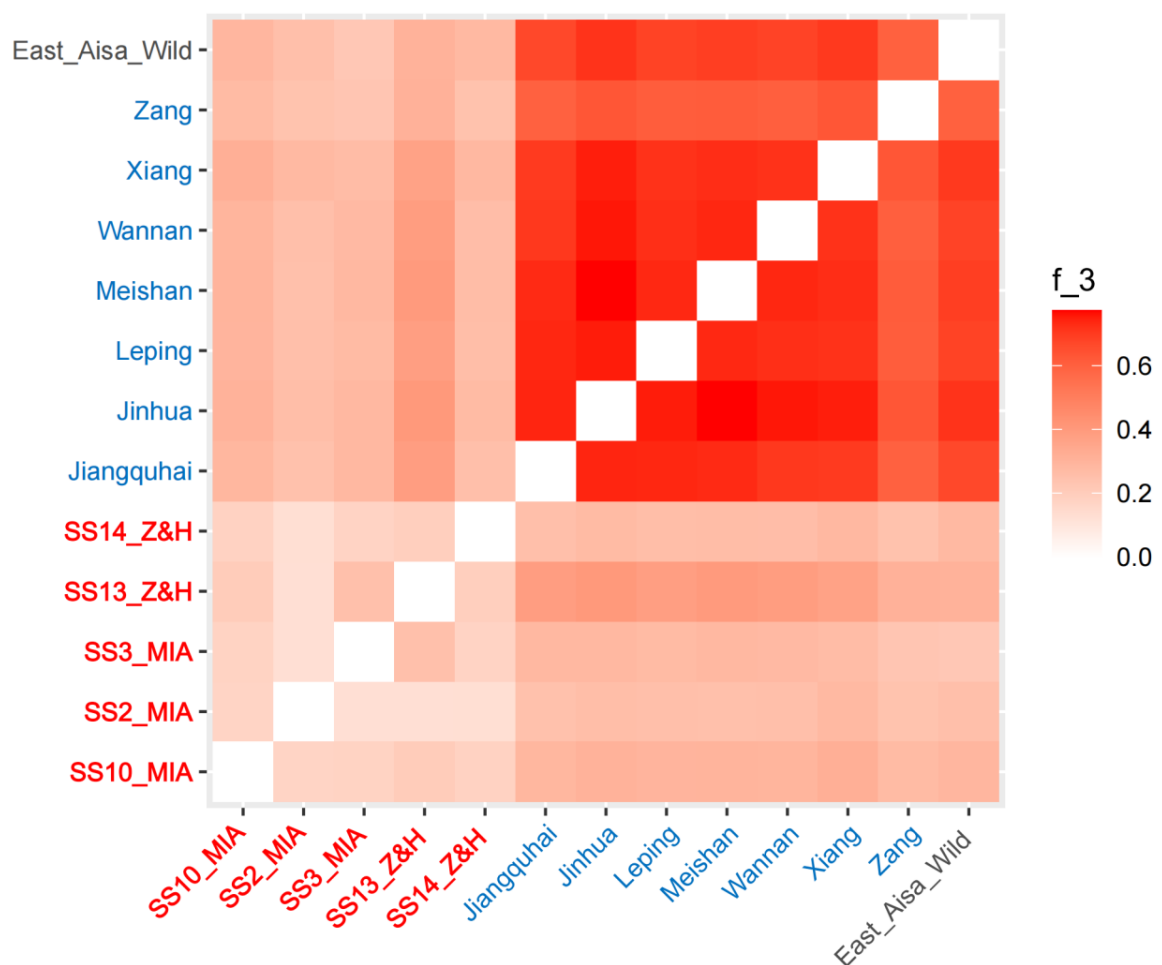


Figure 23 Result of *out-group* f_3 statistics. The IDs of ancient samples are labelled in red font

and modern Chinese pig breeds are ladled in blue font.

The results show that ancient Chinese domestic pigs from MIA and Z&H exhibited higher f_3 values when tested with modern Chinese breeds, indicating they shared more genetic drift. However, the pigs from MIA and Z&H were less closely related compared to the modern ones (Figure 23). One possible explanation for this pattern is that the pigs at the ancient sites had separate origins, yet the populations they represented all contributed to the development of modern breeds. Furthermore, the modern native Chinese breeds and wild boars displayed significantly high f_3 values, suggesting reduced variation in the modern populations.

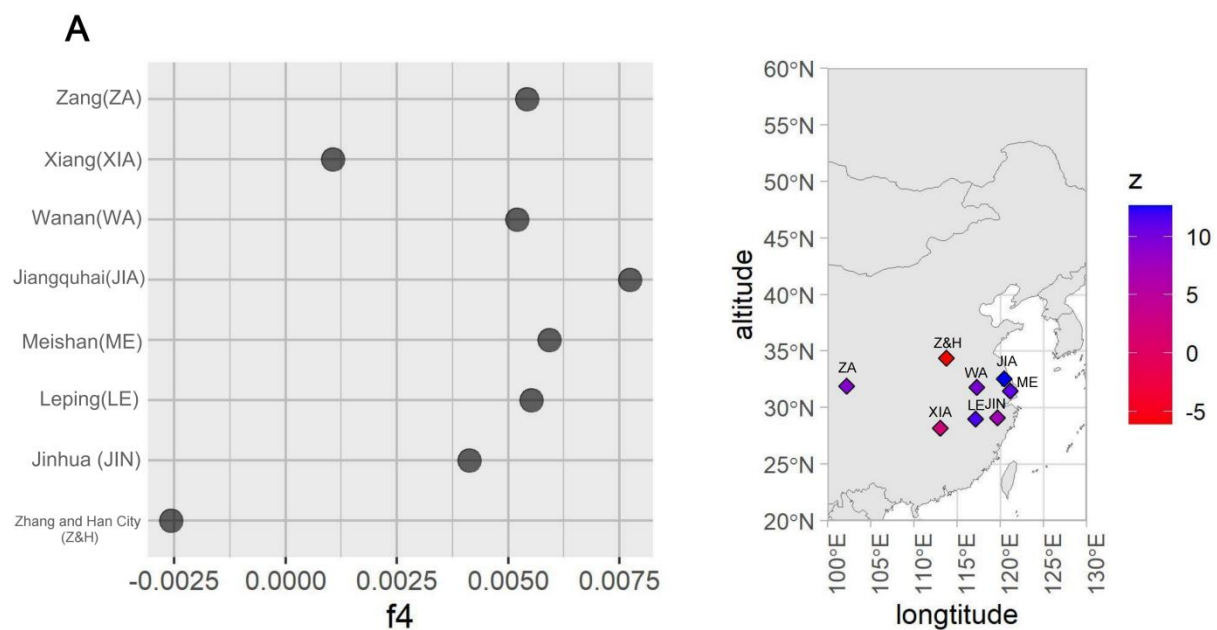
5.4.1.5 ADMIXTURE

To gain insights into the population structure of pigs or wild boars across Eurasia, we conducted an ADMIXTURE analysis (Kenneth, 2011). At $K=2$, pigs and wild boars from China and Western Eurasia are differentiated. At $K=4$, the subgroups of Western Eurasia become distinct. Finally, at $K=6$, the Meishan pig, a native breed in southern China, displays different ancestral components compared to other populations (Figure 24). The Eastern ancestries observed in Western domestic breeds can be attributed to the introgression of Chinese native breeds in the 19th century (Bosse et al., 2014; White, 2011).

As the value of K increases, the ancient Chinese domestic pigs begin to exhibit complex ancestral combinations (Figure 24). This suggests that the ancient Chinese pigs possessed significantly more genetic variation than modern Chinese domestic pigs or Western Eurasian pigs and wild boars.

5.4.1.6 F4-statistics analysis

To investigate the relationships between the ancient Chinese pig populations in MYR and modern Chinese native pigs, we conducted an f4-statistics analysis (Patterson et al., 2012) (Figure 25) using 2,958,161 genomic SNPs. Modern samples were divided based on breeds, while ancient samples were categorized by sites. We observed the introgressions of MIA and Z&H separately to all modern populations by establishing two population combinations: (Warthog, MIA, East Asia wild boar, Modern X) (Figure 25A) and (Warthog, Z&H, East Asia wild boar, Modern X (Figure 25B).



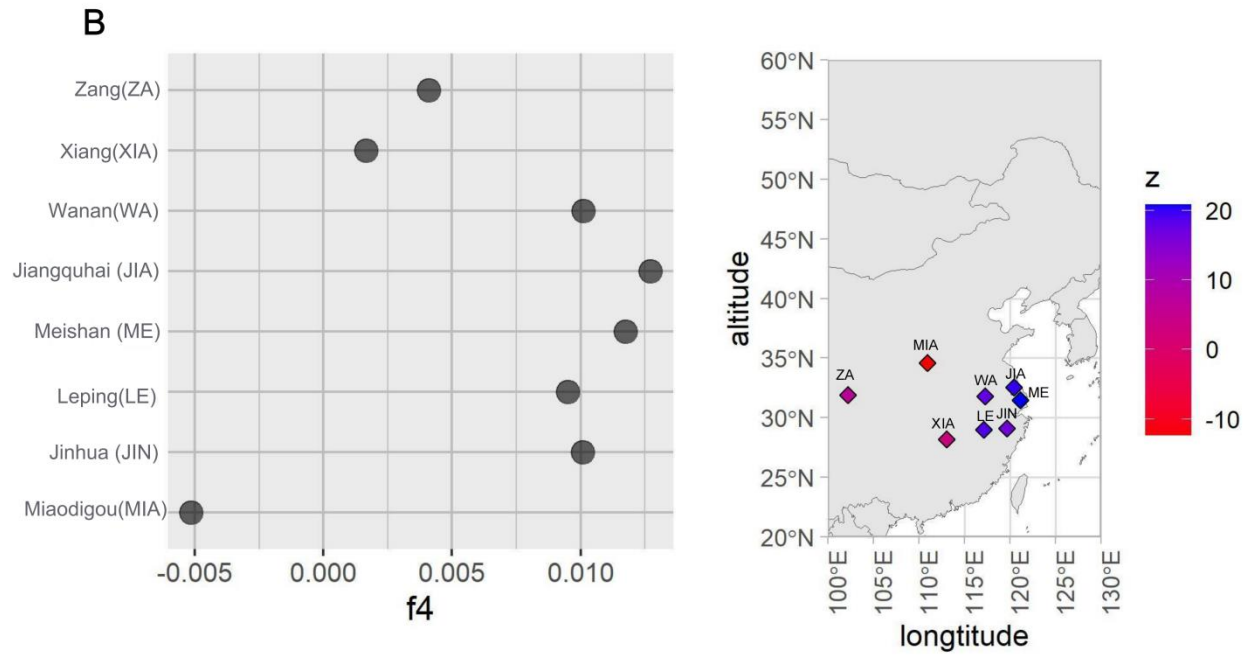


Figure 25 F4 *statistics* results of Chinese domestic pigs . The population combinations were set as (Warthog, MIA, East Asia wild boar, Modern X) (A), and (Warthog, Z&H, East Asia wild boar, Modern X) (B). The scatter plots present the f4 value and maps present the Z score.

Under both population combinations (Warthog, MIA, East Asia wild boar, Modern X) and (Warthog, Z&H, East Asia wild boar, Modern X), the f4-statistics values for modern breeds are consistently positive with significant Z scores (>3). However, MIA and Z&H exhibit negative f4-statistics values when compared to each other relative to the wild boar. This suggests that the populations represented by MIA and Z&H may have both contributed genetically to modern breeds, while within the ancient MYR, the MIA and Z&H pigs were not connected (Figure 25).

5.4.2 Zooarchaeological results

5.4.2.1 Faunal remains of the Zheng and Han City Site and the significance of pigs.

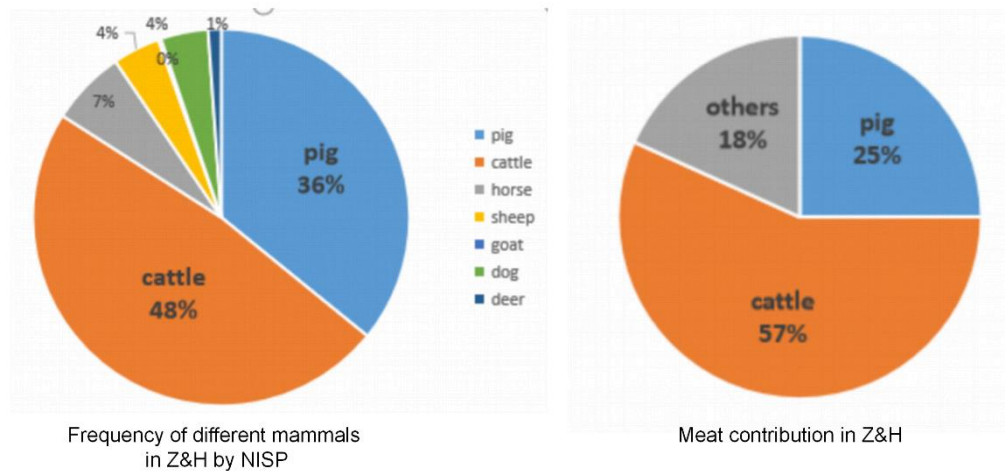


Figure 26 Frequencies of different mammals in Z&H (Left) and Meat contribution in Z&H. The frequencies of animals in the site were calculated based on NISP (Flannery, 1967; Grayson, 1979, 1973). The rate of meat contribution was calculated based on the weight of Bones (Becker, 2001).

In this study, a total of 1,437 samples were analysed, with good bone preservation allowing for the identification of 87.3% of the samples at least to the taxonomic level. However, 22.7% of the samples were too fragmentary to determine their species. The fauna from Zheng and Han City (Z&H) displayed relatively low species diversity. Only one wild species, deer (*Cervoidea sp*), was found, comprising less than 1% of the samples. In contrast, six domestic mammalian species were identified, including dog (*Canis familiaris*), horse (*Equus ferus caballus*), pig (*Sus scrofa domestica*), cattle (*Bos taurus*), sheep (*Ovis aries*), and goat (*Capra hircus*). These species constituted 99% of the total taxa. The most frequently identified animal was domestic

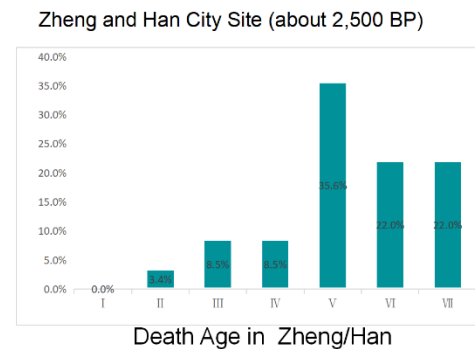
cattle, accounting for 48% of the NISP, followed by domestic pigs at 36% of the NISP. Dogs, horses, sheep, and goats represented 4%, 7%, 4%, and less than 1% of the NISP, respectively (Figure 26).

Based on species identification and NISP statistics, the primary focus of animal exploitation in Z&H was on domestic species, with domestic pigs being a major component of the faunal remains. However, when compared to the MIA site, the significance of domestic pigs in Z&H was notably reduced. At the MIA site, domestic pigs dominated the faunal assemblage, constituting 83.7% and 79.89% of the total fauna in terms of NISP and MNI, respectively (Figure 26).

5.4.2.2 Age of death

A total of 59 well-preserved mandibles from the Zheng and Han City Site were suitable for aging. Among the 59 analysed specimens, none died in stage I; 3.4% died during stage II (N=2); 8.5% died in stage III (N=5); 8.5% died in stage IV; 35.6% died in stage V (N=21); 22% died in stage VI (N=13); and 22% died in stage VII (N=13). The death age of Z&H pigs centered around stage V, comprising approximately 36% of all analysed specimens. This indicates that a significant number of pigs at the site were slaughtered when they were 18 to 24 months old. The concentration of death age during the pigs' prime suggests that this population in the city was indeed domesticated, as the primary purpose of pig farming is to obtain protein and fat, with growth peaking at stage V. Continuing to keep and feed them beyond this point would not significantly increase meat production.

In contrast, the slaughter of pigs at the MIA site occurred at earlier stages and was less centralized. Approximately 11% died before stage I, 24% died in stage II, and 24% died in stage III. The ratios of death age from stage IV to stage VI ranged from 16% to 12%, while around 2% died after stage VII (Figure 27).



I = 0-4 months, II = 5-8 months,
 III = 9-14 months, IV = 14-18
 months, V = 18-24 months, VI = 25-
 36 months, VII = 37+ months

Figure 27 Age of death of pigs from Miaodigou, Yinxu and Zheng and Han City Sites. The determination of death age of pigs was based on the eruption (Silver, 1969) and wear stage of the whole dentition (Grant, 1982; Hillson, 2005). Grant, 1982 has defined seven stages from I to VII, representing the age from 0 to over 37 months.

The Yinxu Site, located in the middle reach of the Yellow River Basin and temporally situated between the Miaodigou and Zhang & Han City sites, offers intriguing insights into pig death ages. In comparison, the death ages of Yinxu pigs were more concentrated in stage V, with over 50% of the individuals slaughtered between 1.5 to 2 years old. The number of pigs beyond stage V was not as high as in the Z&H site (Figure 27).

It appears that the slaughtering ages from the Miaodigou to the Zheng and Han City Site

increased over time. However, even when using the same system (Grant, 1982), numerous factors can affect tooth abrasion and growth, such as environment, diet, breeds, and even secondary influences during the taphonomy process. Sampling bias could also impact the death age structure. Moreover, this study compares only three sites, which makes it difficult to determine with certainty whether an upward trend truly exists. To further investigate this phenomenon, future studies should sample more specimens from different excavation locations representing various contexts.

5.4.2.3 Body mass of the Z&H pigs

We measured the Bd, BT, and Dd of the humerus, along with the length and width of the lower 3rd molar for samples from the Zheng and Han City Site and compared them to those from the Miaodigou Site (Von den Driesch, 1976). The measurements are displayed in Figure 28.

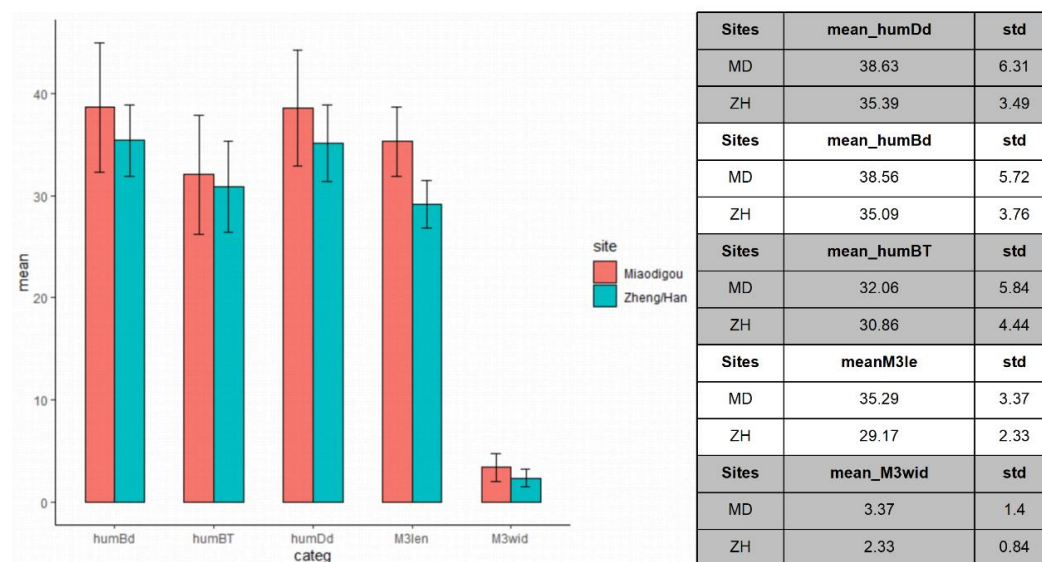


Figure 28 Results of The measurements of animal bones or teeth were processed according to (Von den Driesch, 1976). **humBd**= breadth of humerus's distal end **humBT**=breadth of humerus's trochlea **humDd**=depth of humerus's distal end **M3len**=length of of the 3rd mandible molar **M3wid**=width of the 3rd mandible molar.

For all measurements, the Z&H population consistently displayed smaller mean values and standard deviations compared to the MIA site (Figure 28). This indicates that the Z&H population had a smaller body size. Notably, the body mass of the Z&H pigs deviated less from the mean. This could be attributed to more intensive management of the pigs, where standardized living environments and feeding practices for the herds resulted in less variation in body mass (Becker, 2001; Zeder, 2012).

5.4.2.4 Meat contribution to human diets

The contribution of meat to human diets at the Z&H site was calculated based on the weight of the bones (Becker, 2001). According to the results, cattle were the predominant source, comprising 57% of the total, while pigs were the second-largest source, accounting for 25%—significantly lower than cattle (Figure 26). In contrast, pigs were the primary contributor to the diet of MIA residents, with 85% of the meat, by weight, being attributed to domestic pigs (Liu et al., 2021). This suggests that the protein sources at the Zheng and Han City site were more diverse, and the reliance on pig meat was significantly weaker compared to the Miaodigou Site.

5.5 Discussion

5.5.1 The Origin of the Chinese domestic pigs

Research has shown that domestic pigs have at least two independent origins across Eurasia (Larson et al., 2005; Yu et al., 2013). Our analysis supports this hypothesis. In the phylogeny constructed using whole mitochondrial genomes, pigs from Eastern and Western Eurasia

formed two geographically distinct clades (Figure 21). The PCA (Figure 22), outgroup f_3 -statistics (Figure 23), and ADMIXTURE (Figure 24) exhibited the same pattern, where Asian pigs were genetically distinct from those in the Near East and Europe. Although some European commercial pig breeds possess Asia-derived mitochondrial genomes or nuclear ancestries, this can be attributed to the breeding programs that occurred in the 19th century when the Industrial Revolution in Europe stimulated the demand for meat, leading to the importation of Asian pig breeds to improve meat yield (Bosse et al., 2014; White, 2011).

Regarding the relationship between ancient MYR domestic pigs and modern Chinese native breeds, our results suggest that both populations represented by MIA and Z&H contributed to the formation of modern Chinese domestic pigs. Some individuals of modern breeds, such as Meishan and Jiangquhai, directly share the same maternal ancestries with MYR ancient pigs. The f_3 -statistics results indicate that all modern breeds share some affinities with ancient ones, and the f_4 -statistic further implies introgressions between them (Figure 25).

Interestingly, the ancient pigs from MYR did not display specific geographical or temporal patterns in the mitochondrial phylogenetic analysis. Furthermore, based on the nuclear genome analysis, pigs from MIA and Z&H have weaker genetic connections compared to their associations with modern breeds, as suggested by out-group f_3 -statistics (Figure 24) and f_4 -statistics results (Figure 25).

Over the past few decades, the origin of Chinese domestic pigs has been a question that has

garnered widespread interest. Numerous efforts have been made to identify the locations where domestic pigs first emerged, but pinpointing one or two specific populations or regions as the primary source for modern herds has proven challenging, especially given the great genetic diversity of pigs in East Asia (Cucchi et al., 2011; Jing and Flad, 2002; Larson et al., 2010; Wang et al., 2022; Wu et al., 2007; Xiang et al., 2017b). Several areas, including the Yellow River Basin, Yangtze River Basin, and Mekong River Basin, have been proposed as potential domestication sites (Ai et al., 2015; Jin et al., 2012; Aplin et al., 2011; Wang et al., 2022; Wu et al., 2007; Xiang et al., 2017a; Yang et al., 2011; Zhang et al., 2022).

We hypothesize that the domestication of pigs in ancient China may have involved multiple independent domestication events emerged in multiple geographical areas, with a large and genetically diverse population participating in this process, while frequent introgressions or admixtures among these domesticated populations also occurred. This led to the phenomenon where modern pigs have close relationships with ancient ones, while early domestic populations are less genetically connected. As for why modern pigs are not as diverse as their ancient counterparts, this could be the result of intensified selection processes that occurred at some point in history.

We propose that the study of the origin of Chinese domestic pigs should be divided into two research directions:

- 1) Identifying ancient domesticated populations: The aim here is to understand when, where, and how domestication as a concept or technology was invented in different regions and periods.

Zooarchaeology, stable isotope, and microbe-related diet or disease methods hold more potential in revealing these insights since dramatic changes in pig genomes may not occur at the very initial stage of domestication (Jing, 2003; Zeder, 2012). Instead, some subtle morphological or cultural dietary modifications can be detected. It is important to note that even if we identify the earliest domestic populations, it does not necessarily mean they are the biological ancestors of modern pigs.

2) The formation of modern Chinese domestic pig population: For this research goal, we should view the formation of the modern pig population as a process, investigating when and how various ancestries flowed into the gene pool of modern domestic pigs.

5.5.2 A decreased importance of domestic pigs in the MYR spanning 3,000 years

Pigs have long been important domestic animals in China, serving as a major source of protein. However, this has not always been the case. From the Late Neolithic to the early Iron Age (6,000 – 2,500 BP), the pig's contribution to the whole faunal assembly and diet exhibited a general decreasing trend. During the interval between the Middle and Late Neolithic in the MYR of China (7,000 – 6,000 BP), domestic pigs became the most common animal in archaeological sites, comprising about 70%-80% of faunal remains. This frequency peaked in the late Neolithic MYR, where usually 90% of the identifiable bones belonged to domestic pigs. However, after this period, pig remains dropped back to 70% in the next 500 years and gradually decreased to 30% to 60% in the early Iron Age (Luo, 2012). In our analysis, the Z&H

pigs accounted for about 36% of the NISP, and their diet contribution was only 24% (Figure 26). These results correspond to the decreased importance of domestic pigs in MYR as demonstrated by previous research (Luo, 2012).

We believe that the decline in the pig's proportion was likely caused by the diversification of animal husbandry, where the introduction and prosperity of other domestic taxa from the Near East, such as cattle (Cai et al., 2014; Ren et al., 2022), sheep (Cai et al., 2011, 2007), and goats (Han et al., 2010), reduced pigs' role as a primary food source. This is particularly noticeable for domestic cattle, which were thought to have been first imported about 5,500 BP and thrived around 4,000 BP, coinciding with the decline of pigs in the archaeological record (Luo, 2012). It has been shown that the preference for selecting sacrificial animals in MYR shifted from pigs to cattle around 3,500 BP (Yuan and Flad, 2005). In our research, cattle comprised 48% of the NISP and 57% of the diet contribution, both exceeding the proportion of pigs. Cattle, compared to pigs, offer advantages such as higher protein yield, easier transportation, and more efficient utilization of natural resources surrounding settlements. With all these benefits, it is not surprising that cattle became a major taxon, balancing or even exceeding the presence of pigs. Furthermore, farming multiple species of livestock can significantly increase the adaptability of human societies to risks posed by natural disasters or environmental changes (Joshi et al., 2004, 2004).

5.5.3 Intensified management of domestic pigs

Our results likely reveal a decrease in pig body mass and variation in MYR from 5,500 to 2,500

BP. We measured the Bd, BT, and Dd of the humerus and the length and width of the lower 3rd molar (Von den Driesch, 1976). Regardless of the measurements, the humerus and 3rd molar of Z&H pigs were consistently smaller than those of MIA pigs. At the same time, standard deviations for Z&H were also lower than those for MIA (Figure 28). We suggest that this reflected intensified pig husbandry, which could decrease the size and variation of pig body mass. Intensive management of domestic herds typically leads to a decline in size, particularly for Chinese domestic pigs, where advancements in pig farming could result in a downward trend of body mass in ancient pigs (Becker, 2001; Zeder, 2012).

As for the reduced variation in bone size, we believe that the intensification of animal management can result in higher standardization of diet and living environment for pigs. Under more similar circumstances, the growth of Z&H pigs would consequently show relative synchronization. From 5,500 BP to 2,500 BP, MYR developed from the Neolithic to the early Iron Age. Improvements in agriculture, pig farming knowledge, and division of labour cultivated the conditions for more intensified management of pig herds. Previous research has discovered that pig husbandry intensified following domestication, corresponding with population growth and a shift in socio-economic organization in the Yellow and Yangtze River basins from 7,000 to 2,000 BP (Dong and Yuan, 2020; Zhang, 2021). Our results provide additional evidence to support this hypothesis.

5.6 Conclusion

Our genetic analysis indicated that the populations represented by the domestic pigs from MIA

and Z&H both contributed to the modern native breeds. However, the pigs from MIA and Z&H were less genetically connected, suggesting a more diversified population during the early stage of pig domestication. Based on this, we hypothesized that multiple populations were domesticated in China.

The zooarchaeological analysis suggested that from the late Neolithic to the Iron Age, the importance of domestic pigs experienced a decline, as revealed by the decreased NISP and contribution to diet from MIA to Z&H. The phenomenon we observed might correspond to a trend where, in MYR, the general frequency of domestic pigs dropped from the Neolithic Age. We attribute the decline of pigs to the introduction of other taxa, especially cattle from the Near East, and the subsequent diversification of animal husbandry. Furthermore, the size and variation of body mass were smaller compared to that of MIA pigs, a change that could correspond to the intensification of pig management in the middle reach of the Yellow River Basin.

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Supplementary

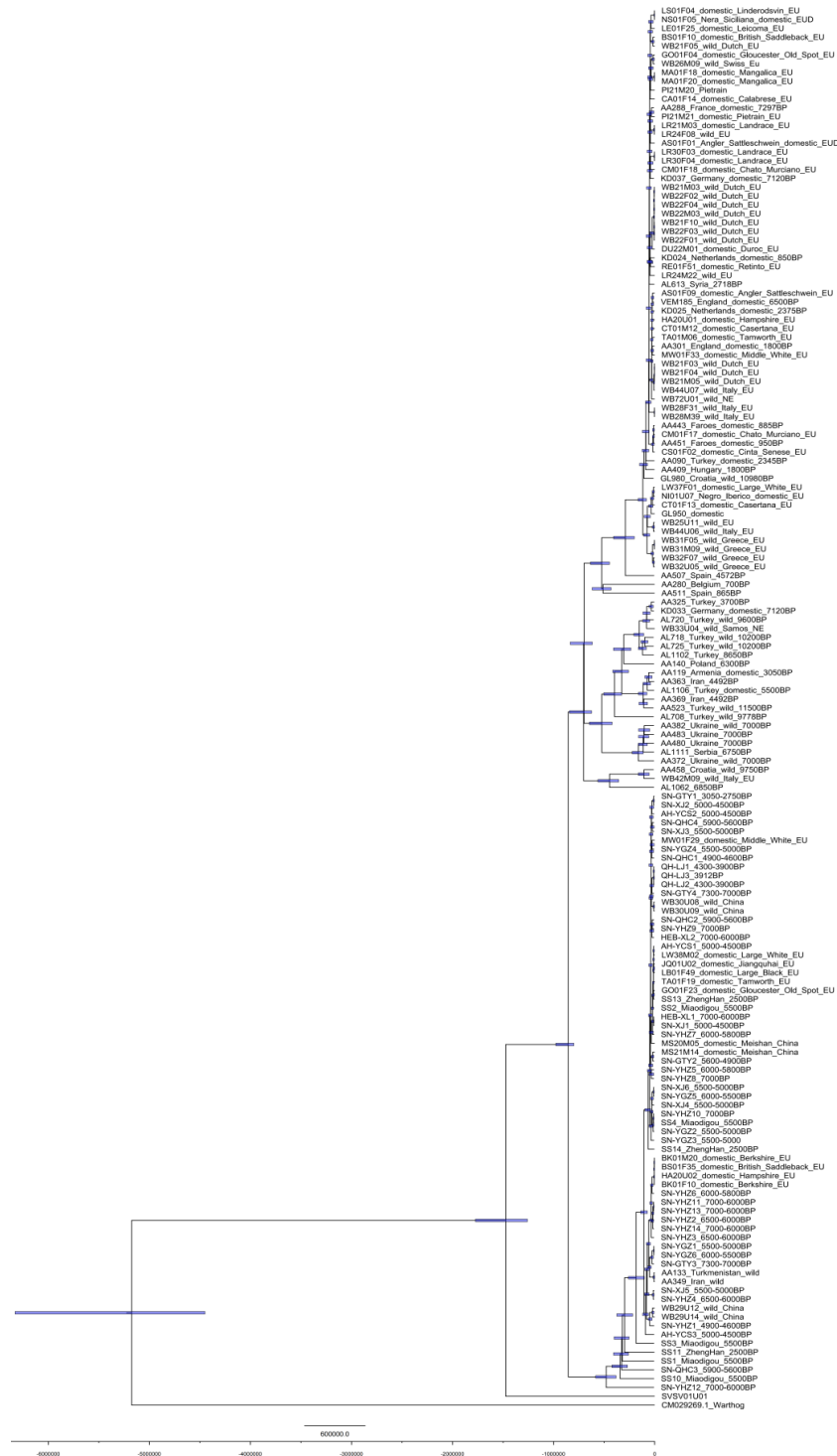


Figure S5 MCC tree constructed using mitochondrial genomes. Ages of tips were used to calculate the time of divergence. Bars on nodes denote the height 95% HPD.

Table S11 Results of outgroup f3 analysis of pigs.

A	B	C	f_3	std.err	Z	SNPs
SS2_MIA	SS2_MIA	West_Wild	0	-1	0	-1
SS2_MIA	SS3_MIA	West_Wild	0.129328	0.004226	30.6	346506
SS2_MIA	SS10_MIA	West_Wild	0.175307	0.00517	33.907	542116
SS2_MIA	SS13_Z&H	West_Wild	0.128678	0.005033	25.564	552152
SS2_MIA	SS14_Z&H	West_Wild	0.130901	0.003842	34.068	507689
SS2_MIA	Jinhua	West_Wild	0.263267	0.006434	40.915	550264
SS2_MIA	Jiangquhai	West_Wild	0.24976	0.006167	40.497	548608
SS2_MIA	Leping	West_Wild	0.256771	0.006111	42.017	570390
SS2_MIA	Meishan	West_Wild	0.255497	0.006013	42.489	574147
SS2_MIA	Wannan	West_Wild	0.256372	0.006293	40.737	556496
SS2_MIA	Xiang	West_Wild	0.282267	0.006574	42.934	554484
SS2_MIA	Zang	West_Wild	0.243746	0.006219	39.193	512172
SS2_MIA	East_Aisa_Wild	West_Wild	0.257063	0.005278	48.709	583336
SS3_MIA	SS2_MIA	West_Wild	0.129328	0.004226	30.6	346506
SS3_MIA	SS3_MIA	West_Wild	0	-1	0	-1
SS3_MIA	SS10_MIA	West_Wild	0.179036	0.004522	39.591	344683
SS3_MIA	SS13_Z&H	West_Wild	0.252886	0.006232	40.577	343359
SS3_MIA	SS14_Z&H	West_Wild	0.177675	0.003953	44.952	321340
SS3_MIA	Jinhua	West_Wild	0.285647	0.006708	42.585	347307
SS3_MIA	Jiangquhai	West_Wild	0.284745	0.006798	41.889	345203
SS3_MIA	Leping	West_Wild	0.275011	0.006415	42.868	361786
SS3_MIA	Meishan	West_Wild	0.286103	0.00681	42.011	364097
SS3_MIA	Wannan	West_Wild	0.279631	0.006822	40.992	351030
SS3_MIA	Xiang	West_Wild	0.268448	0.006591	40.727	352083
SS3_MIA	Zang	West_Wild	0.233426	0.005878	39.712	324132
SS3_MIA	East_Aisa_Wild	West_Wild	0.224415	0.004815	46.61	374281
SS10_MIA	SS2_MIA	West_Wild	0.175307	0.00517	33.907	542116
SS10_MIA	SS3_MIA	West_Wild	0.179036	0.004522	39.591	344683
SS10_MIA	SS10_MIA	West_Wild	0	-1	0	-1
SS10_MIA	SS13_Z&H	West_Wild	0.207419	0.005436	38.159	546491
SS10_MIA	SS14_Z&H	West_Wild	0.183126	0.004234	43.251	505677
SS10_MIA	Jinhua	West_Wild	0.308273	0.006942	44.405	546476
SS10_MIA	Jiangquhai	West_Wild	0.290521	0.006829	42.543	544217
SS10_MIA	Leping	West_Wild	0.299494	0.006763	44.281	564240
SS10_MIA	Meishan	West_Wild	0.300496	0.006788	44.269	567527
SS10_MIA	Wannan	West_Wild	0.297922	0.006867	43.384	551345
SS10_MIA	Xiang	West_Wild	0.3216	0.007001	45.933	549848
SS10_MIA	Zang	West_Wild	0.275268	0.006545	42.055	510084
SS10_MIA	East_Aisa_Wild	West_Wild	0.292773	0.005726	51.132	576733
SS13_Z&H	SS2_MIA	West_Wild	0.128678	0.005033	25.564	552152
SS13_Z&H	SS3_MIA	West_Wild	0.252886	0.006232	40.577	343359

SS13_Z&H	SS10_MIA	West_Wild	0.207419	0.005436	38.159	546491
SS13_Z&H	SS13_Z&H	West_Wild	0	-1	0	-1
SS13_Z&H	SS14_Z&H	West_Wild	0.195065	0.004793	40.697	510412
SS13_Z&H	Jinhua	West_Wild	0.405757	0.009264	43.799	546378
SS13_Z&H	Jiangquhai	West_Wild	0.388632	0.009082	42.794	544073
SS13_Z&H	Leping	West_Wild	0.384726	0.008687	44.289	564604
SS13_Z&H	Meishan	West_Wild	0.400078	0.008775	45.596	566887
SS13_Z&H	Wannan	West_Wild	0.390994	0.009107	42.935	551394
SS13_Z&H	Xiang	West_Wild	0.370233	0.008679	42.656	552768
SS13_Z&H	Zang	West_Wild	0.311552	0.007797	39.96	514015
SS13_Z&H	East_Aisa_Wild	West_Wild	0.307481	0.006343	48.477	580463
SS14_Z&H	SS2_MIA	West_Wild	0.130901	0.003842	34.068	507689
SS14_Z&H	SS3_MIA	West_Wild	0.177675	0.003953	44.952	321340
SS14_Z&H	SS10_MIA	West_Wild	0.183126	0.004234	43.251	505677
SS14_Z&H	SS13_Z&H	West_Wild	0.195065	0.004793	40.697	510412
SS14_Z&H	SS14_Z&H	West_Wild	0	-1	0	-1
SS14_Z&H	Jinhua	West_Wild	0.274716	0.005738	47.878	511065
SS14_Z&H	Jiangquhai	West_Wild	0.25804	0.005563	46.382	508961
SS14_Z&H	Leping	West_Wild	0.262985	0.005308	49.541	530645
SS14_Z&H	Meishan	West_Wild	0.267191	0.005349	49.955	534182
SS14_Z&H	Wannan	West_Wild	0.263991	0.005588	47.24	516351
SS14_Z&H	Xiang	West_Wild	0.284105	0.005717	49.693	515171
SS14_Z&H	Zang	West_Wild	0.246565	0.005389	45.755	475883
SS14_Z&H	East_Aisa_Wild	West_Wild	0.28169	0.005158	54.616	543596
Jinhua	SS2_MIA	West_Wild	0.263267	0.006434	40.915	550264
Jinhua	SS3_MIA	West_Wild	0.285647	0.006708	42.585	347307
Jinhua	SS10_MIA	West_Wild	0.308273	0.006942	44.405	546476
Jinhua	SS13_Z&H	West_Wild	0.405757	0.009264	43.799	546378
Jinhua	SS14_Z&H	West_Wild	0.274716	0.005738	47.878	511065
Jinhua	Jinhua	West_Wild	0	-1	0	-1
Jinhua	Jiangquhai	West_Wild	0.740795	0.011556	64.103	2552705
Jinhua	Leping	West_Wild	0.752961	0.011375	66.192	2616637
Jinhua	Meishan	West_Wild	0.773308	0.011331	68.25	2627301
Jinhua	Wannan	West_Wild	0.758555	0.011824	64.154	2575609
Jinhua	Xiang	West_Wild	0.750405	0.01107	67.787	2576504
Jinhua	Zang	West_Wild	0.633223	0.011423	55.433	2447421
Jinhua	East_Aisa_Wild	West_Wild	0.717079	0.009799	73.182	2665265
Jiangquhai	SS2_MIA	West_Wild	0.24976	0.006167	40.497	548608
Jiangquhai	SS3_MIA	West_Wild	0.284745	0.006798	41.889	345203
Jiangquhai	SS10_MIA	West_Wild	0.290521	0.006829	42.543	544217
Jiangquhai	SS13_Z&H	West_Wild	0.388632	0.009082	42.794	544073
Jiangquhai	SS14_Z&H	West_Wild	0.25804	0.005563	46.382	508961
Jiangquhai	Jinhua	West_Wild	0.740795	0.011556	64.103	2552705

Jiangquhai	Jiangquhai	West_Wild	0	-1	0	-1
Jiangquhai	Leping	West_Wild	0.738787	0.011909	62.037	2599842
Jiangquhai	Meishan	West_Wild	0.73335	0.011701	62.672	2615727
Jiangquhai	Wannan	West_Wild	0.708813	0.011997	59.083	2567251
Jiangquhai	Xiang	West_Wild	0.703407	0.011206	62.77	2568807
Jiangquhai	Zang	West_Wild	0.59976	0.011357	52.81	2438700
Jiangquhai	East_Aisa_Wild	West_Wild	0.670253	0.009899	67.707	2656184
Leping	SS2_MIA	West_Wild	0.256771	0.006111	42.017	570390
Leping	SS3_MIA	West_Wild	0.275011	0.006415	42.868	361786
Leping	SS10_MIA	West_Wild	0.299494	0.006763	44.281	564240
Leping	SS13_Z&H	West_Wild	0.384726	0.008687	44.289	564604
Leping	SS14_Z&H	West_Wild	0.262985	0.005308	49.541	530645
Leping	Jinhua	West_Wild	0.752961	0.011375	66.192	2616637
Leping	Jiangquhai	West_Wild	0.738787	0.011909	62.037	2599842
Leping	Leping	West_Wild	0	-1	0	-1
Leping	Meishan	West_Wild	0.73687	0.011461	64.296	2671649
Leping	Wannan	West_Wild	0.726103	0.011828	61.389	2626194
Leping	Xiang	West_Wild	0.720717	0.01113	64.756	2623476
Leping	Zang	West_Wild	0.613518	0.011328	54.159	2501678
Leping	East_Aisa_Wild	West_Wild	0.684615	0.009792	69.913	2700663
Meishan	SS2_MIA	West_Wild	0.255497	0.006013	42.489	574147
Meishan	SS3_MIA	West_Wild	0.286103	0.00681	42.011	364097
Meishan	SS10_MIA	West_Wild	0.300496	0.006788	44.269	567527
Meishan	SS13_Z&H	West_Wild	0.400078	0.008775	45.596	566887
Meishan	SS14_Z&H	West_Wild	0.267191	0.005349	49.955	534182
Meishan	Jinhua	West_Wild	0.773308	0.011331	68.25	2627301
Meishan	Jiangquhai	West_Wild	0.73335	0.011701	62.672	2615727
Meishan	Leping	West_Wild	0.73687	0.011461	64.296	2671649
Meishan	Meishan	West_Wild	0	-1	0	-1
Meishan	Wannan	West_Wild	0.738722	0.01183	62.446	2638010
Meishan	Xiang	West_Wild	0.728536	0.010926	66.676	2635750
Meishan	Zang	West_Wild	0.616335	0.011301	54.54	2513219
Meishan	East_Aisa_Wild	West_Wild	0.694133	0.009648	71.948	2712999
Wannan	SS2_MIA	West_Wild	0.256372	0.006293	40.737	556496
Wannan	SS3_MIA	West_Wild	0.279631	0.006822	40.992	351030
Wannan	SS10_MIA	West_Wild	0.297922	0.006867	43.384	551345
Wannan	SS13_Z&H	West_Wild	0.390994	0.009107	42.935	551394
Wannan	SS14_Z&H	West_Wild	0.263991	0.005588	47.24	516351
Wannan	Jinhua	West_Wild	0.758555	0.011824	64.154	2575609
Wannan	Jiangquhai	West_Wild	0.708813	0.011997	59.083	2567251
Wannan	Leping	West_Wild	0.726103	0.011828	61.389	2626194
Wannan	Meishan	West_Wild	0.738722	0.01183	62.446	2638010
Wannan	Wannan	West_Wild	0	-1	0	-1

Wannan	Xiang	West_Wild	0.719864	0.0115	62.596	2589430
Wannan	Zang	West_Wild	0.607714	0.011593	52.419	2463760
Wannan	East_Aisa_Wild	West_Wild	0.683422	0.01013	67.466	2674838
Xiang	SS2_MIA	West_Wild	0.282267	0.006574	42.934	554484
Xiang	SS3_MIA	West_Wild	0.268448	0.006591	40.727	352083
Xiang	SS10_MIA	West_Wild	0.3216	0.007001	45.933	549848
Xiang	SS13_Z&H	West_Wild	0.370233	0.008679	42.656	552768
Xiang	SS14_Z&H	West_Wild	0.284105	0.005717	49.693	515171
Xiang	Jinhua	West_Wild	0.750405	0.01107	67.787	2576504
Xiang	Jiangquhai	West_Wild	0.703407	0.011206	62.77	2568807
Xiang	Leping	West_Wild	0.720717	0.01113	64.756	2623476
Xiang	Meishan	West_Wild	0.728536	0.010926	66.676	2635750
Xiang	Wannan	West_Wild	0.719864	0.0115	62.596	2589430
Xiang	Xiang	West_Wild	0	-1	0	-1
Xiang	Zang	West_Wild	0.633128	0.011623	54.47	2457492
Xiang	East_Aisa_Wild	West_Wild	0.706135	0.009784	72.172	2661527
Zang	SS2_MIA	West_Wild	0.243746	0.006219	39.193	512172
Zang	SS3_MIA	West_Wild	0.233426	0.005878	39.712	324132
Zang	SS10_MIA	West_Wild	0.275268	0.006545	42.055	510084
Zang	SS13_Z&H	West_Wild	0.311552	0.007797	39.96	514015
Zang	SS14_Z&H	West_Wild	0.246565	0.005389	45.755	475883
Zang	Jinhua	West_Wild	0.633223	0.011423	55.433	2447421
Zang	Jiangquhai	West_Wild	0.59976	0.011357	52.81	2438700
Zang	Leping	West_Wild	0.613518	0.011328	54.159	2501678
Zang	Meishan	West_Wild	0.616335	0.011301	54.54	2513219
Zang	Wannan	West_Wild	0.607714	0.011593	52.419	2463760
Zang	Xiang	West_Wild	0.633128	0.011623	54.47	2457492
Zang	Zang	West_Wild	0	-1	0	-1
Zang	East_Aisa_Wild	West_Wild	0.601969	0.010492	57.372	2546454
East_Aisa_Wild	SS2_MIA	West_Wild	0.257063	0.005278	48.709	583336
East_Aisa_Wild	SS3_MIA	West_Wild	0.224415	0.004815	46.61	374281
East_Aisa_Wild	SS10_MIA	West_Wild	0.292773	0.005726	51.132	576733
East_Aisa_Wild	SS13_Z&H	West_Wild	0.307481	0.006343	48.477	580463
East_Aisa_Wild	SS14_Z&H	West_Wild	0.28169	0.005158	54.616	543596
East_Aisa_Wild	Jinhua	West_Wild	0.717079	0.009799	73.182	2665265
East_Aisa_Wild	Jiangquhai	West_Wild	0.670253	0.009899	67.707	2656184
East_Aisa_Wild	Leping	West_Wild	0.684615	0.009792	69.913	2700663
East_Aisa_Wild	Meishan	West_Wild	0.694133	0.009648	71.948	2712999
East_Aisa_Wild	Wannan	West_Wild	0.683422	0.01013	67.466	2674838
East_Aisa_Wild	Xiang	West_Wild	0.706135	0.009784	72.172	2661527
East_Aisa_Wild	Zang	West_Wild	0.601969	0.010492	57.372	2546454
East_Aisa_Wild	East_Aisa_Wild	West_Wild	0	-1	0	-1

6 Conclusion

By predominantly utilizing paleogenetic methods in combination with radiocarbon dating and archaeology, this research has investigated four distinct species who are domesticated, semi-domesticated or sharing a commensal relationship with humans, and addressed the anthropogenic influence on the evolution of animals.

6.1 Human-mediated translocation modifying the evolutionary trajectory of animals.

Human-mediated transformation of biogeography is one of the greatest features in Holocene (Capinha et al., 2015). Many species accompanying human migration have left their original range and dispersed globally (Atsenova et al., 2022; Cai et al., 2014; Frantz et al., 2019; Ottoni et al., 2017). The translocation events have deeply influenced the evolution of animals (Capinha et al., 2015). This is especially true to domestic taxa. Based on our analysis, domestic cats derived from African wild cats whose natural range is in the Near East (Driscoll et al., 2007; Ottoni et al., 2017) were introduced to East Asia, overcoming the geographic barriers in Eurasia (Yu et al., 2021).

Although domestication do not necessarily lead to bottleneck effects (Allaby et al., 2019; Frantz et al., 2020; Larson and Burger, 2013; Nei et al., 1975), it does not apply to translocated animals in some cases, especially in the early stage of their arrival. Like domestic cats, the human-mediated dispersal had moved a small part of the original genetic variation of cats to China, living away from their natural range. In the next about 1,000 years after the first arrival,

domestic cats in China were always mainly exhibiting a unitary ancestry and relatively low diversity in contrast to their counterparts in Western Eurasia. Although the traits of *Felis silvestris bieti* were found in the domestic cats in Southwestern China, the admixture between the two cats are thought to be very recent, minor and limited to the edge of Eastern Tibetan Plateau (Yu et al., 2021).

Translocations in another hand can also facilitate admixtures and influence the formation of domestic populations (Larson and Burger, 2013). Our analysis on the ancient Chinese domestic pigs in the Middle Reach of the Yellow River Basin, a natively originated taxa, indicated that with probably different origins, the domestic pigs in two sites have both genetically contributed to the modern Chinese breeds. It is very likely that in history, domestic pigs with various geographic origins in China were frequently admixed. Human-mediated movement of these populations should have played an important role of bringing these populations together and make them encounter each other. Regarding cats, although the dispersal of cats to China had mainly functioned as bottleneck, it is undeniable that the event had also therefore caused minor hybridization of *felis silvestris lybica* and *felis silvestris bieti*.

Despite domestic animals for those semi-domesticated species who has comparatively weak bonding with human societies was also bearing the influence of human mediated translocations. Persian and European fallow deer, which are thought to be suffering from the low genetic diversity after the last glacial, losing adaptability to the environments other than the natural refuge (Baker et al., 2017; Chapman and Chapman, 1975; JANTSCHKE, 1990), had

unbelievably recolonized their pe-glacial range before and even dispersed to a wider area. The Persian fallow deer were brought to Mediterranean region since the Neolithic Age, and their European counterparts also started the journey in the Neolithic and spread to the mainland Europe and even British Isles. Obviously, anthropogenic forces were almost the decisive factor in this process.

In one hand, it could result in bottleneck or founder effects to some animals, especially when the species was dispersed to the area far away from their natural range. In another hand, it would bring different populations or species with geographical separations together, then lead to admixtures, which could have a long-standing impact to the formation the new populations. For those animals, suffering from the threaten of extinction, if their existence is beneficial, human would still translocate them globally making their populations thriving, also it could simply be an illusion of prosperity.

6.2 The correlation of animal evolution and the development between human societies

According to our study, the evolution trajectories of the animals enjoying affinities with human would be synchronized with the development of human societies.

The commensal leopard cats occurred with an accompaniment of the improvement of the Chinese agricultural economy in the late Neolithic when plants and animal farming became the major subsistence. It was the richness of food resources in agricultural settlements that motivated them to fall in a commensal relationship with human (Wirtz and Lemmen, 2003;

Zeder, 2012a, 2012b). Their disappearance was likely a consequence of the breakdown of the Han Dynasty, when the society was systematically deconstructed, population sharply declined, and agricultural economy saw a sudden collapse (Ge, 1991; Li, 2020; Su et al., 2016). Following the long-term social disorder lasting over 600 years, the commensal leopard cats might never come back again. The occurrence of domestic cats, who have replaced leopard cats in human niche, were imported after the recovery of the Chinese society, when the Sui and Tang countries had again unified the mainland East Asia and brought order and stabilization back (Li, 2020; Su et al., 2016). The thriving of economy and the re-establishment of the prosperity of the East-West cultural communication created ideal environment for the introduction of new species.

Similarly the dispersal of fallow deer was also likely synchronized with the trend of cultural communication among human societies, where the recolonization of Europe by European fallow deer was following the human communication trend across Eurasia from the Neolithic to the Medieval Ages, and the regime of Rome and Normans had stimulated this process. The long-distance dispersal of Persian fallow deer to Mallorca was also mediated by the human trade route across Mediterranean Sea established since the 8th century (Michailidou and Dogan, 2008; Williams-Thorpe and Thorpe, 1993).

The pig domestication in China was also associated with the Establishment of agricultural societies, when the improved surplus of food enabled ancient human to tame and feed animals (Dong and Yuan, 2020; Jing and Flad, 2002; Yang et al., 2022). With the advance of agricultural,

pigs in the Yellow River Basin become the most frequently found animals in human settlements, and the management was also gradually intensified (Dong and Yuan, 2020; Yang et al., 2022). However, with the diversification of livestock after the introductions of cattle, sheep and goat from the Near East (Cai et al., 2014, 2011; Han et al., 2010), the importance of pigs regarding their rates of all the taxa and contribution to human diets saw a downward trend.

6.3 Multiple origins and single origin of domestic animals

Globally, domestic pigs were of multiple origins, with different populations of wild boar being domesticated independently in separate regions in the world (Frantz et al., 2019; Giuffra et al., 2000; Jing and Flad, 2002). Our research supports this idea. The results of the thesis demonstrated that the domestic pigs from two sites of the Middle Reach of the Yellow River Basin have all genetically contributed to the modern Chinese pigs while distinct from each other. Meanwhile, the mitochondrial ancestries present no specific geographical patterns. The phenomenon indicates that the occurrence of the Chinese domestic pigs in the Yellow River Basin involved no clear bottleneck effect and might submit to multiple domestication events, even within a relatively small geographic region. In contrast, it is generally accepted that domestic cattle (*Bos taurus*) have a single origin in the Near East and were then spread throughout the world through human migration and trade (Bollongino et al., 2012; Cai et al., 2014). This raises the question of why the two domestic species, have followed different patterns in their origins. We link their difference to the pathways of domestication.

It has been hypothesized that domestication of cattle followed a prey route. Instead of

developing an initial alliance, these animals were the primary prey species that humans had hunted for their meat or fur. Evidently, the prey route originated when people adopted hunting strategies to increase prey availability (Zeder, 2012a). These tactics for game management developed through time and under particular circumstances into herd management and, eventually, the controlled reproduction of managed animals. In this route, humans were the primary agents interacting with animals, certain circumstances would occur prior to the transition from gaming to herding, and specialized hunting and herd management tactics must be created. Considering this, the domestication of cattle was a complex occurrence involving several variables. Given the complicated process, it is hardly unexpected that there was one major domestication centre.

Pigs went into domestication through the commensal pathway, where the wild boars were attracted to the human-associated environment by the agricultural products accumulated (Zeder, 2012a). In other words, the autonomy of pigs plays an important role forming the affinity with humans. Hence, there will be higher probability for pigs in more regions that went into the process of domestication, as long as agriculture took place there. Eventually it has resulted in a phenomenon that there were more many separate domestication centres for pigs.

6.4 Future directions

6.4.1 Human interactions with commensal animals

Commensalism is an important form of symbiosis in ecosystem (Leung and Poulin, 2008). The

domestication of cat, pig, dog and chicken were demonstrated to start from commensalism (Zeder, 2012a, 2012b). Some ancient undomesticated species such as Chinese leopard cats, and hare were also thought to be commensal. We speculate that the commensal relationships are commonly existing between human and various animals especially after the advent of agriculture. Such biological relationship can to a large extent influence the evolution of animals as what has been revealed in this thesis. Particularly, in today's highly urbanized environment, it is of great importance to explore the symbiotic relationship between wildlife and humans in and around cities. This involves understanding the impact of humans on their evolution, how to manage our interactions with them, and the conservation of those animals. To date, rat, mice or red fox are enjoying commensal association with human (Scholz et al., 2020; Yu et al., 2022). Considering its importance, we would like to proceed with finding candidate animals who were/were in commensal association with and analyze how were their evolutionary trajectories were impacted.

A continued focus on the leopard cats can serve as a good starting point to further our understanding the history of the commensal relationship between human and animals. For the next step, we will meticulously trace the new findings on ancient leopard cats and the corresponding archaeological contexts in order to identify any cultural indicators of commensalism. In the meanwhile, we will also continue our study on genomics to determine the selection pattern of commensal leopard cats in order to evaluate the genetic impact of their living in an anthropogenic context.

6.4.2 Human-mediated admixtures

Human-mediated admixture of animals refers to the intentional or unintentional mixing of genetically distinct animal populations through human activities. This can occur through various means, such as the release of non-native species into a new environment, interbreeding of domesticated and wild animals, or intentional crossbreeding for various purposes such as improving animal productivity or creating new breeds.

The Chinese domestic cats have remained a stable population structure for over 1,000 years, while admixtures with the native wild cats still have occurred in the recent decades. The frequent human-mediated translocations of both the Persian and European fallow deer could also have brought the two species or sub-species into genetic interactions especially in the Southeast Anatolia. The admixture of animals can have both positive and negative impacts on biodiversity, ecosystem function, and human well-being. On one hand, it can increase genetic diversity and adaptive potential, but on the other hand, it can lead to the loss of unique genetic traits and even the extinction of certain populations. Therefore, it is important to carefully consider the potential impacts of human-mediated admixture of animals and take appropriate measures to mitigate any negative effects.

Taking the advantages of ancient DNA, we will continue to monitor the occurrence of diverse hybridization events resulting from human interference since ancient periods, examine the mechanisms underlying these events, and assess their impact on the evolutionary history of animals in a long-term context. This allows us to perform a full study of admixture events

mediated by humans and guide the future breeding or conservation works.

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