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Strontium Isotope Analysis of 4th Century Horse-tooth Fragments Recovered from a Daeseong-dong Royal Tomb of the Gaya Confederation

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Abstract: *During the late 4th century CE, the situation on the Korean Peninsula was in a state of flux. Cavalry tactics in warfare, for example, were being actively adopted, and the horse-riding tradition was transmitted to Japan, along with other aspects of culture and practice. Two horse-tooth fragments found in a Daeseong-dong tomb dating to the 4th century Gaya Confederacy, therefore, can be of great significance to animal archaeology. In gross-morphological and ancient DNA (aDNA) analyses, we confirmed that the fragments from Tomb #1 were from horse teeth. To determine the breeding pattern for horses in the 4th century Gaya Confederacy, we subjected the fragments to strontium isotope ($^{87}Sr/^{86}Sr$) analysis. The $^{87}Sr/^{86}Sr$ values were 0.710083±0.000018 (KH-30) and 0.710263±0.000018 (KH-34), respectively, which were found to be mostly in close agreement with the herbaceous plant $^{87}Sr/^{86}Sr$ values representative of the southeastern part of the Korean Peninsula where the Gaya Confederacy and, later, the Silla Kingdom gained the ascendancy. We speculate that it was probably only after Korea was unified under the Silla Kingdom that horse breeding was established throughout the Peninsula by the central government.*

Keywords: Daeseong-dong; Gaya; Horse; Isotope; Korea; Strontium

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Introduction

Recently, the strontium isotope ratio ($^{87}Sr/^{86}Sr$) has been used as an efficient tracer of secular animal mobility and migration patterns. In brief, $^{87}Sr/^{86}Sr$'s spatial distribution varies by geological material (e.g., rocks, soil, groundwater, vegetation), and furthermore, its local compositions are recorded in animal skeletons where ingested strontium substitutes for calcium during bone and tooth mineralization. Strontium isotope data obtained from animal dentine or bone samples, therefore, can be matched to that of vegetation or groundwater in a given area. In this way, it is possible to trace where individual animals were born and raised and to determine, therefrom, if their breeding grounds differed in location (Graustein 1989; Likins *et al*. 1960; Shin *et al*. 2021; Thornton 2011).

In archaeological science these days, $^{87}Sr/^{86}Sr$ data are used to track intra-regional or long-distance migration of animals (Evans *et al*. 2019; Shin *et al*. 2021). Since horses were often bred on islands or mountains but were moved to other places after being raised, $^{87}Sr/^{86}Sr$ analysis data can help researchers to better understand the historical particulars of horse breeding, transport, and movement. In fact, an $^{87}Sr/^{86}Sr$ analysis of horses from 8th century Japan revealed that they had been raised in the East and were then moved into the region around Fujiwara-kyo, the Japanese capital at the time (Gakuhari 2018). However, there have been very few reports on $^{87}Sr/^{86}Sr$ analyses pertaining to the Korean Peninsula, even though Korean horses were very closely related to Japanese ones.

In this paper, we report the outcomes of an $^{87}Sr/^{86}Sr$ analysis on horse-tooth fragments from Daeseong-dong Tomb #1 in Gimhae, South Korea. As is well known among Korean archaeologists, Daeseong-dong was the capital of the Gaya Confederacy (especially Geumgwan Gaya). Kyungsung University Museum's long-term survey, ongoing since the 1990s, has confirmed that Gaya people of the highest ruling class were buried in tombs there; and archaeologically, the Daeseong-dong culture and heritage are regarded as prototypical in terms of ancient horsemanship, iron metallurgy and hard earthenware, all of which were later transmitted to the Japanese archipelago. Horse remains from Daeseong-dong tombs are thus of great significance not only to Korean bioarchaeologists but to Japanese scientists as well.

Materials and Methods

Archaeological Considerations

The ancient tomb ruins of Daeseong-dong on the hill of Gimhae, Gyeongsangnam-do, are a legacy of the legendary King Suro, the founder of the Gaya Confederacy (Figure 1). The tombs have long drawn archaeologists' attention owing to their suspected provenance as royal tombs of Gaya overlords, not to mention their many artifacts, some of which are relevant to the ancient Korean equine heritage that would later be introduced to Japan. Especially, in Tomb #1 (Figure 2A), many specimens of Gaya hard earthenware, which profoundly influenced the establishment of Japan's Sueki ceramics, were identified. Iron relics such as arrowheads, spears, swords, and ingots, along with iron armor, stirrups and ornaments employed in horseback riding, also were discovered in Tomb #1, which re-confirmed that the man had been a warrior-king well experienced in horsemanship and battle.

Inside Tomb #1, animal bones, possibly remnants of a sacrificial burial, also were found. And among them, tooth fragments, recorded as KH-30 and KH-34, were collected and subjected to experimentation (Figures 2B, 2C). In the case of these specimens, there was a significant difference in their respective locations inside the tomb, and so we estimated that the fragments were unlikely to have come from the same horse. A gross-zooarchaeological analysis following an initial morphological analysis, in accordance with the methods of Hilson (1992), Matsui (2007), and Schmid (1972), was performed on them. KH-34 (Figure 2C) was identified as a fragment of an equine mandibular tooth; as for the case of KH-30 (Figure 2B), it also was from an equine tooth, but could not be conclusively identified as either maxillary or mandibular. The horse's estimated age at death (or slaughter), as based on the crown height, was 4 to 5 years.

Ancient DNA analysis

To confirm the animal species for KH-30 and KH-34, we performed an ancient DNA (aDNA) analysis. To guarantee the authenticity of the analysis, we followed the recommendations of Hofreiter *et al*. (2001) and Ho and Gilbert (2010). Specifically, DNA extraction was performed with the SPEX 6750 Freezer/Mill (SPEX SamplePrep, Metuchen, NJ, USA), phenol-chloroform-isoamyl alcohol (25:24:1), and the QIAmp PCR purification kit (QIAGEN, Hilden, Germany), following the method of Hong *et*

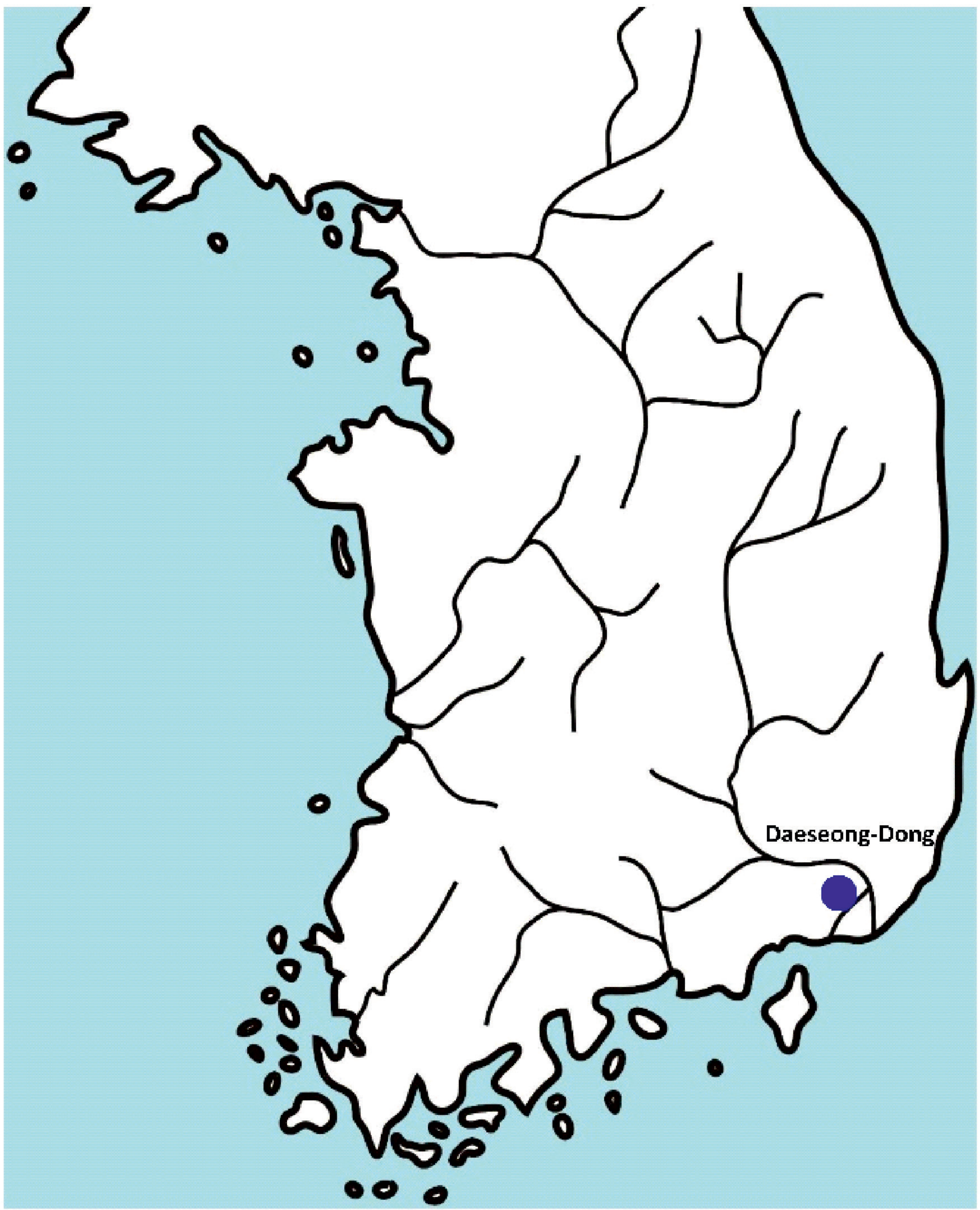

Figure 1: Geographical location of Daeseong-dong archaeological site in Gimhae-si, South Korea.

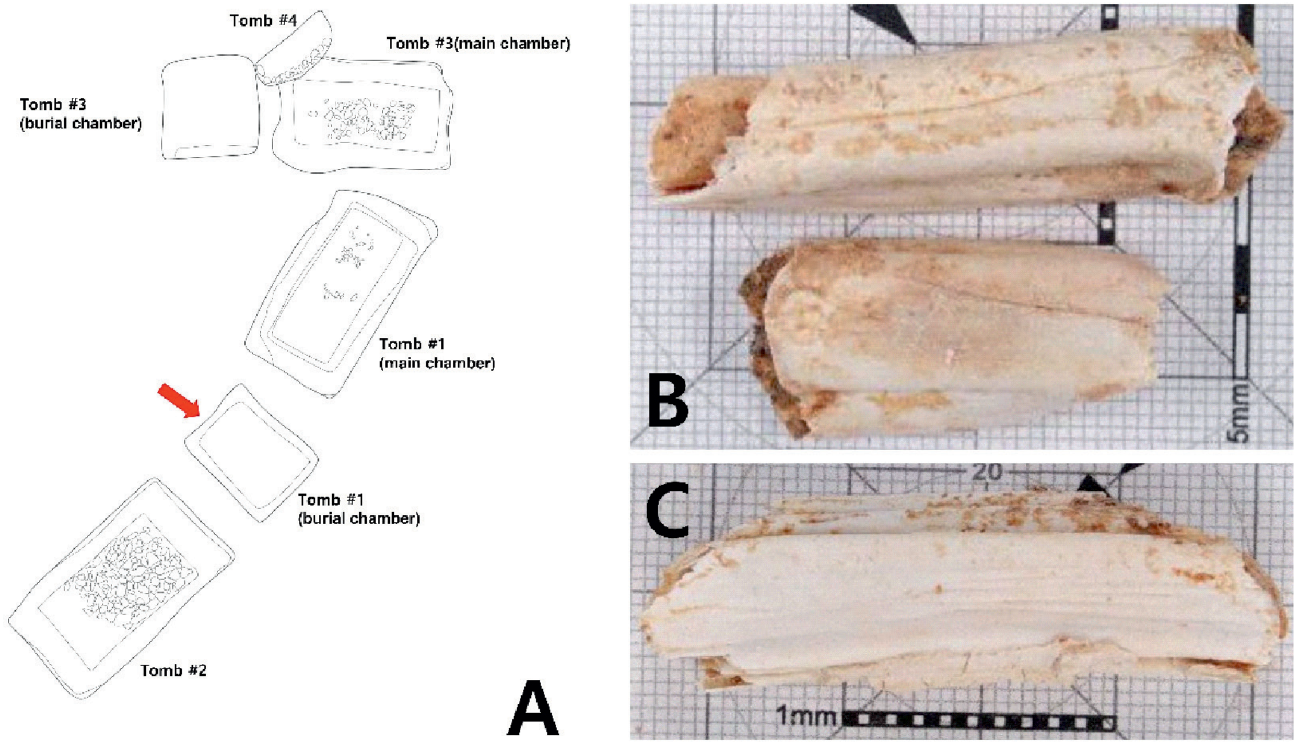

Figure 2: Archaeological context where two different horse teeth used in this study were found. (A) Archaeological context of Daeseong-dong Tomb #1 (Red arrow); tooth fragments KH-30 (B) and KH-34 (C) were used in this study

al. (2018). The extracted DNA (40ng) was treated with uracil-DNA-glycosylase and subsequently mixed with AmpliTaq Gold™ 360 Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) and primer sets (Integrated DNA Technology, Iowa City, IA, USA). PCR amplification was conducted using the SimpliAmp Thermal Cycler (Applied Biosystems, Waltham, MA, USA), applying the conditions summarized in (Table 1). The experiments were performed twice.

The amplified DNA products were separated by gel electrophoresis, revealing specific bands for KH-30 and KH-34. No amplified bands were evident for the negative controls (extraction controls). The extracted DNA was sequenced by the 3730xl DNA Analyzer and the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) (Hong *et al*. 2018). Following the sequencing, the obtained data (Figure 3) were compared with the NCBI/GenBank taxa using NCBI/BLAST tools (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul *et al*. 1997). The NCBI/BLAST results are summarized in Table 2. Since the NCBI/GenBank taxa most closely corresponding to the KH-30 and KH-34 sequences belonged to *Equus caballus*, we could reconfirm our Tomb #1 samples to be equine.

Strontium isotope Analysis

Next, the surfaces of the KH-30 and KH-34 samples were cleaned with a grinding burr preparatory to a high-precision strontium isotope analysis. Small pieces (~10mg) were sonicated, rinsed, and dried at 40°C. Homogenized samples were dissolved in 0.25M HNO3 to minimize the leaching of strontium from detrital materials. Low-blank separation of strontium was achieved by extraction chromatography using Eichrom Sr resin following the methods of Pourmand *et al*. (2014) and Pourmand and Dauphas (2010). The strontium isotope analysis was performed on a Thermo Fisher Neptune Plus™ MC-ICP-MS with an Apex-Q desolvation nebulizer. The determined $^{87}Sr/^{86}Sr$ ratios, as corrected for mass

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                       15302                                                          15364
ERS_NC_001640          TCGAAAACAATCTTCTAAAATGAAGAGTCCCTGTAGTATATCGCACATTACCCTGGTCTTGTA
Daeseong-dong_KH30     .........................................A.....................
Daeseong-dong_KH34     .........................................A.....................

                       15365                                                         15426
ERS_NC_001640          AACCAGAAAAGGGGGAAAACGTTTCCTCCCAAGGACTATCAAGGAAGAAGCTCTAGCTCCAC
Daeseong-dong_KH30     ....................-.........................................
Daeseong-dong_KH34     ....................-.........................................
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Figure 3: The obtained mtDNA sequences from KH-30 and KH34.

bias and isobaric interferences, were 0.710083±0.000018 (KH-30) and 0.710263±0.000018 (KH-34), respectively (Table 3). The final ratios were further adjusted relative to the accepted value of 0.710248±0.000003 (McArthur *et al*. 2001) for Standard Reference Material (SRM) 987 in order to allow for comparison with radiogenic strontium isotope ratios in the literature.

The results of the comparison between the current data from Daeseong-dong Tomb #1 and previously reported $^{87}Sr/^{86}Sr$ data for local herbaceous plant samples in South Korea (Choi *et al* 2023) are summarized in Supplementary Data 1. In brief, the 87Sr/86Sr values of KH-30 and KH-34 are in closest agreement with those recorded from Dalsung, Donggu (Daegu), Ulsan, Haenam, and Haman. The next 10 regions showing high similarity of $^{87}Sr/86Sr$ values are as follows: Gyeongju, Yeongcheon, Goseong, Yeongcheon, Goheung, Namhae, Jeongseon, Cheongsong, Naju, and Buan. The next 15 regions are Cheongdo, Jindo, Dalseong, Happcheon, Yangsan, Hadong, Sangju, Gimcheon, Ulju, Yeosu, Sinan, Geochang, Gangneung, Changwon, and Yeongam. Taken together, it can be said that the above-listed top 30 regions with results similar to those for the horses from the Daeseong-dong tombs fall mostly within the southeastern part of the Korean Peninsula, only a few of them being in eastern or southwestern coastal areas (Figure 4, Supplementary Data 1).

Discussion

The political situation of the Korean Peninsula underwent rapid changes in the late 4th to early 5th century CE, as marked by numerous battles. This period witnessed the active adoption of cavalry tactics in battle, which led to the further transmission of horse-riding customs to Japan, along with other ancient cultures and techniques. The site of the Daeseong-dong tombs, located at the southeastern tip of the Korean Peninsula, is evidence of a more or less fully developed 4th century Gaya Confederacy horse-riding culture. Since the horse remains from Tomb #1 are believed to date to just before the spread of that culture to Japan in the 5th century, the study of horse bones from the Daeseong-dong site holds great significance for zooarchaeology in East Asia broadly.

Table 1: The primers and PCR conditions in this study

	set	*Primer*	*5' to 3'*	*Annealing Temp. (◦C)*	*Length (bp)*
	E.c	E.c-F	TAT ACC ACT CGC AAG CAC CA	57	164
		E.c-R	TTC AGC TTT GGG TGT TGA TG		
PCR Conditions	Pre-denaturation		95°C; 10 min		
	Denaturation		95°C; 30 secs	42 Cycles	
	Annealing		57°C; 30 secs		
	Extension		72°C; 30 secs		
	Final Extension		72°C; 10 min		

Table 2: BLAST searching results indicate coverage and percent identity of each taxon comparing to consensus sequence of Daeseong-dong speciemens mtDNA D-loop (HRS 15302-15426). GenBank accession numbers are also marked

Species	*Total Score (BLAST)*	*Coverage*	*Percent Identity*	*Accession Number*
E. caballus	224	100%	99.19%	MG001448.1
	224	100%	99.19%	KT221838.1
	224	100%	99.19%	KT211218.1
	224	100%	99.19%	JQ340155.1
	224	100%	99.19%	OM222617.1
	224	100%	99.19%	KF038160.1
	224	100%	99.19%	AP013098.1
	224	100%	99.19%	MW534080.1
Equus przewalskii	219	100%	98.39%	NC_024030.1
Equus asinus	195	100%	95.16%	NC_001788.1
Equus grevyi	186	98%	94.26%	NC_020432.2
Canis lupus familiaris	112	100%	80.65%	NC_002008.4
Sus scrofa	88.7	98%	77.87%	NC_000845.1
Bos taurus	75.2	91%	75.21%	NC_006853.1

Table 3: The results of strontium isotope analysis

Sample	*87Sr/86Sr Raw*	*± 95% CI*	*87Sr/86Sr Adjusted*	*± 95% CI*
KH-30	0.710117	0.000018	0.710083	0.000018
KH-34	0.710294	0.000018	0.710263	0.000018

Recently, $^{87}Sr/^{86}Sr$ analysis has yielded important information on horse breeding and movement patterns in East Asia. For instance, in the case of ancient Japan, $^{87}Sr/^{86}Sr$ analysis of animal bones recovered from the site of the 8th century capital, specifically the Fujiwara-kyo ruins, revealed that the radius of activity differed by animal species. For example, dogs were raised near the ruins, whereas pigs or deer seem to have moved about as wild animals, and horses were raised in the far eastern part of the Japanese archipelago, which means that they would have been moved from that area to the Fujiwara-kyo region at some time in their lives, which was a long distance to cover (Gakuhari 2018).

The current $^{87}Sr/^{86}Sr$ analysis of horse-tooth fragments KH-30 and KH-34 from Daeseong-dong Tomb #1 is perhaps especially meaningful in that the breeding pattern employed for horses in Korea has yet to be completely revealed. In our reference to the previous report on samples (n=175) representative of a country-wide distribution of herbaceous plant $^{87}Sr/^{86}Sr$ ratios for South Korea (Choi *et al*. 2023), we could determine whether Daeseong-dong horses had been raised locally or transferred from elsewhere, and if the latter, which outer region specifically had been their point of origin.

Concerning the results of this study (see Figure 4), many breeding pattern possibilities can be considered. The current $^{87}Sr/^{86}Sr$ data obtained for the Daeseong-dong horses were found to be generally similar to the above-noted results representative of herbaceous plants in the southeastern part of the Korean Peninsula (Choi *et al*. 2023), which, in the 4th century was the territory of the Gaya and Silla kingdoms. This means that the Daeseong-dong horses might have been bred somewhere in Gaya or Silla and then moved to a location close to the Daeseong-dong tombs. The individuals buried at sites such as Daeseong-dong Tomb #1 in the 4th century were, as already mentioned, Gaya overlords who had established a powerful state and exerted a controlling influence over of their entire territory. Indeed, the Confederacy showed considerable uniformity in cultural aspects such as the style of tombs constructed and the earthenware made.

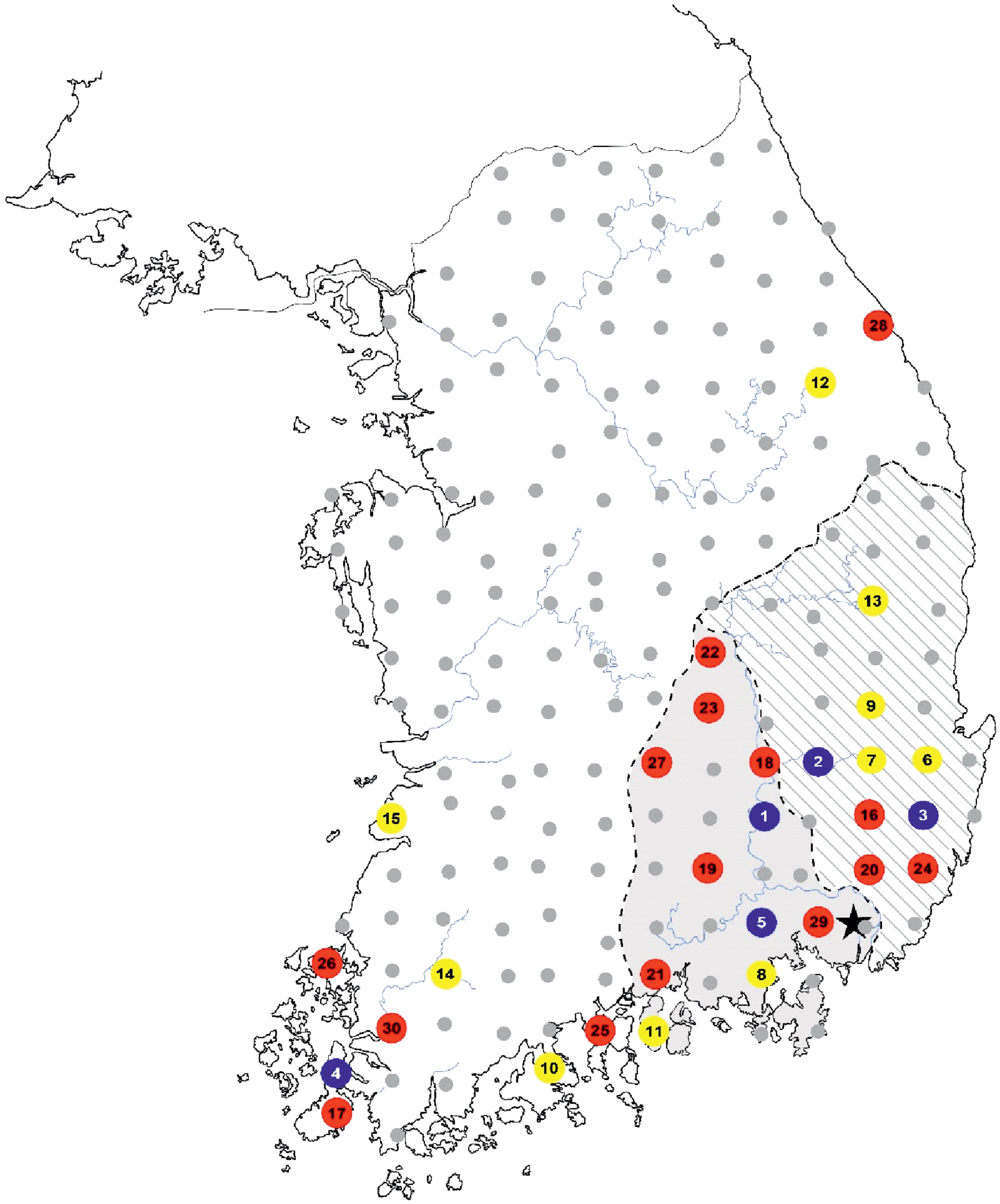

Figure 4: The comparison of strontium isotope ratios between the current data from Daeseong-dong tomb #1 and the local herbaceous plant samples in South Korea [17]. The map is drawn based on the local Herbaceous plant $^{87}Sr/^{86}Sr$ results reported by Choi *et al*. [17]. Gray-shadowed area indicates the possible territory of Gaya Confederacy at the time when the tomb #1 was built. The filled area of oblique line indicates the possible territory of Silla Kingdom at the time when the tomb #1 was constructed. Black star indicates the geographical location of Daeseong-dong archaeological site. The blue dots indicate the locations where the results showing the closest values to our Daeseong-dong sample data. The yellow and red dots then refer to the next ten and fifteen locations with results in the order of the 6th to the 15th and the 16th to 30th, respectively. Each dot in the map is: 1. Dalseong (Daegu); 2. Donggu (Daegu); 3. Ulju (Ulsan); 4. Haenam (Jeonnam); 5. Haman (Gyeongnam); 6. Gyeongju (Gyeongbuk); 7. Yeongcheon (Gyeongbuk); 8. Goseong (Gyeongnam); 9. Yeongcheon (Gyeongbuk); 10. Goheung (Jeonnam); 11. Namhae (Gyeongnam); 12. Jeongseon (Gangwon); 13. Cheongsong (Gyeongbuk); 14. Naju (Jeonnam); 15. Buan (Jeonbuk); 16. Cheongdo (Gyeongbuk); 17. Jindo (Jeonnam); 18. Dalseong (Daegu); 19. Happcheon (Gyeongnam); 20. Yangsan (Gyeongnam); 21. Hadong (Gyeongnam); 22. Sangju (Gyeongbuk); 23. Gimcheon (Gyeongbuk); 24. Ulju (Ulsan); 25. Yeosu (Jeonnam); 26. Sinan (Jeonnam); 27. Geochang (Gyeongnam); 28. Gangneung (Gangwon); 29. Changwon (Gyeongnam); 30. Yeongam (Jeonnam). The gray dots are all marked for the areas that did not rank in the top 30.

The Silla Kingdom, on the other hand, was not under the influence of the Gaya Confederacy. This fact notwithstanding, Choi *et al*. (2023)'s data on the $^{87}Sr/^{86}Sr$ ratios of herbaceous plants for the area under Silla's politico-cultural control, specifically Daegu (Dalseong or Donggu) and Ulsan, showed tendencies similar to those for the Daeseong-dong horse-tooth fragments. In this light, we could not rule out that the Daeseong-dong horses had been raised in those areas and then brought into the region around Daeseong-dong. And given Silla's independence from Gaya, we were presented with the additional, intriguing possibility that movement of horses into the Daeseong-dong area might not have been political tribute but rather, the outcome of trade.

Another example of an area showing highly similar $^{87}Sr/^{86}Sr$ ratios to the Daeseong-dong horse data but that was not politically ruled by the 4th century Gaya Confederacy is the southwestern coast of the Korean Peninsula (Figure 4). Thus, if the horses' breeding grounds were there, they would have been moved into the Daeseong-dong area by sea-going trade. Finally, we should also note that the case of the Baekje Kingdom, which during the 4th century existed on the western part of the Korean Peninsula and includes present-day Seoul, Chungnam, Chungbuk, and Jeonbuk provinces (Figure 4). Since there are no reports of herbaceous $^{87}Sr/^{86}Sr$ data for this area that are similar to the Daeseong-dong horse results, we can posit that the Daeseong-dong horses were not bred in the Baekje area but instead, in the territory of Gaya and/or Silla.

The evidence obtained in this study suggests that Gaya's horses were bred over a wider area than previously assumed. However, horses' transportation distance appears to have been shorter than that during the Fujiwara-Kyo period in Japan (Gakuhari 2018). We attribute this to the earlier-estimated dating for the Daeseong-dong horses and the absence of a unified Korean Peninsula in the 4th century. Our information indicates that although the overlords entombed in Daeseong-dong had built a strong Gaya Confederacy, they had not yet established full-scale operation of ranches for breeding of horses. This may have taken place following the expansion of Silla's power in the 6th century CE, although a complete understanding will require further research.

Conclusion

In this report, we reveal that the horses relevant to two distinct horse-tooth fragments found in Daeseong-dong Tomb #1 might have been raised in the southeastern region of the Korean Peninsula that, in the 4th century, was under the control of either the Gaya or Silla Kingdom. Areas showing herbaceous $^{87}Sr/^{86}Sr$ data similar to that for the Daeseong-dong horses also include regions on the southwestern as well as eastern coasts. We also note that there were no similar $^{87}Sr/^{86}Sr$ results for the western half of the Korean Peninsula, the location of the ancient Baekje Kingdom during the same period. We speculate that it was probably only after Korea was unified under the Silla Kingdom that horse breeding was established *throughout the Korean Peninsula* by the central government.

Conflict of Interests

The authors declare that they have no conflicts of interest in this paper.

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Authors' Contributions

Author contributions are as follows. Conceptualization, design of study, and correspondence: Jong Ha Hong and Dong Hoon Shin; zooarchaeological data analysis: Eun Byul Ko and Jong Ha Hong; archaeological and historical data analysis: Young Min Ko, Yang Su Yi, Ala Go, Jieun Kim, and In Uk Kang

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