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Reconstructing the food structure of ancient coastal inhabitants from Beiqian village: Stable isotopic analysis of fossil human bone

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The Beiqian site located in Jimo city, Qingdao, a few kilometers from the modern coastline was excavated in 2007, 2009 and 2011. A large number of human bones from the early Dawenkou period were unearthed. Through C and N stable isotopic analysis, the food structure of the "Beiqian" ancestors was reconstructed and the influence of terrestrial and marine resources on their lifestyle was discussed. Based on the δ^{13}C and δ^{15}N stable isotopic values of human bone collagen, the analytical results obtained through a ternary mixed model reveal that the food sources of the Beiqian ancestors included about 44.1% marine species (probably shellfish and fish), 34.1% C4 plants (possibly millet), and 21.8% land animals. These results indicate that they lived mainly from fishing and farming, supplemented by hunting or poultry raising. Compared with other sites in the same period, the stable isotope results show that 5000–6000 years ago, the lifestyles of ancestors in the Yangtze River basin, Yellow River basin, Northern coastal area and inland area were very different. The ancestors from the Yangtze River basin focused on rice farming and fishing, whereas the ancestors in the Yellow River basin farmed millet and raised animals. Those in coastal areas relied mostly on farming and marine fishing.

Neolithic age, Dawenkou culture, Beiqian site, food structure

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The food structure of prehistoric ancestors can demonstrate those people's adaptation to their living environment. For example, the utilization of wild versus domesticated seasonal food resources. Although in some cases direct speculation can be made using remnants of food, such information is very limited. Furthermore, remnants more likely reflect the personal preference for a particular food rather than the actual food structure. Carbon (C) and nitrogen (N) stable isotopic analysis of human bone collagen makes it possible to describe the food structure of ancient inhabitants, and provides very useful information including regional lifestyle and reconstruction of social structure. This research approach has recently become of increasing interest in archaeology [1–3].

It has been known that proteins from animal bones may reflect the protein composition from the human diet. This means that the stable isotope ratios from human bone could record the protein composition of an individual's diet in the past. Consequently, stable isotopic analysis can recapitulate the food structure of prehistoric ancestors and the dietary differences between individuals. C stable isotopic analysis is often used to differentiate the C3 (temperate plants such as vegetables, fruits and wheat) and C4 (tropical or dry plants such as millet, maize and sugar cane) plant ecosystems [4], or marine and terrestrial ecosystems [5]. In food chains, animals at upper trophic level have δ^{15}N values of 3%–5% accumulating from animals at lower level as food. Thus stable δ^{15}N isotopic analysis can determine animal

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position in a food chain [6]. Combined C and N stable isotopic analysis can provide information about the plant and meat food sources of ancient humans, and possibly the social economics of those populations lived.

The C and N stable isotope analysis of human bone is now widely used in archaeological science and technology [7–9], including embodiment of rice-growing in southern China, investigation of the north-south transmission route of rice [10], verification of the settlement of northern nomadic tribes and assimilation with Han Chinese [11], and many other meaningful observations. Based on the C and N stable isotopic analysis of human bones excavated from the Beiqian site, this study explores the ancestral food structure and the influence of terrestrial and marine sources on their lifestyle. The Beiqian site is located in Beiqian village, Jimo city, Shandong Province, China, 5 km from the modern coast. Many human bones have been excavated in this site, making it possible to explore the lifestyle of the ancient people from these coastal areas. The purpose of this study was: (1) to reconstruct the food structure of the ancient inhabitants; and (2) using previous literature, to compare the difference in the food structure of the ancient people located elsewhere including the Yangtze River basin, Yellow River basin, Northern coastal area and inland area. These data were then used to explore the food structure and social economics from that period.

1 Materials and method

1.1 Site background

The Beiqian site is located in Beiqian village in the town of Jinkou, Jimo city, approximately 5 km from the coastline, west of the south coast of the Jiaodong Peninsula. Three excavations were undertaken by the Department of Archaeology, Shandong University, Institute of Conservation and Archaeology in Qingdao and Jimo Museum in 2007, 2009 and 2011, respectively. Many relics such as ditches, housing sites, tombs, ash pits, stone tools, potteries, bone, copperwares and mussels were unearthed. Additionally, some multiple second joint burials were first discovered in the Jiaodong area. From the remnants and wares we assume that the Beiqian site had mainly gone through both the Dawenkou early period and the Zhou Dynasty.

1.2 Sample treatment

Twenty human shinbone samples from the Dawenkou period were selected (dating back to about 5500–6100 years): 9 males, 7 females and 4 of unknown gender. The burial number, gender, and other information on the samples are shown in Table 1. According to the experimental method proposed by Ambrose [12], all samples were crushed after de-contaminant treatment on external surfaces, and the bone powder was sieved through a 40–60 mesh sieve and collected into a sand core funnel. The bone powders were soaked and decalcified in 0.1 mol/L HCl solution at 4°C for 3–5 d depending on the samples until no obvious particles remained, replacing daily with fresh acid. Following decalcification, the samples were washed with deionized water to neutralize, and soaked in 0.125 mol/L NaOH solution for 20 h to remove remnants of humic acid. Finally, the samples were washed with water for several times, then placed in 0.001 mol/L HCl solution and heated at 70°C for 48 h, filtered, evaporated and freeze dried to obtain the bone collagen solid. The yields of bone collagen (bone collagen mass/bone powder mass) are shown in Table 1.

1.3 Test analysis

The C content, N content and C, N stable isotope ratios of bone collagen were measured at the Stable Isotope Ratio Mass Spectrometry Laboratory in the Chinese Academy of Forestry. A Thermo Fisher MAT253 isotope ratio mass spectrometer equipped with Flash EA1112 HT elemental analyzer was used to analyze the material. C, N stable isotope ratios are expressed as δ^{13}C (PDB) and δ^{15}N (AIR) and the accuracy of analysis is ±0.1% and ±0.2%, respectively. All the C, N content and δ^{13}C, δ^{15}N results are also listed in Table 1.

2 Results and discussion

2.1 Identification of bone contamination

The pH, temperature or humidity from microorganisms in the soil environment may destroy the original integrity of the bone structure. Such bone diagenesis [13] is known as bone contamination. The correspondence between the food structure of the ancient ancestors and chemical composition of the bones may be changed by bone contamination, so it is necessary to identify whether our human bone samples were contaminated. As indicated in Table 1, with the exception of a few samples, the bone collagen yield was below 10%. This is far less than the average value of modern samples (~20%) [14,15]. However, average C and N contents of all samples are 40.23% and 14.91%, respectively, which is close to modern C content (41%) and N content (15%), suggesting that a burnishing treatment could remove contaminants on the bone surface. In essence the bone collagen could retain the original biological and chemical properties and primarily be considered uncontaminated by the environment. The C/N molar ratio is also an important indicator. If its value falls within 2.9–3.6, the bone collagen can be considered as uncontaminated [16]. Data in Table 1 show that the C/N molar ratio of 20 samples all falls within this range with an average of 3.15, a minimum of 2.95 and a maximum of 3.28, all close to the modern bone collagen value of 3.2. These analyses indicate that although a certain degree of degradation of bone collagen has occurred, the 20

Table 1 Sample information and analytical values

Number	Samples	Gender	Yield of bone collagen (%)	C (%)	N (%)	C/N	^{13}C (‰)	^{15}N (‰)	C4 plant (%)	Shells (%)	Terrestrial animals (%)
1	M4-A	Male	6.09	37.68	14.44	3.04	−9.56	6.36	45.4	13.6	41.0
2	M4-B	Male (elder)	3.38	37.33	14.04	3.10	−9.20	7.38	40.6	31.9	27.5
3	M4-C	Male	6.14	39.32	15.16	3.03	−9.59	7.64	34.8	34.4	30.8
4	M4-D	Female	8.01	39.33	14.91	3.08	−8.03	7.50	50.8	39.3	9.9
5	M4-E	Female	3.53	37.35	14.39	3.03	−8.89	7.87	39.6	41.4	19.0
6	M6	Unknown (youngster)	4.03	41.18	14.66	3.28	−8.34	9.03	35.5	62.9	1.59
7	M22-A	Male	3.73	36.97	14.38	3.00	−9.81	6.52	41.7	15.1	43.2
8	M22-B	Female	1.76	37.51	14.85	2.95	−9.71	6.15	45.6	9.5	44.9
9	M22-C	Female	9.96	40.93	15.13	3.16	−8.70	8.44	36.8	51.6	11.6
10	M22-D	Unknown (child)	10.31	41.10	15.30	3.13	−10.03	7.30	33.3	26.8	39.9
11	M31-A	Male	2.30	41.39	15.21	3.17	−9.89	8.80	22.5	52.1	25.4
12	M31-B	Male	4.59	42.48	15.36	3.23	−9.36	8.67	28.7	52.3	19.0
13	M31-C	Female	2.82	40.07	14.67	3.19	−10.22	8.58	21.1	47.0	31.9
14	M62-A	Male	3.08	41.47	15.17	3.19	−9.33	8.99	26.4	57.7	15.9
15	M62-B	Male	0.57	38.01	13.67	3.24	−8.36	8.16	42.3	48.6	9.1
16	M62-C	Female	4.22	41.64	15.22	3.19	−8.25	8.62	39.7	56.6	3.7
17	M62-D	Unknown (child)	12.53	44.29	15.85	3.26	−9.72	8.66	25.3	50.5	24.2
18	M90-A	Male	7.55	41.64	15.05	3.23	−8.70	9.06	31.8	61.8	6.4
19	M90-B	Female	4.50	42.01	15.33	3.20	−8.59	10.02	23.6	73.4	3.0
20	M139	Unknown (child)	4.75	42.95	15.48	3.24	−10.27	9.14	16.2	55.9	27.9
Average				40.23±2.16	14.91±0.52	3.15±0.10	−9.16±0.66	8.09±0.96	34.1±9.4	44.1±17.5	21.8±14.0

Table 2 Isotope data from different areas in the same period and their possible food structures[a)]

Site	Area	Age (a BP)	Sample amount	^{13}C (‰)	^{15}N (‰)	Plant food sources (%)		Animal food sources (%)			Reference
						C3	C4	Marine shellfish/fish	Fresh water fish	Poultry/hunting	
Sanxing lage	vil-Yangtze River (Southern China)	6500–5500	19	−20.04±0.21	9.69±0.33	27.2±4.3	3.9±1.3	None	68.9±4.5	Small	[25]
Shijia	Yellow River (northwest)	6300–6000	9	−10.03±0.68	8.10±0.45	9.4±5.0	67.8±4.8	None	22.8±6.1		[26]
Zongri	Yellow River (northwest)	5200–4100	24	−10.09±1.07	8.33±0.45	5.7±4.9	67.0±7.2	None	26.1±6.2		[27]
Xigongqiao	Yellow River (East China)	5000–4500	7	−14.97±3.87	8.40±1.32	23.9±22.1	33.6±11.8	None	42.7±15.6		[28]
Beiqian	Coastal areas (East China)	6100–5500	20	−9.16±0.66	8.09±0.96	Small	34.1±9.4	44.1±17.5	Small	21.8±14.0	

a) Taking into account the complexity of food sources as well as limitations of ternary mixed model, there will be uncertainties in the food composition.

samples still maintain the original biological characteristics and can be considered uncontaminated and used for further stable isotopic analysis.

2.2 Food structure analysis of Beiqian ancestors

Previous studies in Japan found that the δ^{13}C and δ^{15}N average values were −25.4 ±1.6% and 1.2 ±2.4% for C3 plants [17], and −10.0 ±0.5‰ and 1.0 ±1.9‰ for C4 plants, which are very close to the δ^{13}C values −26.5% (C3) and −12.5% (C4) from studies in China [18]. In stable isotopic analyses, to describe the food structure of ancient humans, C and N stable isotopes are usually discussed separately. The C stable isotope reflects the ratio of C3 and C4 plants from the diet and the N stable isotope shows the structure of meat (including marine and terrestrial sources).

During the transformation process of food to bone collagen through digestion and absorption, there is a certain degree of enrichment in C and N stable isotopes, generally including 4.5% to 6.1% of δ^{13}C and 3% to 5% of δ^{15}N due to age and food sources [19]. Accordingly, C3 plants [17], C4 plants [17], marine shellfish [20], marine fish [20], terrestrial animals [20], freshwater fish [17] reported in previous literature, and unearthed terrestrial animals (e.g. pig and deer) were selected with a correction of 4.5% for δ^{13}C and 3.4% for δ^{15}N [17]. Their corresponding relationship with the Beiqian human bone collagen is shown in Figure 1. Deer and pig are regarded as the potential primary animal meat sources in the diet, i.e. the terrestrial animals (TM-1). Three pig samples and two deer samples were selected in the test and their C, N stable isotope values were: pigs (−17.5± 2.6‰, 3.8±0.5‰), deer (−21.4±0.8‰, 2.7±1.6‰), total terrestrial animals TM-1 represented by above pigs and deer (−18.5±3.3‰, 3.4±1.1‰). C, N stable isotope data show that for pigs and deer there were a wide variety of food sources. Deer preferentially inhabit meadows, trees or bushes, avoiding human residences and feeding chiefly on herbs, twigs or leaves of trees and even tree bark in winter. These are typically C3 plants, less affected by C4 plants (such as millet) in food sources of ancient Beiqian people. Compared with pigs the δ^{15}N and δ^{13}C in deer bone collagen are relatively low, reflecting the characteristics of deer living on C3 plants. It is reasonable to assume that those deer unearthed from the Beiqian site were probably ancestral prey. However, our pig collagen data are similar to C4 plants, indicating that their main food source was crops (including millet), but that they also ate fruits, shells, rhizomes and most probably some meat from food residues and human feces. Thus as the δ^{15}N values were relatively high, these apparent omnivore characteristics may reflect that pigs were once livestock raised by the ancient people.

Analysis of C and N stable isotopes of animal and plants remaining in the site to obtain the food structure was not done because of the age of the samples, limited amounts and the detection method. Therefore, in the food structure study, we only selected the plant C and N isotope data from the same period reported in [17]. In summary, as deer and pig represent terrestrial meat sources (TM-1) [21], when combined with other literature on plants and marine life, the food structure analysis of ancestors can be roughly reflected. There are still some difficulties in recovering human activities thousands years ago by the current analytical method; however this information is still useful for studying the food structure of prehistoric coastal inhabitants.

Until now, the exact relationship between food protein and the stable isotopic ratios of human bone collagen was not clear. Our isotopic values in the literature may differ from those of the Beiqian site because of factors such as geographical location and climate change. However, the food structure can be generally speculated as in Figure 1. The C and N stable isotope values of human bone collagen generally fall in the range among C4 plants, marine shellfish (MS), marine fish (MF) and terrestrial animals (TM-1). After linear regression the data does not show a significant correlation (R^2 = 0.275, P = 0.2412, N = 20). This suggests that there were a variety of food sources available for Beiqian ancestors (Figure 1).

Based on the δ^{13}C data a simple binary mixed model [21] can be used to calculate the ratio between C3 and C4 plants in the food sources of the ancestors studied. This was particularly suitable for rice and millet mixed farming, such as at the Gouwan site [14]. As for the Beiqian site, according to our stable isotope analysis and excavation information, it can be assumed that the ancestors' food came mainly from fishing and farming. However the ratio between C3 and C4 plants cannot reasonably show their food structure. In Table 1, most of the δ^{15}N isotope values calculated by the binary mixed model are <9%, which is relatively low, suggesting a low proportion of animal resources in food structure. However, a large number of live shellfish remains were excavated from the Beiqian site indicating that the ancestors had

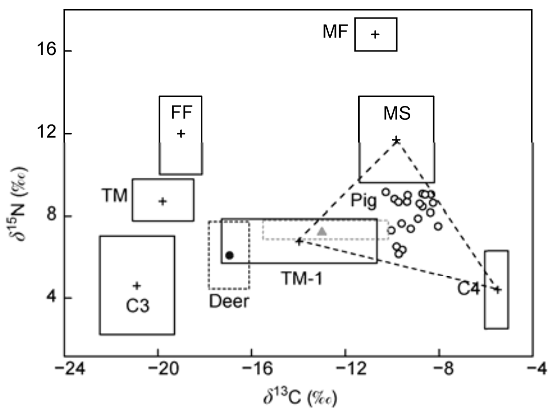

Figure 1 Comparison of δ^{13}C and δ^{15}N isotope values between Beiqian human bone samples and possible food resources. The panes represent the deviation range of food resources including C3 plants (C3), C4 plants (C4), C3 plant-eating land animals (TM), freshwater fish (FF), marine fish (MF), marine shellfish (MS), deer from the excavation site (Deer), pigs from the excavation site (Pig) and terrestrial animals (TM-1) from the excavation site.

broader meat sources, including marine shellfish and/or fish. Based on the absolute ratio between δ^{15}N and δ^{13}C, the binary mixed model may be more useful for analyzing the food structure (especially meat sources) from inland areas. These will have different interpretations compared to the areas that hunt marine fish, especially shellfish as meat sources.

In this study, a ternary hybrid model was used. Three points that possibly correlate with food sources were selected to form a triangle in Figure 1. This food structure was expressed by percentage content of the three food sources. Compared with the binary mixed model, this method can examine the δ^{13}C and δ^{15}N stable isotopes simultaneously. By increasing the number of components, the result will be closer to a more realistic situation based on our data. In mathematics, that is the transformation from binary linear function into plane ternary function.

In the Beiqian site, carbonized grains of millet, a large number of shells, domesticated pig bones and deer bones were unearthed, suggesting that the lifestyle of ancestors included farming, fishing, livestock and hunting. The stable isotopic analysis of human bones show that their food structure was based on C4 plants (probably millet), marine food (may be sea shellfish and fish) and terrestrial animals. Therefore, according to the analysis in the site, we can select three variables, C4, shellfish (MS) and terrestrial animals (TM-1), to study the food structure using the ternary mixed model. It should be noted that the terrestrial animals TM-1 variable (Figure 1) selected here is a statistical average of stable isotope data from pigs and deer in the site.

After rigorous mathematical derivation of the formula (a more detailed summary of the mathematics is listed in Appendix), triangular co-ordinates including farming (C4), marine fisheries (shellfish, MS) and livestock (land animals, TM-1) were defined, according to the lifestyle of Beiqian ancestors. The results showed that the food structure mainly included approximately 34.1% C4 plants (possibly millet), 44.1% marine sources (shellfish and fish in the sea) and 21.8% terrestrial animals (Table 1). Between individuals there was a large range. For example, the consumption span of C4 plants was 34.6% (from 16.2% to 50.8%), shellfish reached 63.9% (from 73.4% to 9.5%), while terrestrial animals was up to 41.9% (from 3.0% to 44.9%), indicating that individual eating habits, or abundant food sources, also determine the individual food structure.

The Beiqian site was located on a plain, only a few kilometers away from the coast, providing ideal farming and fishing conditions for the ancestors. From our data, millet farming, shellfish collecting and fish hunting all accounted for more than 35% in the reconstructed food structure. These were regarded as the two dominant plant and animal food sources, which agrees with the excavated shells and fish bones found from the Beiqian site. The shellfish and fish sources possess seasonal and perishable characteristics, while millet and other crops are easily stored and can be supplied all year round. Although a large number of domesticated animal bones were found in the site, demonstrating that for example poultry farming already existed, the isotopic data indicate that terrestrial animal food sources accounted for ~22%, less than shellfish and other major meat sources. Based on the ternary mixed model, the results show that Beiqian ancestors lived primarily from fishing and farming, supplemented by poultry farming and hunting.

It should be noted that our ternary mixed model has some limitations. For example the type of food selected may influence our conclusions. We therefore regard it as improvement of the binary mixed model. Combining our model with our excavation data revealed that most domesticated animal bones from the Dawenkou period were pig bones, and 90% of those pigs were >1 year old. In addition, more than 50% of those pig bones were >2 years old. This information suggests that domesticated pigs had been very common in that period, and had existed in very large numbers. Also we can assume that most pigs were slaughtered before maturity. In summary, the lifestyle of Beiqian ancestors focused on fishing, farming and poultry farming, supplemented by hunting in the Dawenkou period.

2.3 The food structure of ancestors from different areas

Stable isotope analysis of human bones unearthed in the Yellow River basin indicates that millet farming and poultry farming started to popularize about 7000 years ago [22,23]. In the Yangtze River basin, rice farming had developed earlier, and human bone isotope analysis shows that the meat source of ancestors mainly came from fishing and hunting activities and that poultry farming was not common [24,25]. The data in Table 2 show the published studies from the same period including the sites of Sanxing village in Jintan city, Jiangsu, in the Yangtze River basin [25], Shijia in Lintong city, Shanxi, in the Yellow River basin [26], Beiqian in Qingdao city, Shandong, or relatively later Zongri in Tongde city, Qinghai [27] and Xigongqiao located between Yellow River and Yangtze River in Tengzhou city [28]. These five sites can be divided into the Yangtze River basin, Yellow River basin, and northern inland and coastal area. The isotopic analysis of those sites could reveal differences in the food structures of ancient ancestors from different areas and show any similarities or differences in their lifestyles.

The ancestors from the Sanxing village site [25] show that isotope values fall between C3 plants and TM in Figure 2. According to the on-site information these values are considered to be between those of C3 and freshwater fish (FF). The δ^{13}C value is the lowest in the five sites and shows the evident characteristics of C3 plants which would suggest rice cultivation. The δ^{15}N value is the highest, suggesting that meat sources mainly came from fish rather than land animals, and it indicates that fishing was more popular in the Yangtze River basin because of its well-developed drainage system. In the same period, the δ^{13}C values from the Shijia site [26] and later the Zongri site located in Yellow

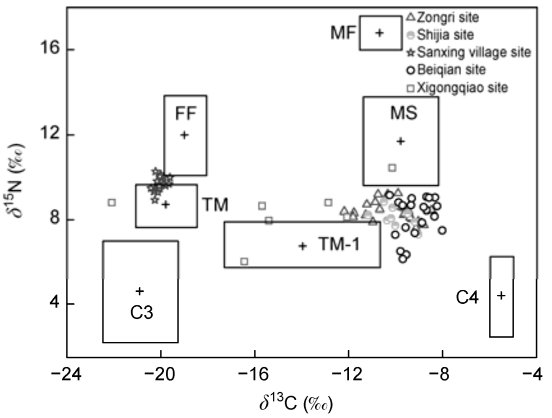

Figure 2 Comparison of the δ^{13}C and δ^{15}N isotope ratios in human bone samples and possible food resources including the Zongri, Shijia, Sanxing village, Beiqian and Xigongqiao sites. Other symbols are described in Figure 1.

River basin [27] were ~ −10.0%, reflecting characteristics of C4 plants, possibly belonging to millet farming. The δ^{15}N was slightly lower than that from the Yangtze River basin. Considering the geographical features there, we suggest that the main food sources were likely to have been poultry farming and hunting. In Figure 2, these two sites are closer to the location point of C4 plants, indicating that the animal food sources may be in a secondary position in the food structure with millet farming as the main food source. As for the Xigongqiao site [28] located between the Yangtze River and Yellow River basin (now located in the south of Shandong Province), the distribution of the δ^{13}C and δ^{15}N values is wide (Figure 2). However, the values of δ^{13}C were located at positions between those of C3 and C4 plants. Additionally, the values of the δ^{15}N were lower than those from the Yangtze River basin, but equivalent with those from the Yellow River basin. It can be speculated that the farming in the Tengzhou site included both rice and millet, with the integration of North-South farming. Farming was the main lifestyle and meat sources came from hunting or poultry.

The relationship between different sites through the information reflected by stable isotopes shows that the Beiqian site falls in the same area with the two sites located in the Yellow River basin (Figure 2). Thus we can conclude that the plant food sources were all from C4 plants. However, we previously mentioned that the meat sources of the Beiqian ancestors, particularly marine shellfish or fish, were different from those from the Zongri and Shijia sites. Therefore, the ternary mixed model is limited and requires additional on-site excavation information, particularly in the analysis of local meat sources. Overall, the farming type in the Yellow River basin and coastal sites was mostly of C4 plants from millet farming, and the main meat source in inland areas was mainly hunting or poultry farming. In comparison, coastal areas show fishing as the main farming type. Figure 2 also shows that it is difficult to distinguish the specific food sources between northern inland and coastal ancestors by the analysis of δ^{13}C and δ^{15}N values, because of the similar δ^{15}N value of marine fish (shellfish) and C4 plants. It is necessary to take into account the excavation information to get more reasonable conclusions. Furthermore, ternary mixed models may be more applicable for the northern coastal ancestors that lived on millet farming and fishing, reflecting the proportion between meat and plants in the food structure.

According to our analyses, the results from the ternary mixed model show that the diet characteristics of ancestors in Sanxing village, located in the Yangtze River basin, were mostly from fishing (69%) and C3 rice (27%). The ancestors of the Shijia site, inland of the Yellow River lived on C4 plants (67%–68%), followed by poultry/hunting/freshwater fishing (26%–23%), with C3 plant sources <10%. At the Xigongqiao site located between the Yellow River and the Yangtze River, poultry farming accounted for 43% of food sources, while C3 plants (rice) and C4 plants (corn) mixed cultivation accounted for ~57% (Table 2).

Based on our comparative analysis of the stable isotope ratios of 5000–6000-year-old human bones of ancestors unearthed in the Yellow River basin, Yangtze River basin, surrounding inland and coastal areas, we conclude the following: (1) the ancient inhabitants living in the Yangtze River basin were living mostly on a fishing-based diet, which was supplemented by the cultivation of rice. In this region, poultry farming was not common; (2) millet crops were widely cultivated in inland areas of the Yellow River basin, with poultry as the main source of meat; (3) located between the Yangtze River and the Yellow River, the Huaihe River basin was at the intersection area of rice and millet farming. At this location arable farming of crops and poultry provided the main sources of food; and (4) in northern coastal areas the inhabitants mainly lived from millet farming and marine fishing. In addition, at these locations poultry farming had also become popular.

3 Conclusions

The stable isotopic analysis of human bones of ancient Beiqian people showed that the food structure of these Dawenkou ancestors included mainly farming of millet and marine shellfish. This diet was supplemented by poultry farming and/or hunting. Compared with other sites in the same period, ~5000–6000 years ago, the diet at coastal sites was different from those in the Yangtze River, Yellow River and inland area. The Yangtze River basin mainly focused on millet farming and fishing, whereas the Yellow River basin focused on arable and poultry farming, and coastal areas relied on farming and marine fishing.

We note that the reliability of our ternary mixed model depends on the stable isotope data of C3, C4, marine and freshwater fish and terrestrial animals. When collecting our stable isotope data, available referenced data were limited.

The C and N stable isotope data from the Japanese literature may bring some deviations due to geographical differences. For example, some literature has pointed out that the deviation of the $\delta^{15}N$ values of marine fish between the Japan Sea and the inland sea is ~2%–4%, and additionally 1%–2% deviation may also occur between different food chains. Additionally other conditions, such as geography and climate, can affect the biological characteristics of plants and animals, and thus their stable isotopic values. The ternary mixed model has only taken into account three factors and still has some limitations in exploring the complicated food structures of the ancient ancestors. However, it is a valuable tool and potentially useful for any future studies.

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Supporting Information

Appendix: Ternary mixed model

The supporting information is available online at csb.scichina.com and www.springerlink.com. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.