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Ancient mitochondrial genome reveals matrilineal genetic inheritance of Chinese goats

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ABSTRACT

As one of the most important domestic animals in ancient China, the origin, diffusion and matrilineal inheritance of goats have been important issues of archaeological research. In this study, we successfully extracted mitochondrial whole genome sequences from 77 samples of goat remains excavated from 16 sites in China, which date back from the Late Neolithic (4300-3800 BP) to the Ming Dynasty (600-400 BP). The results of ancient DNA analysis indicated that the Chinese goat matrilineages began to expand 7000-6000 years ago. The discovery of sub-lineages A2 and B2 suggests that they may have evolved or derived in China. The expansion of lineage A and the decline in the number of lineage B provide important evidence for the eastward migration of humans from the western part of the Eurasian continent. Furthermore, this study confirms that ancient Chinese goats had contributed genetically to the modern goats of China, and that the Chinese goats are genetically related to goats in South and Southeast Asia. Mitochondrial genome analysis of ancient Chinese goats not only provides an important resource for future analyses and research, but also offers new perspectives for the origin and diffusion of domestic goats.

1. Introduction

The domestication of wild animals had played a crucial role in the revolutionary transition from hunter-gatherer to agriculture-based societies (Wen and Zhao, 2021). As one of the earliest domesticated animals, the goat (*Capra hircus*) made significant contributions to the development of human societies, not only as a stable source of high-quality animal protein and products such as skins and hairs, but also as a contributor to the formation and development of national cultures via its vital role in religious rituals and divinations (Cai et al., 2021). Therefore, it is of great significance to explore the issues related to the origin, diffusion and matrilineal inheritance of goats. Archaeological evidence suggests that domestic goats originated in the Fertile Crescent of the Near East about 10,000 years ago, and then spread around the world along with human migration (Zeder, 2008). As informed by molecular biology studies, the direct ancestor of the domestic goats is the wild goats (i.e., Bezoar, *Capra aegagrus*) (Ajmone-Marsan et al., 2014).

A large number of goat remains and archaeological artefacts associated with goats such as goat figurines, goat pens, and other remains

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have been unearthed from many Neolithic archaeological sites in China. The earliest dated domestic goats identified by morphological analysis have been unearthed from the Shimao site in Northern Shaanxi which dates back to the Late Longshan period (4300-3800 BP) (Hu et al., 2017). During the Shang and Zhou dynasties, goats appeared in abundance in Northern China, and gradually spread throughout the region (Zuo, 2017). It is hypothesized that domestic goats may have spread to Northwestern China during the Late Longshan period around 4000 years ago, and were introduced to the Central Plains in the middle of China during the Erlitou culture around 3700 years before present (Cai et al., 2021). The results of ancient genome studies show that the Chinese goat originated in West Iran, departed from Iran during the Chalcolithic period (about 6000–7000 BP), and spread to Northwest China around 4000 years ago. However, the dispersal of goats into China and the historical dynamics after their entry into China remain largely uncertain. In addition, the number of sites included in previous studies is small, and as for domestic animals, more attention has been paid to the nuclear genome. By comparison, ancient goat mitochondrial matrilineages have received insufficient attention. Furthermore, previous matrilineal studies have focused on mitochondrial DNA (mtDNA) fragments, ignoring the more informative mitochondrial whole genome sequences (Meadows et al., 2011). It is worth noting that mitochondrial DNA has been widely used in molecular identification of animals and has been proven successful in species identification for many animals (Hong et al., 2020).

It is now widely accepted that seven maternal lineages of goats existed: A, B, C, D, F, G, and T, with the F and T lineages being primarily wild goats. Modern wild goats have all of the seven lineages (Colli et al., 2015). An investigation into the geographic distributions of different ancient goat lineages in the Fertile Crescent found that the six lineages of domestic goats (A, B, C, D, F and G) had a more distinct distribution pattern when domestication started in the Neolithic, which disappeared after the Neolithic period (Daly et al., 2018). The mitochondrial lineage structure of modern goats exhibits a less obvious distribution pattern, with lineage A dominant at the highest frequency and declining in frequency throughout Eurasia from west to east. Lineage B is mainly found in Asia, with a small amount in South Africa. Lineages C and D are distributed throughout Europe and Asia. Lineage G is mainly distributed in the Near East and North Africa.

In this study, 77 examples of ancient goat remains excavated from a total of 16 sites in China dating back from the Late Neolithic to the Ming Dynasty were subjected to ancient mitochondrial genome study. In light of published data as well as the findings of this study, it is expected that this research can reveal (1) the origin and diffusion, (2) the historical dynamics, and (3) the genetic continuity of Chinese goat maternal lineages.

2. Materials and methods

2.1. Archaeological sites and sample collection

A total of 77 samples were collected from 16 sites in China, spanning the Late Neolithic to the Ming Dynasty. Of the 77 samples, 55 were morphologically identified as goats, 12 as sheep, and 10 as sheep/goat. A brief introduction to the 16 sites is included in the Supplementary material, the geographic locations of the sites are shown in Fig. 1, and the samples are summarized in Table 1.

To investigate the genetic relationship between Chinese goats and goats from other parts of the world, we also collected a sample of goat femur dating back to 1300 years ago from the North Caucasus region of Russia, which was coded as KA01G (Zheng et al., 2020).

2.2. DNA extraction, library construction and high-throughput

An electric grinding tool and sterilized disposable drills were used to remove 2 mm off the surface of bone samples to prevent external contamination. Then, the cleaned bone samples were immersed in a 10 % chloramine solution for 15 min, followed by decontamination with DEPC water and 5 min immersion in 70 % ethanol. Finally, UV-light irradiated the samples until they were dry. The next day, the bone samples were ground into powder using an electric grinding tool, and 200 mg of each sample was collected. DNA extraction followed the methods of Yang et al. (1998) and Dabney et al., (2013), using the QIAquick® PCR Purification Kit combined with ultrafiltration tubes (Millipore) and the MinElute® PCR Purifcation Kit to obtain the extraction solution. For library construction, we adapted the experimental method developed by the Max Planck Institute in Germany (https://www.protocols.io/view/a-z-of-ancient-dna-protocols-for-shotgun-illumina-36wgq529xgk5/v2/guidelines) and the instruction of NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® reagent kit

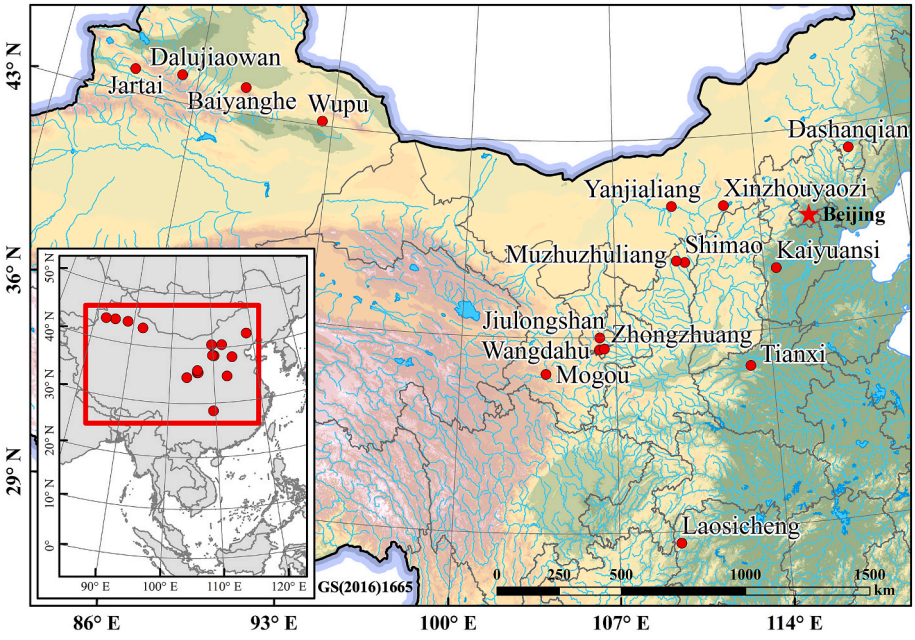

Fig. 1. Location of archaeological sites.

Table 1
Information on the samples.

Site	Lab Code	Archaeological Code	Element	Age (yBP)	Morphology
Baiyanghe	FB03S	2016FBM8: G	Tooth	3300–2800	Sheep/Goat
Baiyanghe	FB06S	2017FBIM2(F1)	Tooth	3300–2800	Sheep/Goat
Dalujiaowan	DL02S	2015XSDL-M1-2	Tooth	2700–2000	Goat
Dashanqian	DSQS7	97KDIH239⑤	Tooth	~3000	Sheep
Dashanqian	DSQS10	97T305③	Tooth	~2300	Sheep
Dashanqian	DSQS14	96KDIT418G2:18	Tooth	~3000	Sheep
Jartai	NJ08S	2016NJT12③:59	Humerus	3600–3000	Goat
Jiulongshan	JLS05S	YJM11:D2-21	Tooth	~2500	Goat
Jiulongshan	JLS06S	YJM2:D2-5	Tooth	~2500	Goat
Jiulongshan	JLS08S	YJM5:D2-5	Tooth	~2500	Goat
Kaiyuansi	KYS09S	TS09E05⑨b:56	Tibia	~1050	Sheep
Kaiyuansi	KYS15S	TG18⑥b:227	Mandible	~850	Sheep/Goat
Kaiyuansi	KYS26S	TS09E06L2:131	Ossametatarsalia	~750	Sheep/Goat
Kaiyuansi	KYS31S	TS09E06L2:257	Ossametatarsalia	~750	Sheep/Goat
Kaiyuansi	KYS36S	TG19④a:102	Ossametatarsalia	~650	Sheep
Kaiyuansi	KYS37S	TS09E05⑨a:28	Ossametatarsalia	~650	Sheep
Kaiyuansi	KYS38S	TS09E05⑨a:38	Ossametatarsalia	~650	Sheep/Goat
Kaiyuansi	KYS43S	TS09E05⑧a:150	Tibia	~400	Sheep/Goat
Kaiyuansi	KYS04G	TG18⑥b:108	Metacarpale	~850	Goat
Kaiyuansi	KYS06G	TS09E06⑦a:250	Scapula	~900	Goat
Kaiyuansi	KYS08G	TG18⑤b:185	Scapula	~750	Goat
Kaiyuansi	KYS10G	TS09E06L2:674	Scapula	~750	Goat
Kaiyuansi	KYS12G	TG19④a:71	Skull	~650	Goat
Laosicheng	LSC02S	13YLG10:13846	Mandible	600–400	Goat
Laosicheng	LSC03S	13YLG10:13927	Mandible	600–400	Goat
Laosicheng	LSC04S	13YLG10:13988	Mandible	600–400	Goat
Laosicheng	LSC05S	13YLG10:14308	Mandible	600–400	Goat
Laosicheng	LSC06S	13YLG10:14311	Mandible	600–400	Goat
Laosicheng	LSC07S	13YLG10:14449	Mandible	600–400	Goat
Laosicheng	LSC08S	13YLG18:2268	Mandible	600–400	Goat
Laosicheng	LSC09S	13YLG18:2268	Mandible	600–400	Goat
Mogou	MG03S	2010LCMH11①	Tooth	3600–3400	Sheep
Muzhuzhuliang	MZG20	H87:D2	Humerus	2740-2680 cal	Goat
Muzhuzhuliang	MZG28	IK13:6	Humerus	~2700	Goat
Muzhuzhuliang	MZG29	IH149:D3	Mandible	~2700	Goat
Muzhuzhuliang	MZG34	IH84③:09	Mandible	~2700	Goat
Muzhuzhuliang	MZG38	IH84③:09	Tibia	~2700	Goat
Shimao	SM24S	G2:D39	Tooth	4300–3800	Sheep/Goat
Shimao	SM28S	F7:D30	Tooth	4300–3800	Sheep/Goat
Shimao	SMG1	G2:D41	Humerus	4300–3800	Goat
Shimao	SMG4	G2:D38	Mandible	4300–3800	Goat
Shimao	SMG5	F7:D29	Mandible	4300–3800	Goat
Shimao	SMG7	F7:D41	Mandible	4300–3800	Goat
Shimao	SMG10	F7:D45	Humerus	4300–3800	Goat
Shimao	SMG11	Y1:D22	Humerus	4300–3800	Goat
Tianxi	GTM01G	2015GTM230:1–19	Metacarpale	438-350 cal	Goat
Tianxi	GTM02G	2015GTM230:5–24	Humerus	524-435 cal	Goat
Tianxi	GTM03G	2015GTM230:7–16	Humerus	473-308 cal	Goat
Tianxi	GTM04G	2015GTM230:9–10	Ilium	525–308	Goat
Tianxi	GTM06G	2015GTM230:2–27	Tibia	525–308	Goat
Tianxi	GTM08G	2015GTM230:6–24	Femur	525–308	Goat
Tianxi	GTM11G	2015GTM230:14–2	Tibia	525–308	Goat
Wangdahu	WDH03S	PWM1:D10	Tooth	~2500	Sheep
Wangdahu	WDH05S	PWM4:D12	Tooth	~2500	Sheep/Goat
Wangdahu	WDH06S	PWM1:D14	Tooth	~2500	Sheep
Wangdahu	WDH07S	PWM1:D23	Tooth	~2500	Sheep
Wangdahu	WDH08S	PWM7:D30	Tooth	~2500	Goat
Wupu	HW01G	78HWANM15:3	Astragalus	3300–3000	Goat
Xinzhouyaozi	BG1	LBM10-1	Tooth	~2500	Goat
Xinzhouyaozi	BG5	LBM30-5	Tooth	~2500	Goat
Xinzhouyaozi	BG6	LBM30-6	Tooth	~2500	Goat
Xinzhouyaozi	BG7	LBM31-3	Tooth	~2500	Goat
Xinzhouyaozi	BG8	LBM38-2	Tooth	~2500	Goat
Xinzhouyaozi	BG9	LBM44-1	Tooth	~2500	Goat
Xinzhouyaozi	BG10	LBM28-6	Tooth	~2500	Goat
Xinzhouyaozi	LBM5	LBM11-9	Tooth	~2500	Sheep
Xinzhouyaozi	LBM6	LBM28-5	Tooth	~2500	Sheep
Yanjialiang	YJL01G	06BJY:24	Humerus	~650	Goat
Yanjialiang	YJL02G	06BJY:25	Humerus	670-625 cal	Goat
Yanjialiang	YJL03G	06BJY:50	Ulna	670–625	Goat
Yanjialiang	YJL04G	06BJY:94	Scapula	670–625	Goat
Yanjialiang	YJL05G	06BJY:232	Tooth	670–625	Goat
Yanjialiang	YJL06G	06BJY:4939	Radius	670–625	Goat
Yanjialiang	YJL08G	06BJY:6786	Humerus	670–625	Goat

(*continued on next page*)

Table 1 (*continued*)

Site	Lab Code	Archaeological Code	Element	Age (yBP)	Morphology
Yanjialiang	YJL09G	06BJY:6703	Scapula	670–625	Goat
Yanjialiang	YJL10G	06BJYH58:381	Humerus	670–625	Goat
Zhongzhuang	ZZ01S	PZM1:D23	Tooth	~2500	Goat

with minor modifications. Mitochondrial capture was performed on iGeneTech Bioscience. Finally, paired-end whole-genome shotgun sequencing was performed using the Illumina Hiseq X Ten platform.

2.3. Data processing

Raw data were processed using the PALEOMIX v1.3.7 pipeline (Schubert et al., 2014). Firstly, adapter sequences were identified and removed using AdapterRemoval v2.2.0 (Schubert et al., 2016). During the processing, reads below 35 base pairs (bp) in length were filtered out, and bases with a quality below 20 were discarded. The paired-end data was then merged. Using BWA v0.717 (Li, 2013) and NC_005044.2 as the reference sequence, the data were aligned with the aln algorithm. Reads with a mapping quality less than 25 were discarded. PCR duplicates were discarded using the MarkDuplicates command in Picard v2.20.0 (Sacco et al., 2017). All local realignments around indels were performed using GATK v3.7.0 for base quality rescaling and end trimming (McKenna et al., 2010). Sequencing quality and mitochondrial coverage were assessed using Qualimap v2.2.1 (Okonechnikov et al., 2016). Mitochondrial DNA consensus sequences were extracted using ANGSD v0.931 (Korneliussen et al., 2014), htsbox-r312 (github.com/lh3/htsbox), Schmutzi v1.5.7 (Renaud et al., 2015), and MIA v1.0 (mapping iterative assembler) (github.com/mpieva/mapping-iterative-assembler), respectively, from which the fasta files were obtained. The fasta files were then integrated and aligned, and corrected manually. All the base sites were compared and contrasted, and "N" was assigned where conflicts occurred, from which the mitochondrial DNA consensus sequences were finalized and obtained.

2.4. Authenticity and reliability of the sequencing results

In accordance with the ancient DNA contamination prevention protocol, all pre-PCR procedures were conducted in a dedicated ancient DNA laboratory at Jilin University, while the post-PCR steps were carried out in a separate and geographically distant laboratory. Prior to each experiment, the workspace was exposed to ultraviolet light for 30 min. Throughout the experiment, pipettes and the super-clean table were regularly wiped with bleach. All personnel in the laboratory wore protective clothing, including sterile disposable caps, masks, and gloves. Additionally, all disposable consumables used during the experiments were DNA-free grade. To detect any potential contamination, blank controls were included at each stage of DNA extraction and amplification, all of which yielded negative results.

Usually, ancient DNA samples show a higher frequency of C > T or G > A mutations at the ends due to damage caused by hydrolysis and oxidation (Briggs et al., 2007). After evaluating the sequencing data using MapDamage v2.2.1 (Jónsson et al., 2013), we found that they corresponded to the damage pattern of ancient DNA (Fig. S1), ensuring the authenticity and reliability of the ancient DNA data. As shown in Fig. S1, for some samples the instruction of NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® reagent kit with minor modifications was applied during library construction, where the reagents excluded the typical 5′ mutations. Therefore, it can be seen that some samples lack 5′ C > T mutations in Fig. S1 (Cai et al., 2022).

2.5. Data analysis

To analyze ancient goat samples in this study in a broader spatial and temporal context, we constructed a database against published ancient and modern goats mtDNA sequence data. We downloaded the mitochondrial genome sequences of goats from NCBI GenBank (https://www.ncbi.nlm.nih.gov/), used the sequence NC_005044.2 as the reference sequence and compared the data with our samples using MUSCLE v3.8.1 (Edgar, 2004).

We divided all sequences into 6 regions (codon region at positions 1, 2, 3, rRNA region, tRNA region, and control region). With the sheep (*Ovis aries*) mitochondrial genome sequence NC_001941.1 as the outgroup, jModelTest 2.1.1 (Darriba et al., 2012) was used to carry out statistical selection of best-fit models of nucleotide substitution in each of the six regions, against the Bayesian Information Criterion (BIC). The Bayesian phylogenetic tree was constructed with BEAST v2.6.7 (Bouckaert et al., 2014) using a strict molecular clock model. Tracer v1.7.2 (Rambaut et al., 2018) was adopted to test all effective sample sizes greater than 300. Information of the Bayesian phylogenetic tree was summarized with TreeAnnotator v2.6.0 (BEASTdoc, 2017), and we set the first 25% of the samples as the burn-in, which would be discarded at the start of the run. The obtained phylogenetic tree was displayed, annotated and managed by the iTOL (Letunic and Bork, 2021).

Bayesian skyline plots were generated by BEAST v2.6.7, using a strict molecular clock model, and Bayesian skyline with a piecewise-linear tree model, running for 80,000,000 iterations of sampling every 8000 iterations. We used Tracer v1.7.2 to test all effective sample sizes greater than 300, indicating good convergence.

Haplotypes were assigned to sequences by DnaSP v6.0 (Rozas et al., 2017), and different haplotype frequencies were calculated using Arlequin v3.5.2.2 (Excoffier and Lischer, 2010). Finally, PopART v1.7 (Leigh and Bryant, 2015) was used to construct the median-joining network.

The temporal network of the haplotypes was created with TempNet script (Prost and Anderson, 2011) on R v4.1.2 (R Core Team, 2022).

3. Results

Mitochondrial genome sequences were extracted from a total of 78 samples, and the mitochondrial genome coverage of all samples ranged from 1.267X-1440.924X, with an average coverage of 199.018X and a median coverage of 46.093X. The mitochondrial genome coverage of all samples was greater than 1X, and the number of sites covered by the mitochondrial genome was greater than 10,000 (Table 2). The Mitotoolpy tool (Peng et al., 2015) in conjunction with phylogenetic tree results was used to detect the haplotype of each sample.

To determine the lineage of ancient goats in this study, we carried out a phylogenetic analysis of mitochondrial whole genome sequences extracted from the 77 ancient Chinese goat samples and one ancient Caucasian goat sample, as well as the previously published 82 mitochondrial genome sequences of modern goats. The sheep mitochondrial genome sequence was used as outgroup, and a Bayesian phylogenetic tree was created (Fig. 2, Supplementary Table S1). The results showed that the 77 ancient Chinese goat samples were assigned to four lineages, A, B, C, and D. Among them, 52 (68%) were from lineage A, 15 (19%) from lineage B, 3 (4%) from lineage C, and 7 (9%) from lineage D. The ancient Caucasian goat was assigned to lineage A.

The ancient goat samples excavated from 16 sites were categorized into four groups in chronological order: Late Neolithic Age (5000-4000 BP), Xia, Shang, and Western Zhou Dynasties (4000-2771 BP), Spring and Autumn and Warring States periods (2771-2221 BP), Tang to Ming Dynasties (1319-336 BP), and the lineage frequencies of samples in each site were counted (Fig. S2).

We counted the lineage frequencies of all ancient and modern

Table 2
Key results.

Lab Code	Raw Reads	Mapped Reads	Average Fragment Length (bp)	Mean Coverage of mtDNA (X)	Number of Sites Aligned to Reference	Duplication Rate	Lineage	DNA Identification
SM24S*	10,504,244	1,759,747	56.23	73.535	16,551	99%	A	Goat
SM28S*	3,821,646	495,724	47.43	342.737	16,540	80%	A	Goat
SMG1*	19,002,090	113,301	61.96	11.676	15,537	97%	B	Goat
SMG4	98,507,514	572	60.74	1.747	12,393		A	Goat
SMG5	106,222,202	445	54.7	1.267	10,989		D	Goat
SMG7	120,637,820	4238	62.56	13.055	16,547		B	Goat
SMG10	151,221,832	7440	47.57	14.234	16,539		A	Goat
SMG11	110,203,942	1961	82.0	8.198	16,458		C	Goat
MZG20	1,806,395,686	78,441	62.25	28.929	16,350		A	Goat
MZG28	88,906,650	1182	62.92	3.588	15,965		A	Goat
MZG29	100,032,564	5454	67.32	19.015	16,527		A	Goat
MZG34	76,878,720	3303	59.76	8.851	16,584		A	Goat
MZG38	91,203,224	8151	51.57	17.503	16,565		A	Goat
MG03S*	2,413,496	1,033,216	69.57	1056.892	16,588	70%	A	Goat
DSQS7	49,936,936	13,401	86.52	42.409	16,571		A	Goat
DSQS10	74,668,730	34,740	97.11	191.446	16,643		A	Goat
DSQS14*	10,744,232	5,383,057	108.11	899.938	16,528	97%	C	Goat
BG1	86,305,729	58,721	76.27	38.515	16,606		B	Goat
BG5*	2,165,988	805,439	67.04	113.391	16,597	97%	A	Goat
BG6*	5,536,232	2,386,709	79.11	1146.995	16,583	90%	A	Goat
BG7*	2,499,082	956,850	75.16	806.334	16,639	81%	B	Goat
BG8	46,979,572	4010	56.66	10.849	16,553		A	Goat
BG9*	1,187,520	331,417	50.39	261.597	16,562	78%	A	Goat
BG10*	3,531,306	1,460,142	64.77	560.142	16,574	90%	A	Goat
LBM5	19,312,892	2108	86.16	9.748	16,517		A	Goat
LBM6*	12,759,244	3,910,318	74.86	1440.924	16,592	91%	A	Goat
YJL01G	1,031,253,952	318,354	67.35	824.955	16,624		A	Goat
YJL02G	2,010,923,364	228,753	66.45	565.991	16,608		A	Goat
YJL03G*	8,038,478	3,470,340	76.69	81.736	16,590	99%	A	Goat
YJL04G	28,635,882	6151	67.58	18.243	16,562		A	Goat
YJL05G*	3,122,952	827,314	68.73	253.001	16,568	93%	D	Goat
YJL06G	35,943,294	1590	57.52	4.298	16,111		A	Goat
YJL08G	17,793,640	4866	61.54	13.052	16,573		A	Goat
YJL09G	42,006,712	7616	60.59	20.675	16,577		A	Goat
YJL10G	21,360,098	4193	56.7	10.129	16,498		A	Goat
HW01G*	1,859,378	544,420	51.1	318.528	16,524	77%	A	Goat
NJ08S*	6,372,736	1,157,204	69.35	630.452	16,623	87%	A	Goat
FB03S	28,659,266	12,790	87.12	45.155	16,615		D	Goat
FB06S	30,905,274	4666	55.82	11.692	16,522		A	Goat
DL02S	19,772,760	13,542	76.48	47.031	16,609		A	Goat
JLS05S	94,588,052	1558	49.59	3.643	14,409		C	Goat
JLS06S	128,814,342	29,708	54.5	78.477	16,630		D	Goat
JLS08S	28,903,804	2678	78.73	10.669	16,509		A	Goat
WDH03S	68,406,218	13,996	77.76	57.572	15,587		A	Goat
WDH05S	102,372,122	20,112	83.92	87.966	16,594		A	Goat
WDH06S	1,062,698,944	328,029	65.99	807.532	16,614		A	Goat
WDH07S	112,520,316	6439	52.4	19.041	16,623		B	Goat
WDH08S	81,671,792	2119	49.55	5.049	15,129		A	Goat
ZZ01S	99,648,942	4061	47.83	12.332	16,243		B	Goat
GTM01G	108,833,906	2888	54.91	6.157	16,153		B	Goat
GTM02G	104,196,044	3994	73.88	14.075	16,639		B	Goat
GTM03G	1,072,735,008	177,271	83.28	198.94	16,573		A	Goat
GTM04G	113,327,202	1519	70.78	3.178	15,955		B	Goat
GTM06G	101,926,690	36,039	102.38	79.135	16,643		B	Goat
GTM08G	103,524,780	23,619	105.34	42.428	16,643		B	Goat
GTM11G	101,920,410	17,044	73.72	57.792	16,641		B	Goat
KYS09S	11,826,068	5653	56.21	14.348	16,607		A	Goat
KYS15S*	7,573,256	326,648	84.83	150.175	16,582	99%	A	Goat
KYS26S*	9,964,000	4,410,551	62.63	319.261	16,561	98%	A	Goat
KYS31S*	1,942,330	428,750	71.22	128.13	16,551	93%	A	Goat
KYS36S	11,819,624	1953	54.3	5.161	16,440		B	Goat
KYS37S*	6,415,924	1,604,800	64.61	84.895	16,565	99%	A	Goat
KYS38S*	8,819,084	3,756,370	68.36	99.03	16,565	99%	A	Goat
KYS43S	17,759,468	17,042	58.81	50.594	16,611		D	Goat
KYS04G*	2,328,920	648,950	63.05	18.714	16,437	99%	A	Goat
KYS06G*	3,995,420	1,545,232	68.29	121.795	16,493	98%	D	Goat
KYS08G*	2,469,196	701,161	79.45	90.641	16,572	98%	D	Goat
KYS10G*	6,212,876	1,202,697	75.92	200.58	16,573	96%	A	Goat
KYS12G	28,460,854	1432	71.73	5.381	16,313		A	Goat
LSC02S*	3,059,582	1,071,888	101.85	40.709	16,635	99%	B	Goat
LSC03S*	8,493,460	3,093,994	123.35	1031.635	16,643	95%	B	Goat
LSC04S*	2,521,072	1,136,121	139.16	312.629	16,643	96%	A	Goat
LSC05S*	6,739,118	1,772,823	74.63	1048.179	16,612	85%	A	Goat

(*continued on next page*)

Table 2 (*continued*)

Lab Code	Raw Reads	Mapped Reads	Average Fragment Length (bp)	Mean Coverage of mtDNA (X)	Number of Sites Aligned to Reference	Duplication Rate	Lineage	DNA Identification
LSC06S*	6,668,346	81,099	59.62	35.024	16,548	87%	A	Goat
LSC07S	198,704,020	12,503	92.81	62.259	16,596		A	Goat
LSC08S*	2,649,184	865,374	69.31	185.441	16,609	95%	A	Goat
LSC09S*	913,670	17,272	92.07	31.957	16,501	64%	A	Goat
KA01G	57,556,792	15,131	69.88	34.468	16,600		A	Goat

Note: * represents samples that have been used with the mitochondrial capture technique.

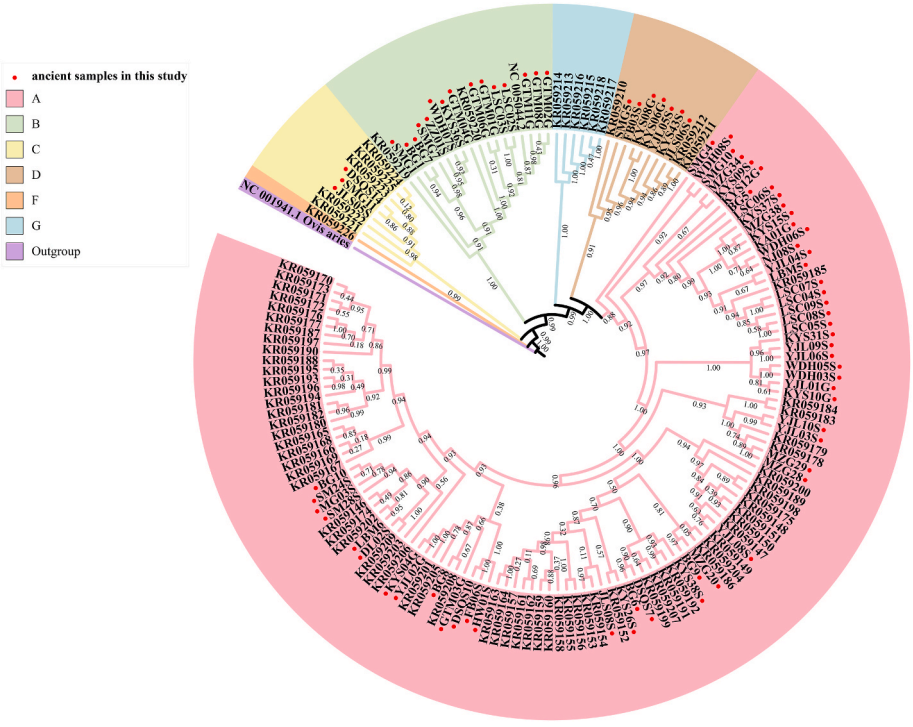

Fig. 2. Bayesian phylogenetic tree created on mitochondrial genome sequences.

domestic goat samples from different regions in the database (Fig. S3). The ancient samples come from West Asia, Central Asia, Europe, and China; and the modern samples from West Asia, Central Asia, Europe, Africa, Northern China, Southern China, South Asia, and Southeast Asia. As shown by the results, there are four divergent lineages, A, B, C, and D for ancient and modern Chinese goats, while the G, F, and T lineages are not observed in either ancient or modern samples; modern Northern Chinese goat samples can be categorized into four lineages, A (68%), B (19%), C (4%), and D (9%), with the frequency of lineage A being higher than that of lineage A in the ancient Chinese samples, and the Southern China samples including only two lineages, A (69%) and B (31%). Although the A lineage ranks first in both modern Northern and modern Southern Chinese goats, the frequency of modern Northern Chinese A lineage is higher than that of A lineage in the modern Southern Chinese goats, while the frequency of modern Southern Chinese B lineage is higher than that of B lineage in the modern Northern Chinese goats. Ancient European samples had only A lineage, and modern European samples included A (87%), B (2%), C (9%), and G (2%) lineages. Relatively few modern African, Southeast Asian, and South Asian samples were analyzed, of which all five African samples are categorized into the A lineage, the three Southeast Asian samples into the B lineage, and the seven South Asian samples into the A (71%) or B (29%) lineages. Given that Chinese goats originated from West Asia, we further divided the West Asian samples chronologically into Neolithic, Chalcolithic, Bronze, Iron, and Modern periods (Fig. S4). It was shown that there are five lineages, A (22.4%), B (16.3%), C (8.2%), D (22.4%), and G (30.7%) in West Asian samples dating back to the Neolithic Age, with lineage G having the highest frequency, followed by A and D. From the Chalcolithic Age onwards, the A (70%) lineage increased in frequency and became dominant, and the B lineage gradually disappeared; the modern West Asian samples include only the A (82%) and G (18%) lineages.

In order to unveil the genetic evolutionary relationships among ancient Chinese goats in different periods, we selected the complete control region sequences (15431–16443, total 1213 bp) from the mitochondrial genome sequences of 77 ancient Chinese goat samples in this study to construct the temporal network (Fig. 3, Supplementary Table S2). The ancient samples were divided into 4 periods in chronology: Late Neolithic Age, Xia, Shang, and Western Zhou Dynasties, Spring and Autumn and Warring States, Tang to Ming Dynasties. The results showed that a total of 65 haplotypes were attributed to lineages A, B, C, and D. Among them, due to the small number of samples from the Late Neolithic period (i.e., only 8 samples from the Shimao site), no genetic continuity of haplotypes was observed. MG03S (haplotype 57) and DSQS7 (haplotype 33) from the Xia, Shang, and Western Zhou Dynasties are connected by vertical solid lines with BG10 and BG6 from the Spring and Autumn and Warring States, respectively, indicating that an obvious genetic continuity existed among the haplotypes. MZG34 and MZG38 from the Spring and Autumn and Warring States share haplotype 47 and are connected by a vertical solid line with KYS37S and YJL04G (sharing haplotype 47) from the Tang to the Ming Dynasties, showing an obvious genetic continuity for haplotype 47.

It is obvious from the above goat matrilineal descent analysis that

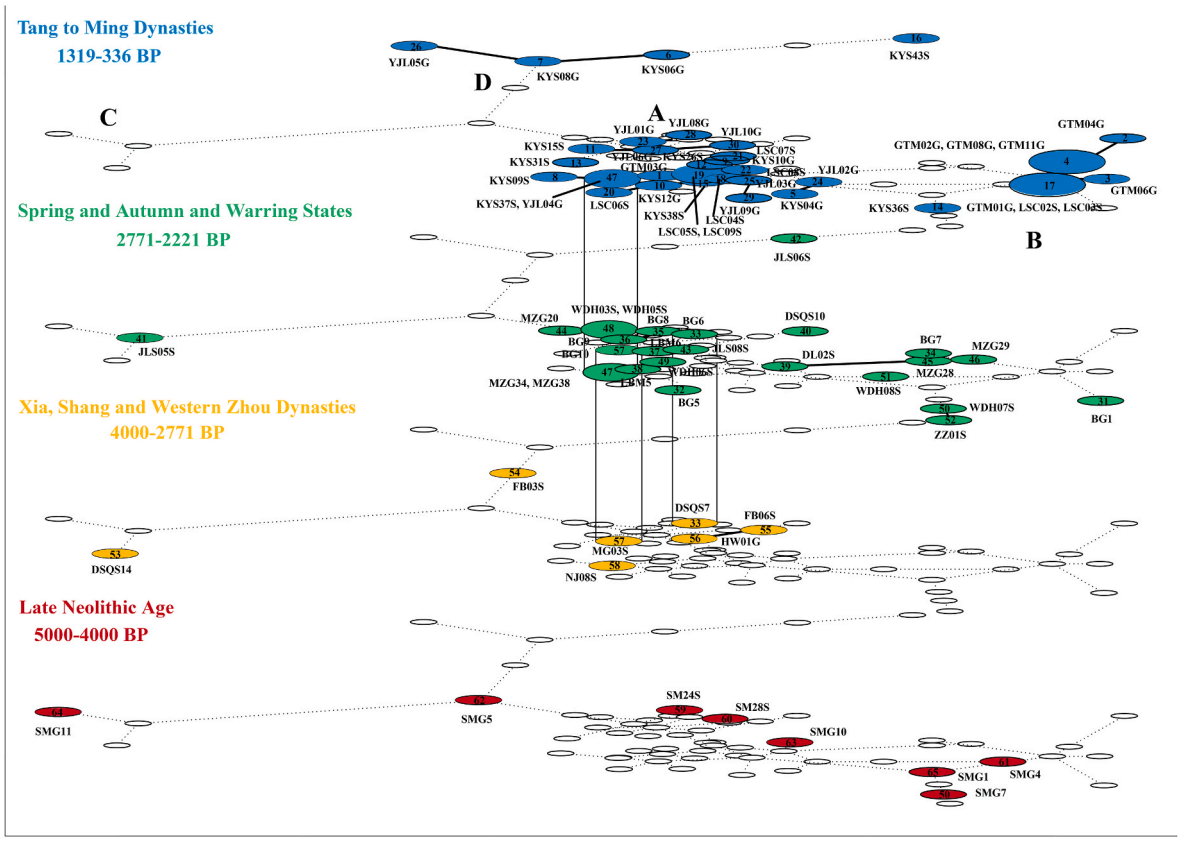

Fig. 3. Temporal network of haplotypes for complete control region of ancient Chinese goats from 4 periods. The size of the colored circles is proportional to the number of samples, and numbers represent different haplotypes. Colorless circles denote haplotypes absent within the time period. Haplotypes in different time periods shared between different times are connected by vertical solid lines.

both ancient and modern Chinese goats include four lineages, A, B, C, and D. In order to reveal the origins and diffusion dynamics of the four lineages, we analyzed lineages A and B with the highest frequency, respectively.

We categorized the A lineage goats geographically into 10 groups: ancient West Asia, ancient Europe, ancient Central Asia, modern Northern China (Northern China), modern Southern China (Southern China), modern Europe (Europe), modern West Asia (West Asia), modern South Asia (South Asia), ancient Northern China, Laosicheng (LSC, the only sampled ancient specimen from southern China), based on which a median-joining network was created (Fig. 4A, Supplementary Table S3). The results showed that lineage A could be further divided into three sub-lineages (A1, A2, A3), and both sub-lineages A1 (66.5%) and A3 (11.7%) share a center-builder haplotype with ancient Northern Chinese samples, suggesting that ancient Northern China goats genetically contributed to the matrilineal inheritance of modern domestic goats in China. Sub-lineage A2 (21.8%) includes Chinese goat samples, with 11 samples from ancient Northern China (KYS12G, KYS09S, KYS37S, KYS15S, YJL04S, BG5, LBM5, MZG34, MZ38S, NJ08S, WDH06S), six samples from the Laosicheng site, and some modern samples from Northern and Southern China. Samples from both Laosicheng and ancient Northern China share a center-builder haplotype with the A2 sub-lineage. We inferred the population historical dynamics of the Chinese A and A2 lineages respectively, according to the Bayesian skyline plot (BSP) where the X-axis denotes time, the Y-axis denotes the effective population size, and the solid line represents the median of the estimation, with the shaded portion representing the range of posterior densities that reached the 95th percentile. According to the Bayesian skyline plot of the Chinese A lineage goat population (Fig. 4B–Supplementary Table S4), it is assumed that the Chinese A lineage matrilineal goat population was in a relatively stable state in 7000 BP, while the effective population experienced two expansions during 7000-6000 BP and 3500-2000 BP and reached a stable state during 6000-3500 BP and from 2000 BP up to the present, respectively. As shown in the Bayesian skyline plot of A2 lineage goats (Fig. 4C–Supplementary Table S5), the A2 lineage maternal population was in a relatively stable state in 4000 BP, whereas the effective population underwent two expansions during 4000-2700 BP and 800-250 BP, respectively, and remained stable during 2700-800 BP and from 250 BP up to now.

Lineage B goats were divided into nine groups geographically: ancient West Asia, ancient Central Asia, modern Southeast Asia (Southeast Asia), modern South Asia (South Asia), modern Europe (Europe), modern Northern China (Northern China), modern Southern China (Southern China), ancient Northern China, and Laosicheng (LSC, the only sampled specimen from ancient southern China), on which median-joining network was developed (Fig. 5A, Supplementary Table S6). Consistent with previous research findings (Han et al., 2010), the B lineage can be divided into two sub-lineages (B1, B2), where the B2 sub-lineage (47.5%) includes all samples from the Tianxi and Laosicheng sites, all samples from South and Southeast Asia, one modern European sample, and some samples from Southern China and Northern China, with samples from the Tianxi site, modern Southern China and Northern China sharing the center-builders haplotype with sub-lineage B2, proving its genetic contribution to modern Chinese, South Asian, and Southeast Asian goats. Samples from ancient Northern China and those from ancient West Asia, ancient Central Asia, modern Northern China, and modern Southern China clustered together under sub-lineage B1 (52.5%), showcasing that its haplotype contributes genetically to the gene pool of modern Chinese goats. We inferred the population historical dynamics of the B lineage population and the Chinese B lineage population according to the Bayesian skyline plot (BSP). As can be

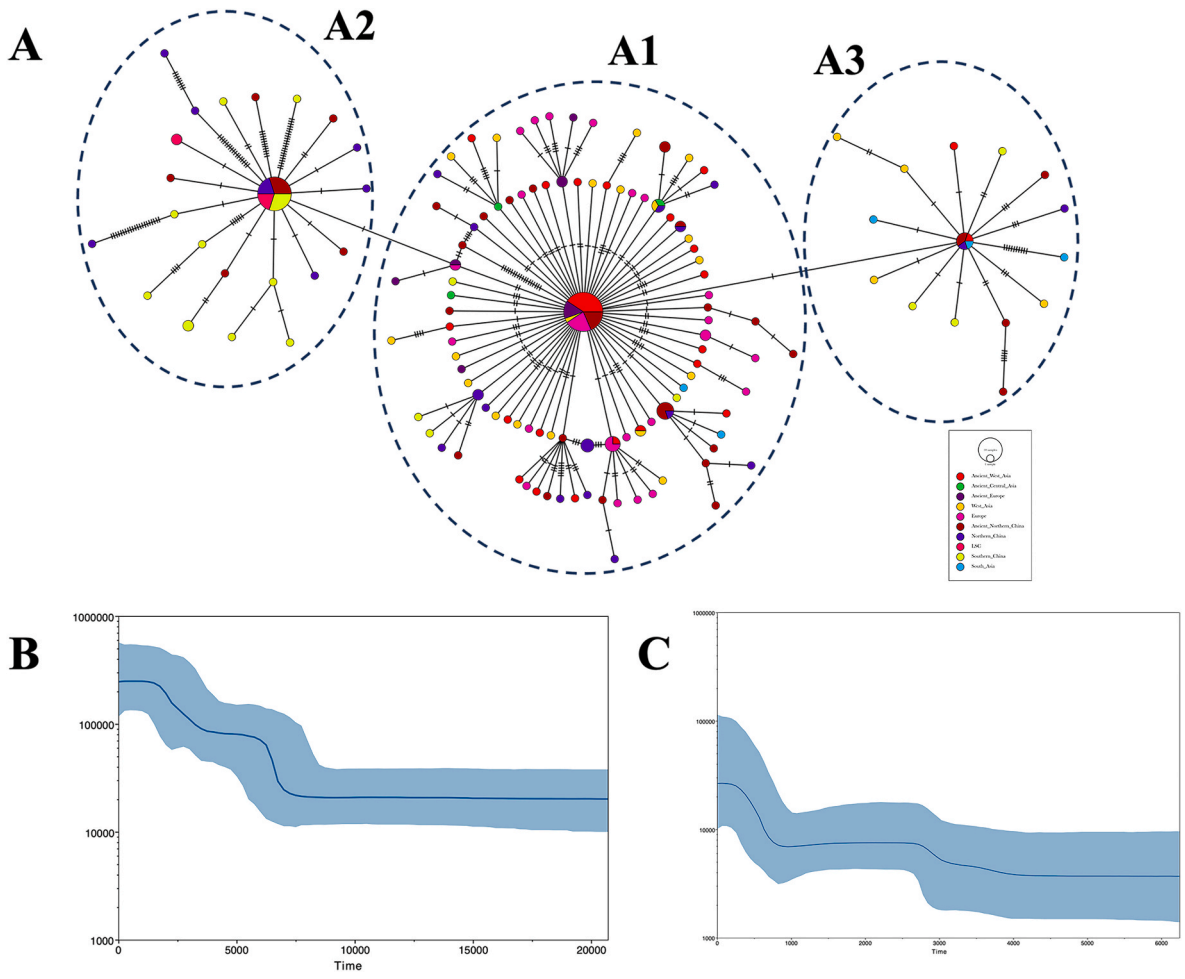

Fig. 4. (A) Median-joining network of mitochondrial genome for lineage A goats; (B) Bayesian skyline plot of mitochondrial genome for Chinese lineage A goats; (C) Bayesian skyline plot of mitochondrial genome for lineage A2 goats.

observed from the Bayesian skyline plot of the B lineage (Fig. 5B–Supplementary Table S7), the B lineage matrilineal population was in a relatively stable state around 10,200 BP, and the effective population underwent an expansion during 10,200–9500 BP, coinciding chronologically with goat domestication (Zeder, 2008). After that, the B lineage matrilineal population remained stable during 9500-2500 BP, and its effective population kept declining from 2500 BP. The Bayesian skyline plot of the Chinese B lineage population (Fig. 5C–Supplementary Table S8) shows that the B lineage matrilineal population was in a relatively stable state about 4200 BP, then its effective population experienced an expansion of relatively small magnitude 4200-3200 BP, it remained stable during 3200-800 BP, its effective population experienced a relatively slight decline in 800-500 BP, and reached a stable state 500 years ago.

4. Discussion

Most of the samples in this study were morphologically identified as goats, with some of them determined as sheep or sheep/goat due to poor preservation and incomplete physical characters. However, this study successfully identified all the samples as goats by performing ancient DNA analysis. It was found that among the 78 samples in this study, lineage A had the highest frequency, consistent with the dominance of lineage A in modern goats. Further, the discovery of the four lineages, A, B, C, and D in the earliest samples excavated from Shimao site indicates that all the four lineages had been introduced into China 4000 years ago.

4.1. Matrilineal origin and diffusion of Chinese goats

Previous research has pointed out that Chinese goats were introduced from abroad and domesticated in the Fertile Crescent of West Asia. In another paper we published, based on the results of whole genome analysis, we inferred that Chinese goats derived mainly from an ancestral population in the eastern part of the Fertile Crescent, and departing from Iran around 7000-6000 BP (Chalcolithic Age) arrived in Northern China about 4000 years ago (Cai et al., 2020).

Shown by Fig. 4B, the population underwent expansion 7000-6000 years ago, coinciding chronologically with the departure of goats from Iran. In addition, Zheng et al., based on nuclear genome analysis, inferred that the expansion of the East Asian goat population occurred about at 6000 BP (Zheng et al., 2020). This indicates that goats expanded and migrated to China 7000-6000 years ago. Apart from that, Fig. 4A shows that among the three sub-lineages of lineage A, ancient West Asian samples of sub-lineages A1 and A3 share a center-builder haplotype, further verifying the West Asian origins of lineage A. Sub-lineage A2 only comprises Chinese goats, and Fig. 4C shows that the sub-lineage A2 population began to expand 4000 years ago, close to the time when goats entered China. Therefore, it can be hypothesized that the A2 sub-lineage may have formed after its entry into China or formed on the way to China and developed later in China, which is similar to the situation for cattle (Cai et al., 2017).

As the lineage B goats are distributed mainly in Asia, some researchers have speculated that the lineage B may have originated in China and then identified two sub-lineages (B1 and B2) within the B

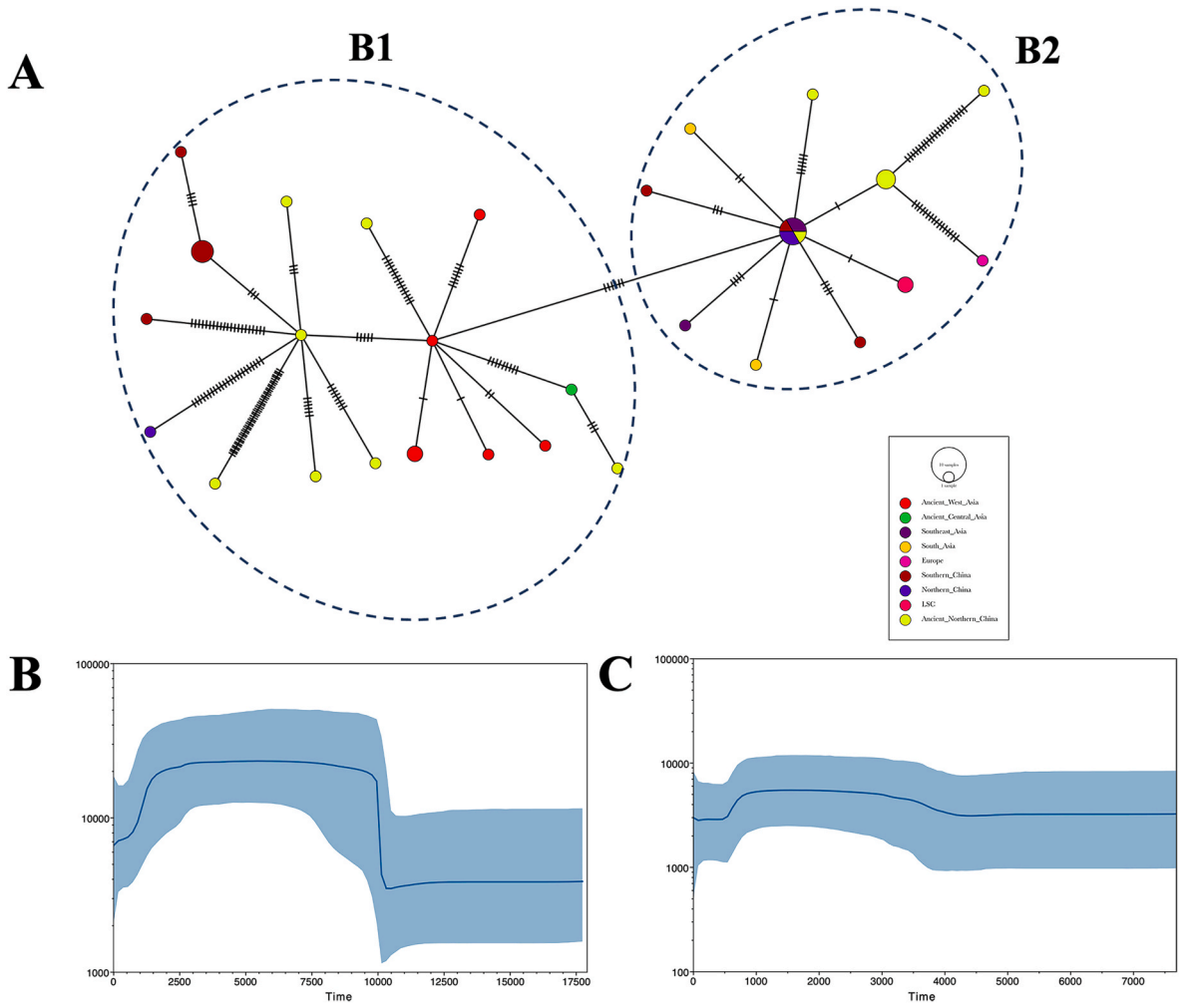

Fig. 5. (A) Median-joining network of mitochondrial genome for lineage B goats; (B) Bayesian skyline plot of mitochondrial genome for lineage B goats; (C) Bayesian skyline plot of mitochondrial genome for Chinese lineage B goats.

lineage (Chen et al., 2005; Han et al., 2010). As shown in Fig. 5A, among the sub-lineages (B1 and B2), sub-lineage B1 includes Neolithic West Asian samples, indicating that the lineage B of goats already existed in the Eastern Fertile Crescent, West Asia in the Neolithic period, much earlier than the appearance of goats in China. Moreover, as suggested by Fig. 5B, the lineage B expanded about 10,000 years ago. Therefore, it can be inferred that the sub-lineage B1 originated in West Asia. After the Neolithic, lineage B appeared in West and Central Asia at a very low frequency, while it appeared frequently among the ancient Chinese population. In the small number of the sub-lineage B2 samples, no other ancient samples were found except for all the samples from the Tianxi and Laosicheng sites, while except for the one modern European sample, the rest of the sub-lineage B2 samples were distributed in China, South Asia, and Southeast Asia. We inferred that (1) the sub-lineage B2 may have formed and spread to Southeast and South Asia after the spread of lineage B goats to China; (2) Since the Laosicheng site is located in southern China and Tianxi in middle China, and no sub-lineage B2 samples were found among ancient Northern Chinese samples, it is possible that the lineage B goats may have formed the sub-lineage B2 after arriving in the South Asian subcontinent and Southeast Asia, and then spread to the southern part of China.

4.2. Matrilineal historical dynamics of Chinese goats

In the Eurasian continent, as an important intersection for human activities and civilization exchanges, human migrations have affected the frequency of Chinese goat lineages and population structure. From Fig. 4B, we can see that the Chinese lineage A goats underwent expansion around 3500-2000 BP and existed in the western part of the Eurasian continent at the highest frequency. With the large-scale eastward human expansion 4000-3000 years ago (Shao, 2012), a large number of lineage A goats may have been brought to China. Due to the west-to-east expansion of the lineage A, we can see from Fig. 5B that the population size of the B lineage declined around 2500 BP. As discussed above, the lineage B developed in East Asia and was mainly distributed in East Asia, Southeast Asia, and South Asia. Therefore, it is inferred that the eastward expansion of lineage A gradually replaced the lineage B in the eastern Eurasian continent, resulting in the decline of lineage B. As a result, the population size of the lineage B Chinese goats decreased around 800 BP as well (Fig. 5C). It is worth noting that the frequency of lineage B in Southern China is higher than that in Northern China (Fig. S3B). According to previous findings, the goat populations diverged in Southern and Northern China after their entry into China. The above results suggest that the substitution of A lineage by B lineage occurred more in Northern China while Southern China was less affected.

Sub-lineage A2 underwent an expansion 800 years ago (Fig. 4C), which is supposed to be associated with increasing human migrations and the development of agriculture and animal husbandry at that time. While the Chinese lineage A population was in a stable state 2000 years ago, it is presumed that the expansion of sub-lineage A2 in China replaced part of the sub-lineage A1 and A3 goats, accounting for the

relatively stable total sample size of lineage A 800 years ago.

4.3. Matrilineal genetic continuity of Chinese goats

After entering into China, thanks to their great adaptability to the ever-changing environment which enabled their survival in harsh conditions, goats have been widely distributed since ancient times with human migrations (China National Commission of Animal Genetic Resources, 2011; Zuo, 2018). The matrilineal inheritance between modern goats and ancient goats remains an important issue to be explored in this study. Based on the results displayed in the temporal network, we found that genetic continuity already existed among ancient Chinese goat populations in different periods. As shown in Fig. 3, three haplotypes had genetic continuity in lineage A samples dating back to different periods. Fig. 4A shows that both sub-lineages A1 and A3 share center-builder haplotypes with ancient Northern Chinese samples, implying that ancient Northern Chinese goats contributed to the matrilineal inheritance of modern domestic goats in China. Samples unearthed from the Laosicheng site and ancient Northern Chinese samples in the sub-lineage A2 share center-builder haplotypes with the modern Southern and Northern Chinese samples, evidencing the genetic contribution of ancient goats to the matrilineal inheritance of modern goats. In addition, the samples from the Laosicheng site include only sub-lineage A2 samples, and about two-thirds of the modern Southern Chinese A lineage samples fall into sub-lineage A2 while one-third of the modern Northern Chinese A lineage samples belong to sub-lineage A2. Considering the expansion history of sub-lineage A2, it can be inferred that the sub-lineage A2 diffused into Southern China after their formation, and that after the divergent development of the goat populations in Southern and Northern China, the Southern populations were less affected by the Western Eurasian population, with sub-lineage A2 existing at a high frequency until now.

Among the lineage B samples, the ancient sub-lineage B2 samples, those excavated from Laosicheng (representing the situation in Southern China) and Tianxi sites (showcasing the situation in Northern China) are of a short duration. There are samples from the Tianxi site that share the center-builder haplotype of sub-lineage B2, suggesting that it had a matrilineal genetic contribution to the modern goats from Northern and Southern China. In sub-lineage B1, ancient Chinese samples and the modern samples from Southern and Northern China clustered together, indicating the genetic contribution of its ancient samples to modern goats (Fig. 5A).

5. Conclusion

In this study, we performed ancient DNA analysis on goat remains excavated from a total of 16 sites dating back from the Late Neolithic to the Iron Age about 3900-450 BP. Phylogenetic analyses, principal component analyses, median-joining network, Bayesian skyline plotting, and lineage frequency calculation were carried out, and it was revealed that the Chinese goat matrilineages began to expand 7000-6000 BP. The discovery of sub-lineages A2 and B2 indicated that the two sub-lineages may have been formed or developed in China. The expansion of lineage A and the decline in the number of lineage B provided evidence for the eastward human migration from western Eurasia. At the same time, this study confirmed the important genetic contributions made by ancient Chinese goats to modern Chinese goats, as well as the genetic relatedness between Chinese goats and goats in South Asia and Southeast Asia. Whole mitochondrial genome sequencing analysis on ancient Chinese goats in this study not only offers an important resource for future analyses and research, but also provides new insights into the origin and diffusion of domestic goats.

Data availability statement

All novel sequences have been deposited in GenBank of NCBI (https://www.ncbi.nlm.nih.gov/)under the accession numbers PP554741-PP554818. All aligned mitochondrial bam files reported in this paper have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA017145) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

CRediT authorship contribution statement

Guangjie Song: Writing – original draft, Formal analysis, Data curation. **Xinyan Zhang:** Formal analysis, Data curation. **Jianen Cao:** Writing – review & editing, Supervision, Resources. **Songmei Hu:** Writing – review & editing, Supervision, Resources. **Quanjia Chen:** Writing – review & editing, Supervision, Resources. **Wenyan Li:** Writing – review & editing, Supervision, Resources. **Linheng Mo:** Writing – review & editing, Supervision, Resources. **Yongqiang Wang:** Writing – review & editing, Supervision, Resources. **Jie Zhang:** Writing – review & editing, Supervision, Resources. **Xuemei Yan:** Writing – review & editing, Supervision, Resources. **Cunshi Zhu:** Writing – review & editing, Supervision, Resources. **Juan Wang:** Writing – review & editing, Supervision, Resources. **Ruilin Mao:** Writing – review & editing, Supervision, Resources. **Yu Jiang:** Writing – review & editing, Supervision, Resources. **Dawei Cai:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jas.2024.106026.

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