

East Asian Gene flow bridged by northern coastal populations over past 6000 years

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This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Liu et al. East Asian Gene flow bridged by northern coastal populations over past 6000 years

In the presented manuscript, the authors provide a detailed analysis of the fine-scale population genetic history of East Asia. The analysis is novel with respect to the period of history and region/s under investigation. I don't have major complaints about the methodology or statistical design of the manuscript. That said, I do have several comments which I believe should be addressed in order to make the manuscript trackable, especially for those unfamiliar with the demographic history of East Asia such as myself.

Minor Comments

– When describing historical periods (particularly for the first time) please include their age ranges i.e., the Kofun period, Yayoi period, Dawenkou cultural period, and Jin Dynasty, discussed throughout your manuscript.

– When discussing a number of geographical landmarks, such as the Yellow River Basin and the Shandong region, it would increase the readability of your paper by referencing them in Figure 1. In general I found it cumbersome to follow.

– Similarly, placing the location of your newly sequenced genomes within the geographical context of published east Asian aDNA in a Figure would also greatly increase your manuscript readability for non-specialists.

Line 69: The phrase, "In this study," made it sound like the current manuscript. Change wording to more clearly reflect the authors of ref 10.

Lines 141-143: Indicate which figures correspond to the analysis results described in text.

Line 145: Specify that in PCA, "They formed clusters distinct from ..."

Figures

Fig 1. (A) It would be helpful to place the heading "Shandong sites" above the DWK, LS, CD headings just to reinforce that this is the region of analysis. For regions described in the text, i.e., "Yellow River Region" it would be helpful to have these indicated in the map. (C - D) it does not appear that the color and shapes match those shown in (A). For instance, the green star for Yixi in (A) is not present in the list of green shape/color legend in (C). If this is incorrect, then can the authors please make the correct correspondence more clear.

Reviewer #2

(Remarks to the Author)

Liu et al has newly generated and analysed whole genome data of 85 individuals dated to between 6000 and 1500 years ago. This study found that the genomic formation of ancient populations in Shandong has involved north-south gene flow from 7700 years ago; inland-coastal-island gene flow in East Asia since Neolithic time in Shandong. In particular, this study highlighted the historical gene flow from mainland East Asia (Shandong) to the Ryukyu Islands which is of interests to multiple disciplines. It is highly recommended for publication after revision. Find detailed comments below.

1. It would be great to accurately define the Yellow River Basin, as the Yellow River runs through thousand kilometers in China, and mark Yellow River in the map of figure 1.
 2. Line 107-108, "this dynamic period" → define 'this'
 3. Line 110, uncomplete sentence "spanning a sixth of the coastline China"
 4. Line 125, "contamination levels higher than 5% for uniparental markers", why examine contamination level for uniparental markers?
 5. Line 164, "Early Neolithic and Dawenkou cultural period". It would be good to clarify in general in the text on the period of Dawenkou/Shandong as Dawenkou and Longshan are part of Neolithic period in Shandong.
 6. Line 178, does the affinity with NEA contradict the affinity with SEA? What would be possible explanation of conclusion from both north and south directions "this coastal region was impacted by populations from across mainland East Asia" at line 194-195.
 7. Line 225, "This observation suggests a decreased level of influence from Yangshao populations towards the coastline." Not sure if this conclusion stands solidly using qpAdm proportion as the supporting evidence.
 8. Line 271, conclusion of "interaction within the Shandong region rather than interactions with populations outside of the Shandong region". I am not sure that I am convinced by the presented evidence as the mentioned YR ancestry in Shandong is from the Middle Yellow River zone (outside of Shandong, Ning et al 2020), which is the 'first wave of introgression from inland to coastal populations' as far as I understand (given at line 266, it is written that "the proportion of ancestry in the Longshan Shandong populations that derived from Yellow River populations was estimated to 46-49%."). Suggest the authors either add more evidence or rephrase.
 9. Section "Shandong coastal East Asians to Ryukyu": this is a very intriguing finding!
In Cooke et al 2021 paper, the authors modeled (~3000 year old) Yayoi as admixture between Jomon and WLR_BA_o/HMMH_MN/Lokomotiv_EN. Has the authors tested Shandong-related ancestry in Yayoi, or using Yayoi as the only basal Japanese source + the other ancient Shandong population when modelling ancient historical Japanese populations?
 10. In general, the evidence for the alignment between cultural patterns and biological change presented in this manuscript is quite vague, though it was discussed at Line 413-414. Suggest rephrase Line 413-424
 11. I would expect the authors could add more robust analyses regarding the conclusion of "NEA, SEA gene flow to Shandong before 7000 years ago" based on samples from Xiaojingshan, and related possible explanations in the Discussion part. There are only f-statistics supporting this conclusion, but exact ancestry modelling results are missing.

It is also north worthy that the data quality of newly reported Xiaojingshan samples is rather low, with no contamination information perhaps due to low data quality. Hence to make this conclusion more robust, it might be necessary to boost up the data quality of Xiaojingshan to make sure that the observation is robust. Or explain the used published Xiaojingshan data in a clearer way in the text.
- Meanwhile, it would be great to see qpAdm models for Xiaojingshan, for instance, the authors could use previously published ancient East Asians before 7000 years ago as one of the ancestral sources, and report the exact proportions from NEA, SEA and any other possible ancestral sources in 7700BP Xiaojingshan.
12. Regarding the genetic connection between Shandong and Ryukyu islands, are there any related linguistic evidence around this (e.g. Ryukyuan languages mentioned in Robbeets et al 2021)? Could the authors find or add related information about that regarding the newly discovered genetic connections versus linguistic connections, and explain if this gene flow has accompanied with the dispersal of agriculture as well?

Reviewer #3

(Remarks to the Author)

Lie et al. generated genome-wide data from 85 ancient individuals excavated from 11 sites across the Shandong region. These genomes were co-analyzed with other ancient and present-day datasets. The study identifies population interactions between inner and coastal East Asia and suggests a potential impact of coastal East Asians on the insular population of the

Ryukyu Islands in Japan. The dataset has significant cross-disciplinary value for the broader research community, particularly for those working on East Asian genomics. However, I have several concerns regarding their analysis and interpretations.

1. My major concern is the over-interpretation of results without sufficient statistical confidence. The authors applied f4-tests to identify extra genetic affinities between a Shandong population (e.g. one of the DWK populations) and a Yellow River population relative to an earlier population from the Shandong region (e.g. one of the Early Neolithic populations). There are various combinations of Yellow River (4 populations) and earlier Shandong populations (3 for Shandong_EN; 3 for Dawenkou or DWK; 2 for Longshan or LS; 6 for Chinese dynasty or CD) used in the tests. However, the statistically significant signals are often sporadic; genetic affinity is evident only when a specific set of Yellow River and Shandong populations is used. This indicates either variability in the genetic affinity with Yellow River populations across contemporaneous Shandong populations or variation in the earlier populations. The only results that are convincing and correctly interpreted are the f4-tests presented in Table S6, which clearly demonstrate extra genetic affinities between post-agricultural ancient Japanese and ancient Shandong populations compared to pre-agricultural Japanese (i.e., hunter-gatherer Jomon), as most of the Shandong populations tested as P3 exhibit $Z < -3$. In this case, I fully agree with the authors' assertion that these ancient Japanese could have some affinity with the Shandong populations.

Additional comments that are linked to this first major concern:

L175-178: The results from the f4-tests do not seem to robustly support the connection between Xiaojingshan and non-Shandong populations. Firstly, the Z-scores are not consistently significant. Secondly, in most cases, statistical significance is only supported by either one of the P1 populations (Xiaogao or Boshan), rather than both. I recommend using a more stringent cut-off of $Z < -3$ or $Z > 3$ for statistical significance. If this stricter threshold is applied, the signals become even more sporadic. Overall, the results do not appear robust enough to support the proposed connection. Additionally, why are the signals of genetic affinities so different between the P1 populations? Please clarify the source of this variability, e.g. differences in their genetic ancestry between Xiaogao and Boshan.

L179-183: In Table S11, Xiaojingshan and BQ should be included in the form of f4-tests in the title of this table. Additionally, why is Bianbian not included in this analysis despite being included in other f4 tests (e.g., Table S8)? The genetic affinity of DWK with SEA varies significantly among the three DWK populations. For each DWK population, 3 Shandong_EN and 28 SEA and NEA populations were tested as P1 and P3 in the form of f4(P1, DWK; P3, Mbuti), resulting in a total of 84 f4-tests. Only two tests show significant affinities between FJ and SEA, in particular when Xiaogao was used as P1. There are more significant signals from the other two DWK populations, GS and BQ; however, these signals are not consistent across the three Shandong_EN. In the case of the BQGroup, there are significant signals from nearly half of the Amur River populations (NEA) tested in f4(Boshan/Xiaogao, BQGroup; Y, Mbuti). The connection between DWK and SEA suggested by the authors strongly depends on the choices of P1 and P3, which raises concerns about its robustness.

L186-191: Before testing any differences in the genetic affinity between Shandong_EN and DWK, please run f4 analyses using the forms (Boshan/Xiaogao/Xiaojingshan, Boshan/Xiaogao/Xiaojingshan; SEA/NEA, Mbuti) and (GSGroup/FJGroup/BQGroup, GSGroup/FJGroup/BQGroup; NEA/SEA, Mbuti) to examine intra-population variation in their affinity with SEA and NEA.

L191-195: Given the differential genetic affinities observed between the DWK and Shandong_EN populations, there may be variations in genetic ancestry within each of these groups. This needs to be thoroughly clarified, as the authors use an earlier population to assess the impacts of non-Shandong populations on a later population.

L209-212: I appreciate the authors providing detailed results from their f4-tests. However, the significant genetic affinities with the Yellow River populations appear sporadic and seem to depend heavily on the specific Shandong_EN and Yellow River populations chosen. Thus, the results do not convincingly demonstrate that the DWK populations have additional genetic affinity with the Yellow River populations compared to the Early Neolithic populations. There may be variation in genetic ancestry among the four Early Neolithic populations, among the Yellow River populations, and among the three DWK populations. Additionally, the variation could be due to low-coverage of data. The authors need to clarify these key questions before drawing any conclusions from this analysis.

L216-224: Why did the authors fix the source of the Early Neolithic to Xiaojingshan? What rationale supports this choice? What results would be obtained if other Early Neolithic populations were used in modelling? Additionally, could you model the FJGroup using a two-way admixture of the Early Neolithic and YR_LN, similar to the model used for the BQGroup? It is unclear why one of the DWK populations was modelled with another DWK population. I cannot see any evidence in Table S11 or the other results supporting a close genetic relationship between FJ and BQ.

L240-243: This extra genetic affinity is observable in the CZYGroup only when Xiaojingshan and YR_LN are used as P1 and P3. The other 15 combinations of P1 and P3 do not support a stronger genetic affinity between the CZYGroup and the Yellow River populations compared to Shandong_EN. It is unclear if this is due to low data coverage in the CZYGroup or if the CZYGroup is more genetically similar to Shandong_EN than to another Longshan population, YJC. In either case, the f4-tests do not seem to support this statement. There should be differences in genetic ancestry between CZY and YJC, although the admixture modelling presented in Figure 4 does not support this idea. All of these results are confusing, and the authors need to provide a clear explanation for these discrepancies.

L256-L261: If this was the authors' interpretation, I would consider that the Yellow River populations share a similar number of alleles with both Shandong_EN and the CZYGroup, as 15 out of the 16 f4-tests do not support any statistically significant

extra affinity between the Yellow River populations and the CZYGroup (see my previous comment). This lack of confidence in the interpretation arises from the weak statistical significance of the genetic link between the CZY and Yellow River populations. Even for the other Longshan population, YJC, how do the authors explain that their extra genetic affinity with the Yellow River populations is not observable when Boshan is used as P1?

L261-267: The results from qpAdm contradict those from the f4-tests, as the genetic affinity with the Yellow River populations differs significantly between the CZYGroup and YJCGroup (Table S8).

L285-288: I don't think the results support this conclusion. With 2 Longshan populations and 4 different Yellow River populations tested, there are 8 f4-tests for each of XZ and YX. However, only two tests for XZ and one for YX show significant affinity with specific Yellow River populations. There are more tests without statistical support for this relationship than those with support. The authors need to identify the sources of this variability before drawing any conclusions.

L338-340: To determine if this genetic affinity is truly unique to Nagabaka_historic, please test f4 with the forms (Kofun, 6000-1500 BP Shandong populations; Nagabaka_historic, Mbuti) and (Kofun, Nagabaka_historic; 6000-1500 BP Shandong populations, Mbuti). If any Shandong populations still show significant differences, the authors should model Nagabaka_historic as an admixture between Kofun and additional ancestry that exhibits significant affinities with Nagabaka_historic compared to Kofun in the f4-test.

2. When performing admixture modelling using qpAdm, please provide not only tail probabilities, which represent the fit of the tested model, but also nested p-values for competing models (e.g., between three-way and two-way admixture models or between two-way and single-ancestry models). These nested p-values are critical for quantitatively assessing model fit, helping to determine whether a two-way admixture sufficiently explains the genetic makeup of a target population or if a three-way admixture better fits the data. To calculate this p-value, you can use the following command in R: `1 - pchisq(chisq_model1 - chisq_model2, df = dof_model1 - dof_model2)`. Additionally, please include error bars in the bar plots presenting the qpAdm results, as this would make it easier to visualize the data compared to referencing the text and supplementary tables separately.

Additional comments that are linked to this second major concern:

L292-294: Please replace CZYGroup with BQGroup and GSGroup or with Xiaojingshan and Yellow River populations, and model the admixture in each of the CD populations. The authors modelled the genetic ancestry of a target population using an earlier population and additional ancestry, but all these sources trace their origins back to Shandong_EN and Yellow River ancestry, with varying proportions of each. This approach will clearly illustrate how Yellow River ancestry evolved over time.

L347: In the paper reporting the tri-ancestry structure of Japanese populations, WLR_BA_o and HMMH_MN were used to represent the northern inland population, as both show a stronger affinity with Amur River populations than with Yellow River populations. However, as demonstrated by Ning et al. (2020), the West Liao River populations exhibit heterogeneous genetic ancestry over time. Please use WLR_BA_o and HMMH_MN as the northern inland population in your modeling.

L349-353: Surprisingly, the genetic affinity between Nagabaka_historic and the CZYGroup is weaker than that between Nagabaka_historic and the YJCGroup. Considering this and the other points I've commented on, the genetic ancestry could differ between these two Longshan populations. Please conduct a more thorough analysis of any genetic heterogeneity within the different cultural populations in the Shandong region. Additionally, the authors should use the results from the f4-tests to screen potential sources for testing in qpAdm, as the current results are quite confusing. Furthermore, the authors should provide nested p-values for three-way versus two-way admixture models for Nagabaka_historic. This will help determine whether a two-way admixture model sufficiently explains the genetic ancestry of Nagabaka_historic or if a three-way admixture model offers a better explanation. This approach should also be applied to the northern inland populations represented by WLR_BA_o and HMMH_MN.

Minor comments:

I found it strange that supplementary tables are presented in descending order. Can you please correct this?

Table S7: I believe there is a typo in the label for P2. It should be "CZY" instead of "GZY."

L69: Please specify which study you are referring to here. It seems like it could be reference 10, but clarifying this will help avoid any confusion.

L71: "main island" should be plural.

L73: Reference 10 is not the only study that has demonstrated population stratification in the Japanese archipelago, particularly the differences between the Ryukyu and main islands. Please include additional references, such as Yamaguchi-Kabata et al. (2008; <https://doi.org/10.1016/j.ajhg.2008.08.019>), Okada et al. (2018; <https://doi.org/10.1038/s41467-018-03274-0>), and Sakaue et al. (2020; <https://doi.org/10.1038/s41467-020-15194-z>).

L78-80: Please cite a reference on the Neolithic Shandong data.

L162-165: In the ADMIXTURE plots (Fig. 2C), could you please add labels indicating which groups correspond to the different Shandong populations (i.e., Early Neolithic, DWK, LS, or CD)?

L168-170: Please clarify which results you are referring to that support the genetic affinity between the Early Neolithic and Amur River populations. Adding the appropriate citation to these results would provide clarity.

L360-363: Again, a simple way to prove this is to use f4(Kofun, 6000-1500 BP Shandong populations; Nagabaka_historic, Mbuti) and f4(Kofun, Nagabaka_historic; 6000-1500 BP Shandong populations, Mbuti).

L367-368: This dating should be revisited once the authors have addressed the other comments on admixture modelling of Nagabaka_historic.

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

The authors have addressed my comments well. I do not have further comments.

Reviewer #3

(Remarks to the Author)

I would like to express my appreciation to the authors for their efforts in addressing the comments I provided on the original manuscript. While my primary concerns regarding the f4 analyses have been largely resolved, I have additional comments on some of the points I previously raised. In particular, I remain entirely unconvinced by the proposed connection between the Shandong populations and Nagabaka_historic.

Firstly, I appreciate the authors' efforts in conducting the analyses I suggested in my original comments, specifically the f4-tests in the forms of: f4(Kofun, Shandong populations; Nagabaka_historic, Mbuti) and f4(Kofun, Nagabaka_historic; Shandong, Mbuti). However, based on the results of these tests, I do not find clear support for additional genetic affinities between the Shandong populations and Nagabaka_historic when compared to the Kofun.

As demonstrated in a previous study cited in this manuscript (Cooke et al. 2023), the genetic structure of Nagabaka_historic is best explained by the tripartite ancestry model used for the Kofun, which includes Jomon, Northeast Asian (represented by WLR_BA_o and HMMH_MN), and East Asian (represented by Han) ancestry, albeit in different proportions. Therefore, if there were indeed a significant genetic connection between the Shandong populations and Nagabaka_historic, I would expect the Kofun to serve as the most appropriate reference for identifying any additional connections between the Shandong and Nagabaka_historic, which is absent in the Kofun.

Because of this, while the authors have presented evidence suggesting differential affinities to Shandong populations between the Kofun and Nagabaka_historic, I find that the data presented do not convincingly establish a unique genetic connection between the Shandong populations and Nagabaka_historic. Upon closer examination of Table S10, these signals of genetic affinities appear to be somewhat inconsistent, varying depending on the specific Shandong populations used. This variability raises questions about the robustness of the results and the authors' interpretations.

Secondly, I again appreciate the authors' discussion of the estimated admixture timing in Nagabaka_historic (between 1600–1400 years BP) and its contextualization with the dynastic period in the Japanese archipelago (Lines 446-452 in the revised main text). However, it is crucial to emphasize that this timing corresponds to the Aceramic period in the Ryukyu Islands, including the site where the bones associated with Nagabaka_historic were excavated, before the dynastic period began. The cultural transitions in the Ryukyu Islands are distinct from those on mainland Japan. As Yamagiwa (2022) discusses in "Early human cultural and communal diversity in the Ryukyu Islands," the dynastic period (or Ryukyu Kingdom as phrased in the text) in the Ryukyus only began around the 11th or 12th century AD, much later than the period referenced in the manuscript.

Moreover, there is very limited—if not entirely absent—archaeological evidence supporting a direct connection between the Ryukyu Islands and China during the Aceramic period, which adds uncertainty to the genetic results and interpretations. This lack of archaeological corroboration weakens confidence in the analysis and the conclusions drawn in this context.

Given these concerns, I recommend that the authors reconsider the claims related to the gene flow from Shandong to the Ryukyu Islands unless more compelling evidence can be provided. Alternatively, removing these claims would help maintain the manuscript's focus the Shandong region where the data and story are stronger and more conclusive.

Minor comments:

- The claim regarding the lesser impact of YR in coastal DWK and a higher impact in inland DWK, as presented in Figure 4, requires further clarification. It is not clear where these different sites are located geographically, and given that there are only three groups within this period, it may be premature to draw strong conclusions about geographic variation. Specifically, the data indicate one inland group and two coastal groups. Even if this pattern holds, the data presented appear too limited to confidently support such a conclusion. I recommend that the authors either provide additional evidence to strengthen this claim or revise the argument to reflect the current limitations of the data.

- In Lines 480-486 of the rebuttal letter, the explanation raises a question: if a two-way admixture between GSGroup (one of the DWK populations) and YR is proposed, why do the qpAdm results not support this admixture for TLGroup and XZGroup? I suggest further clarification or additional evidence to explain this discrepancy, as relying only on DATES may not be sufficient without qpAdm support.

- Why did the authors present selected combinations of the tests by qpAdm in Table S5?

- In Lines 486-494 of the rebuttal letter, the authors successfully modeled additional gene flow from YR in the YXGroup, and given the similar DATES results for TLGroup and XZGroup, they suggest that this scenario could apply to those groups as well, despite qpAdm modeling not being successful. However, this conclusion may be somewhat optimistic without further evidence. To strengthen this argument, I recommend providing additional evidence that YXGroup is genetically similar to TLGroup and XZGroup, and exploring in more detail why the admixture scenario worked for YXGroup but not for the other two groups. This would help clarify whether the proposed conclusion is fully supported by the data.

- In Lines 290-306 of the main text, the qpAdm results do not support additional YR contribution for TLGroup and XZGroup. Given this, I would recommend caution in concluding that these groups have recent YR ancestry based on gene flow alone, as this could potentially weaken the robustness of your results. To strengthen the argument, it may be beneficial to either provide additional evidence for this gene flow or reconsider how this aspect is presented to ensure it aligns with the qpAdm findings.

Version 2:

Reviewer comments:

Reviewer #3

(Remarks to the Author)

I would like to thank the authors for their efforts in addressing the concerns I raised in my previous comments to the first round of revision. I appreciate the new simulation analysis, which enhances the validation of their results from the rotating qpAdm models. However, I was unable to fully evaluate the details of this simulation in the revised manuscript, as the methodology remains unexplained in the Materials & Methods section. I also sought this information in the papers cited in the rebuttal letter (Loosdrecht et al. 2018; Bai et al. 2024), but was again unable to find the necessary details. If the authors intend to retain this analysis in the manuscript, rather than just presenting it as their response, I recommend that the authors should include a thorough explanation of the methodology in the manuscript.

I remain unconvinced by the authors' response regarding the absence of archaeological evidence for the timing of admixture. While paleogenomic data can indeed offer valuable insights where historical records are sparse, it's crucial to recognise that the Aceramic period is marked not by a total absence of archaeological artifacts but specifically by the sudden disappearance of pottery use. Given that this period spans nearly 2,000 years, it raises significant questions about the extent to which any inferred genetic connections between the Southern Ryukyu Islands and the Shandong region can be supported without corresponding archaeological evidence.

One specific point I noticed is that the authors presented, in Table S5(b), a three-way admixture model, involving YR_LN, YJCGGroup (a Shandong population), and Jomon, that significantly better explains the genetic makeup of Nagabaka_historic than a two-way admixture model of YJCGGroup and Jomon. This is supported by a nested p-value of 0.04. This finding is noteworthy when compared with the Kofun genetic ancestry, which is sufficiently explained by a three-way admixture model involving YR_LN, AR/WLR, and Jomon. Therefore, the key difference between genetic ancestry of Kofun and Nagabaka_historic populations appears to lie in the origin of the second ancestry component—Shandong populations in one case versus AR/WLR in the other.

The primary evidence supporting a link between the Southern Ryukyu Islands and Shandong during the Aceramic period is the admixture timing estimated under the two-way admixture model of YJCGGroup and Jomon. However, given the potential limitations of this model, I am cautious about interpreting this result as strong evidence of a connection specifically during the Aceramic period.

Additionally, I would like to point out that the literature cited in the revised manuscript to support this connection does not appear to be relevant to the Aceramic period in the Ryukyu archipelago. Instead, these sources document interactions during the Ryukyu Kingdom, which began in the 14th-15th centuries AD, significantly later than the period estimated by the authors. If the authors wish to make a strong claim about a connection between these regions during the Aceramic period, it is essential to identify and cite literature that specifically addresses this era. The current references do not adequately support such a claim.

Overall, the claim of gene flow from the Shandong region to the Southern Ryukyu Islands around 1400-1600 BP would benefit from a more cautious framing. I recommend the following revisions to clarify the interpretations in the manuscript:

1. Emphasise the three-way admixture model: Highlight the three-way admixture model involving YR_LN, YJCGGroup, and Jomon, as this seems to most accurately represent the genetic profile of the Nagabaka_historic population based on the rotating qpAdm analysis.

2. Reconsider the use of DATES in the two-way admixture model: For Nagabaka_historic, please reconsider the inclusion of the DATES analysis under the two-way admixture model (YJCGGroup and Jomon). Since DATES is generally less suited for admixture models with three distinct ancestral sources, it may lead to less reliable results in this case.

3. Revise Figure 4B to depict potential connections more tentatively: Rather than specifying gene flow dating from Shandong to Nagabaka, consider using a dotted or dashed line to indicate a potential connection between the regions. This

will allow you to illustrate the possible relationship without implying a precise date, as the DATES results may be affected by model misspecification.

Version 3:

Reviewer comments:

Reviewer #3

(Remarks to the Author)

Thank you for addressing all the points raised during the previous round of the review process. I appreciate the thoroughness and clarity of your revisions. The changes made significantly enhance the quality and clarity of the manuscript. I have no further suggestions or concerns.

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REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Liu et al. East Asian Gene flow bridged by northern coastal populations over past 6000 years

In the presented manuscript, the authors provide a detailed analysis of the fine-scale population genetic history of East Asia. The analysis is novel with respect to the period of history and region/s under investigation. I don't have major complaints about the methodology or statistical design of the manuscript. That said, I do have several comments which I believe should be addressed in order to make the manuscript trackable, especially for those unfamiliar with the demographic history of East Asia such as myself.

Thank you for recognizing the design and statistical analysis of our study, and we appreciate your valuable suggestions on the details, which we have now revised them according to your suggestions.

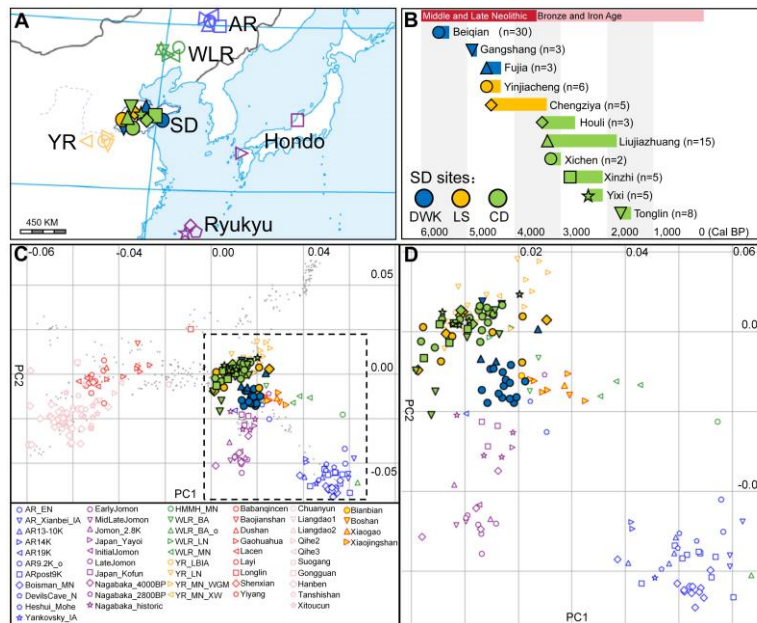
Minor Comments

– When describing historical periods (particularly for the first time) please include their age ranges i.e., the Kofun period, Yayoi period, DaWenKou cultural period, and Jin Dynasty, discussed throughout your manuscript.

Thank you for your comments. We have added chronological ranges for the cultures mentioned through the whole manuscript as “DaWenKou cultural period (~6,000-4,600 BP), LongShan cultural period (~4,600-4,000 BP), Jin Dynasty (~1,750-1,500 BP), Yayoi period (~2,300-1,750 BP), Kofun period (~1,750-1,400 BP)” when they firstly noted in this study. Modifications have been marked in blue in the text.

– When discussing a number of geographical landmarks, such as the Yellow River Basin and the Shandong region, it would increase the readability of your paper by referencing them in Figure 1. In general I found it cumbersome to follow.

Thank you for the suggestion on how to increase clarity. We added multiple labels to Figure 1A to improve the readability of our study, pointing out the Yellow River Basin (YR), the Shandong region (SD), and others.



– Similarly, placing the location of your newly sequenced genomes within the geographical context of published east Asian aDNA in a Figure would also greatly increase your manuscript readability for non-specialists.

Thanks for pointing how to improve our manuscript’s readability. Following your suggestion, we added the geographical locations of relevant published ancient East Asians in Figure 1A to provide a better overview of the data used in this study.

Line 69: The phrase, “In this study,” made it sound like the current manuscript. Change wording to more clearly reflect the authors of ref 10.

Thanks for your comments. We replaced the previous phrase with “In previously published studies” in Line 70.

Lines 141-143: Indicate which figures correspond to the analysis results described in text.

Thanks for your suggestion. We added the corresponding figure citations for the PCA (Fig. 1C-1D), Umap (Fig. S2A) and t-sne (Fig. S2B) analyses as “Using cluster analyses (PCA²⁷, Umap²⁸, t-sne²⁹), we found that the Shandong individuals are located near ancient and present-day northern East Asians, including previously sampled Early Neolithic Shandong populations (9,500-7,500 BP) (Fig. 1C-1D, Fig. S2A-2B)” in Lines 147-150. These modifications are marked in blue in the text.

Line 145: Specify that in PCA, “They formed clusters distinct from ...”

Thanks for your suggestion. We added “in the PCA” and completed the sentence as you suggested: “They formed clusters distinct from that observed for ancient humans from the Japanese archipelago, West Liao River, and Amur River regions in the PCA (Fig. 1C-1D)” in Lines 151-153. Modifications are marked in blue in the text.

Figures

Fig. 1. (A) It would be helpful to place the heading “Shandong sites” above the DWK, LS, CD headings just to reinforce that this is the region of analysis. For regions described in the text, i.e., “Yellow River Region” it would be helpful to have these indicated in the map. (C - D) it does not appear that the color and shapes match those shown in (A). For instance, the green star for YiXi in (A) is not present in the list of green shape/color legend in (C). If this is incorrect, then can the authors please make the correct correspondence more clear.

According to your suggestion to Figure 1A, we added a subheading to emphasize that the newly published individuals in this study are from populations in the Shandong region and to indicate important geographic landmarks (e.g., the Yellow River Basin); we added the heading “SD sites” in Figure 1B to reinforce that this is the region of analysis; We also apologize for not making it clear how the colors and shapes of the markers in Figure 1A-1B relate to their counterparts in Figure 1C-1D. We have now added to the figure notes of Figure 1C-1D as “Color and shape of markers for the new published ancient Shandong populations of this study are the same as in (A) and (B)”.

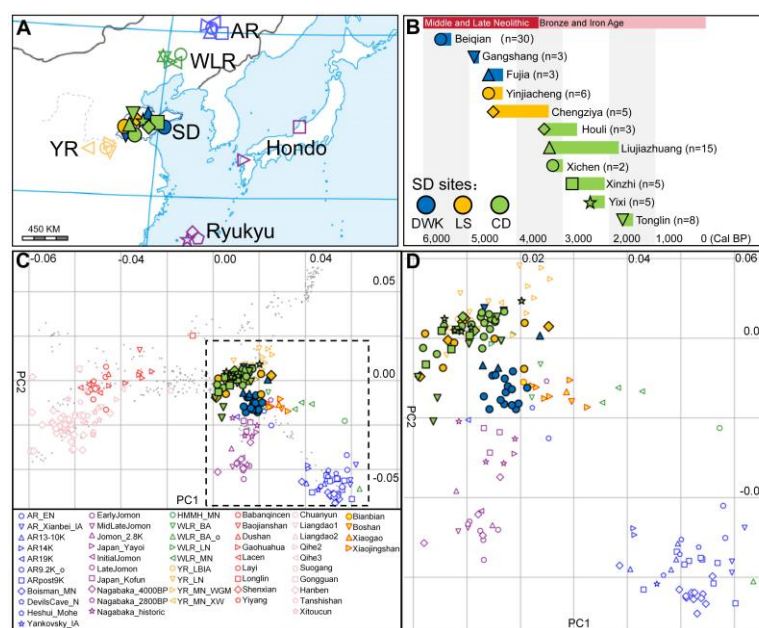
Reviewer #2 (Remarks to the Author):

Liu et al has newly generated and analysed whole genome data of 85 individuals dated to between 6000 and 1500 years ago. This study found that the genomic formation of ancient populations in Shandong has involved north-south gene flow from 7700 years ago; inland-coastal-island gene flow in East Asia since Neolithic time in Shandong. In particular, this study highlighted the historical gene flow from mainland East Asia (Shandong) to the Ryukyu Islands which is of interests to multiple disciplines. It is highly recommended for publication after revision. Find detailed comments below.

Thank you for recognizing the importance of this study, and we are also very grateful for your many important suggestions. Based on these suggestions, we have made changes to make our paper more readable and more rigorous.

1. It would be great to accurately define the Yellow River Basin, as the Yellow River runs through thousand kilometers in China, and mark Yellow River in the map of figure 1.

Thanks for your suggestion. We marked the Yellow River populations found in the Yellow River Basin in Figure 1A so that readers can locate the region more easily (now labeled as YR).



2. Line 107-108, “this dynamic period” → define ‘this’

Thanks for your suggestion. We apologize for not specifying a specific chronological range for this period, which we indicated in the text with the phrasing: “To study changes in coastal populations from the Shandong region during the dynamic period spanning from the early Neolithic period to the Jin Dynasty” in Lines 108-109. Modifications are marked in blue in the text.

3. Line 110, incomplete sentence “spanning a sixth of the coastline China”

Thanks for pointing out the grammatical error. We adjusted “spanning a sixth of the coastline China” as a subordinate clause after the sentence describing the Shandong region as “we collected 85 individuals from 11 sites dating from 6,000 to 1,500 BP from Shandong, which spans a sixth of the coastline of China” in Lines 111-112. Modifications are marked in blue in the text. Additionally, we reviewed the entire manuscript for additional grammatical errors, and hope we have caught them all.

4. Line 125, “contamination levels higher than 5% for uniparental markers”, why examine contamination level for uniparental markers?

Thanks for your question. Uniparental markers are informative of contamination levels of the sample. This is because uniparental markers are expected to be haploid, so identifying heterozygous sites are thus indicative of contamination. In this study, we estimated the contamination level of the data using X chromosomes (males) and mitochondrial genome (males and females), and used 5% as a threshold to distinguish potentially heavily contaminated samples. To clarify this, we have now rephrased the sentence: “We estimated the contamination level using X chromosomes (males) and mitochondrial genomes (males and females)^{23, 24}. For all sampled individuals but one of the 78 with at least 40,000 SNPs (TL4773_d_k) and two of the low-coverage individuals (BQ4625_d_low_k, YX4790_d_low), we estimated contamination levels lower than 5%” in Lines 126-130.

5. Line 164, “Early Neolithic and DaWenKou cultural period”. It would be good to clarify in general in the text on the period of DaWenKou/Shandong as DaWenKou and LongShan are part of Neolithic period in Shandong.

Thanks for your suggestion. To clarify that both the DaWenKou and LongShan cultures belong to the Neolithic period; we added the sentence: “Newly sampled Shandong populations were associated with one of three periods: the DaWenKou cultural period spanning the Neolithic period dating to 6,500 - 4,600 BP (Shandong_DWK), the LongShan cultural period spanning the Neolithic period dating to 4,600-4,000 BP (Shandong_LS), and the early Chinese dynastic period spanning 3,500-1,500 BP (Shandong_CD, Fig. 1A-1B)” in Lines 138-142. We also examined the remainder of the paper for similar confusing language, and revised appropriately. Modifications are marked in blue in the text.

6. Line 178, does the affinity with NEA contradict the affinity with SEA? What would be possible explanation of conclusion from both north and south directions “this costal region was impacted by populations from across mainland East Asia” at line 194-195.

Thanks for your comments. The affinity with NEA (aAR/FE) does not necessarily contradict the affinity with SEA (aSC), as both NEA and SEA populations can interact with Shandong populations, as observed in both (1) F4: the most f4 (Bianbian/Boshan/Xiaogao/SD9K/aYR/aLR/aSC, Xiaojingshan; aAR/FE, Mbuti) < 0 ($-16.1 < Z < 0.4$) and f4 (Bianbian/Boshan/Xiaogao/SD9K/aYR/aLR, Xiaojingshan; aSC, Mbuti) < 0 ($-7.9 < Z < 1.6$) and (2) qpAdm analyses: Xiaojingshan can only be modeled by a 3-way model with 74.2% ancestry related to Early Neolithic Shandong

ancestry related to Bianbian, Xiaogao, Boshan (SD9K), 9.8% ancestry related to Early Neolithic populations from Fujian (Fujian_EN), and 16.0% ancestry related to Amur River populations younger than 14,000 years ago (ARpost14K) (Table S5b). These evidences support that the Xiaojingshan individuals share connections to both NEA and SEA populations. We have now clarified the result: “In an f4-analysis assessing whether any Early Neolithic Shandong individuals share excess ancestry with other ancient East Asians, we observed that the 7,700 BP Xiaojingshan individuals share additional alleles with northern East Asians (aAR), i.e. most f4 (Bianbian/Boshan/Xiaogao/SD9K/aYR/aLR/aSC, Xiaojingshan; aAR/FE, Mbuti) < 0 ($-16.1 < Z < 0.4$), and southern East Asians (aSC), i.e. most f4 (Bianbian/Boshan/Xiaogao/SD9K/aYR/aLR, Xiaojingshan; aSC, Mbuti) < 0 ($-7.9 < Z < 1.6$) relative to older Shandong individuals dating to ~9,000 BP and other northern East Asians (except for AR9.2K_o, the individual shares some genetic affinity with ancient Shandong populations)” in Lines 181-190 and “Using rotational qpAdm strategy with to further parse the Xiaojingshan admixture, Xiaojingshan can only be modeled by a 3-way model with 74.2% ancestry related to Early Neolithic Shandong ancestry related to Bianbian, Xiaogao, Boshan (SD9K), 9.8% ancestry related to Early Neolithic populations from Fujian (Fujian_EN), and 16.0% ancestry related to Amur River populations younger than 14,000 years ago (ARpost14K) (Table S5), confirming that Xiaojingshan shows additional genetic influences from northern and southern East Asian ancestries outside of the Shandong region” in Lines 190-197. We also elaborated the related content in main text: “This suggests that as early as the Early Neolithic, there was already some interaction with northern and southern East Asian populations from other regions of East Asia” in Lines 197-199.

7. Line 225, “This observation suggests a decreased level of influence from Yangshao populations towards the coastline.” Not sure if this conclusion stands solidly using qpAdm proportion as the supporting evidence.

Thanks for your suggestion. We have now revised the related evidence to show the difference in Yellow River ancestry in DaWenKou populations that sampled distant or close to the coastal line. (1) In the PCA (Fig. 1C-1D), we observed that the three DaWenKou Shandong populations, from the relatively inland GangShang site (GSGroup) to the relatively coastal BeiQian (BQGroup) and FuJia (FJGroup) sites, are shifted to away from Early Neolithic Shandong populations (Shandong_EN), and toward Yangshao-related Yellow River (YR) populations. Specifically, the inland GSGroup cluster with YR populations, whereas the coastal BQGroup and FJGroup fall between the YR and Early Neolithic Shandong populations (Fig. 1C-1D) (2) the proportion calculated by qpAdm of YR ancestry in 6,000-4,600 BP Shandong populations vary, with the relatively inland GSGroup showing the highest levels of YR ancestry (~ 50 - 71%, Fig. 4A, Table S5), followed by the relatively coastal BQGroup (~13%, Fig. 4A, Table S5) and FJGroup (~0 - 13%, Fig. 4A, Table S5). (3) In the ADMIXTURE analysis, the GSGroup shows a higher proportion of YR component (orange) compared to other DaWenKou populations (Fig. 2C). We also revised these into main text as :

203 “In the PCA (Fig. 1C-1D), we observed that the three DaWenKou Shandong
204 populations, from the more inland GangShang site (GSGroup) to the more coastal
205 BeiQian (BQGroup) and FuJia (FJGroup) sites, are shifted away from the Early
206 Neolithic Shandong populations (Shandong_EN), and toward Yangshao-related
207 Yellow River (YR) populations. It can be observed that the three populations from the
208 DaWenKou period distributed along this axis (from Shandong_EN to YR) show
209 different affinities to YR populations. Specifically, the relatively inland GSGroup
210 cluster with YR populations, whereas the coastal BQGroup and FJGroup fall between
211 the YR and Early Neolithic Shandong populations (Fig. 1C-1D).

212
213 To further determine whether DaWenKou populations show additional YR ancestry
214 relative to Early Neolithic Shandong populations, we employed a rotational qpAdm
215 strategy to estimate ancestral components found in the DaWenKou populations. A total
216 of 11 representative East Asian populations (e.g. ARpost14K, aFujian_EN) were
217 rotated as potential source ancestries for the three DaWenKou groups (Table S5). Using
218 this strategy, only the population shows highest fitness to the model will be considered
219 as an ancestral source population (Tail_prob > 0.05, each ancestral proportion >
220 standard error, pnest < 0.05). With the rotational qpAdm strategy, we found that the
221 DaWenKou populations (BQGroup and GSGroup) can be modelled as a mixture
222 ancestry related to Early Neolithic Shandong populations (Shandong_EN, ~29 - 87%)
223 and YR populations (YR, ~13 - 71%), while FJGroup can be “1-way” modelled as
224 Shandong_EN (Tail_prob = 0.08) or BQGroup (Tail_prob = 0.47) (Fig. 4A, Table S5).

225
226 Consistent with previous observation that the three sampled DaWenKou population
227 shows different affinity to the YR populations, the proportion calculated by qpAdm of
228 YR ancestry in 6,000-4,600 BP Shandong populations vary, with the GSGroup showing
229 the highest levels of YR ancestry (~ 50 - 71%, Fig. 4A, Table S5), followed by
230 BQGroup (~13%, Fig. 4A, Table S5) and FJGroup (~0 - 13%, Fig. 4A, Table S5). In
231 an ADMIXTURE analysis, the GSGroup shows a higher proportion of YR component
232 (orange) compared to other DaWenKou populations (Fig. 2C). Collectively, these
233 patterns suggest that ancestry related to YR populations impacted populations in the
234 Shandong region during the DaWenKou cultural period. The inland geography of the
235 GSGroup relative to the coastal location of the BQGroup and FJGroup suggest that
236 such impacts decrease with proximity to the coast. ” in Lines 205-238.

237
238 8. Line 271, conclusion of “interaction within the Shandong region rather than
239 interactions with populations outside of the Shandong region”. I am not sure that I am
240 convinced by the presented evidence as the mentioned YR ancestry in Shandong is from
241 the Middle Yellow River zone (outside of Shandong, Ning et al 2020), which is the
242 ‘first wave of introgression from inland to coastal populations’ as far as I understand
243 (given at line 266, it is written that “the proportion of ancestry in the LongShan
244 Shandong populations that derived from Yellow River populations was estimated to
245 46-49%.”). Suggest the authors either add more evidence or rephrase.

Thanks for your comments. We have now explicitly provided supporting evidence for concluding the YR ancestry in LongShan populations is from previous introgression from YR to early Shandong populations instead of an additional introgression occurred recently. Particularly, while LongShan populations possesses YR-related genetic components (as suggested by ADMIXTURE, and affinity to YR populations can also observed in PCA), the Longshan population cannot be modelled directly by a mixture of Shandong and YR ancestries (no 2-way model can be passed using rotational qpAdm, Table S5). Instead, the LongShan population can only be modeled by a DaWenKou population (GangShang) possess high level of YR component (68-79%) and another Shandong population with low level (i.e., BeiQian, BQGroup) or without (i.e., Xiaojingshan) YR components. Also, the lack of additional connections to YR population of the LongShan population can be observed in f4 (Shandong_DWK, Shandong_LS; YR, Mbuti) ~ 0 ($-2.3 < Z < 3.3$, with only one “Z-value” > 3 when the Shandong_DWK was GSGroup, Table S6), further supporting this conclusion.

We have now clarified the abovementioned information as:

“We first found that the genetic influence of YR populations persisted in Shandong populations of the Late Neolithic who are associated with the LongShan cultural period (YJCGroup and CZYGroup). In a PCA, similar to the DaWenKou GSGroup, both YinJiaCheng and ChengZiYa individuals clustered with Yellow River individuals (Fig. 1C-1D). In an ADMIXTURE analysis, individuals from the YJCGroup and CZYGroup show a component related to inland YR populations, and the proportion of YR component in these two groups is within the range of that of the three DaWenKou populations (GSGroup, BQGroup, and FJGroup) (Fig. 2C). We then explored whether this YR components was introduced from additional introgression from YR populations using f4. We found that f4 (Shandong_DWK, Shandong_LS; YR, Mbuti) ~ 0 ($-2.3 < Z < 3.3$, with only one “Z-value” > 3 when the Shandong_DWK was GSGroup, Table S6), suggested that LongShan populations did not share more genetic connections with YR populations.

Using the rotational qpAdm analysis (Table S5), we found that both LongShan populations can only be modeled as a mixture of ancestry related to the GSGroup (68 - 79% YR) – the DaWenKou population with elevated YR ancestry – and another DaWenKou population (the BQGroup) or an Early Neolithic Shandong population (21-32%, Fig. 4A, Table S5).

Interestingly, our result suggests different patterns of LongShan admixture and the DaWenKou admixture. The LongShan admixture cannot be modeled using the “Shandong-related ancestors” and the “YR population”, but only as mixtures of the two Shandong ancestries, further suggesting a stronger continuity between the LongShan and the older populations. Overall, our results support LongShan Shandong populations maybe mixtures of the DaWenKou ancestors from both the more coastal and inland of Shandong region, with no additional influence from the YR populations.” in Lines 245-272.

9. Section “Shandong costal East Asians to Ryukyu”: this is a very intriguing finding! In Cooke et al 2021 paper, the authors modeled (~3000 year old) Yayoi as admixture between Jomon and WLR_BA_o/HMMH_MN/Lokomotiv_EN. Has the authors tested Shandong-related ancestry in Yayoi, or using Yayoi as the only basal Japanese source + the other ancient Shandong population when modelling ancient historical Japanese populations?

Thanks for your comments. We have tested Shandong-related ancestry in the Yayoi using F4 analyses, and no additional Shandong population ancestry components were found in the Japanese populations of the Yayoi period using f4-statistics such as f4(YR/WLR/AR, BQGroup; Yayoi, Mbuti) ~ 0 ($-2.4 > Z > 0.2$). Regarding the Shandong-ancestry in Yayoi, the data quality of the Yayoi (the SNPs = 27,874) is very poor compared to the Jomon individuals (the SNPs >650,000), which may lead to inaccuracy in the modeling, thus this population is excluded from the qpAdm analysis.

10. In general, the evidence for the alignment between cultural patterns and biological change presented in this manuscript is quite vague, though it was discussed at Line 413-414. Suggest rephrase Line 413-424

Thanks for your comments. We have now rephrased this part and focused on the changes in shifts in genetic structure over time instead of the alignment between cultural and biological patterns as: “The different patterns of gene flow that occurred during the DaWenKou, LongShan, and early dynastic cultural periods show that the history of how the genetic structure of the Shandong populations was formed between 6,000 and 1,500 BP” in Lines 418-420.

11. I would expect the authors could add more robust analyses regarding the conclusion of “NEA, SEA gene flow to Shandong before 7000 years ago” based on samples from Xiaojingshan, and related possible explanations in the Discussion part. There are only f-statistics supporting this conclusion, but exact ancestry modelling results are missing.

It is also north worthy that the data quality of newly reported Xiaojingshan samples is rather low, with no contamination information perhaps due to low data quality. Hence to make this conclusion more robust, it might be necessary to boost up the data quality of Xiaojingshan to make sure that the observation is robust. Or explain the used published Xiaojingshan data in a clearer way in the text.

Meanwhile, it would be great to see qpAdm models for Xiaojingshan, for instance, the authors could use previously published ancient East Asians before 7000 years ago as one of the ancestral sources, and report the exact proportions from NEA, SEA and any other possible ancestral sources in 7700BP Xiaojingshan.

Thanks for your comments. We have now added ancestry modelling result to further support this conclusion, as the reviewer suggested. Specifically, Xiaojingshan can be modelled as a mix of ancestries related to Shandong populations, SEA populations (aFujian_EN) and NEA populations (ARpost14K) in the qpAdm analysis (as we

discribed in the comments #6 by this reviewer, L149-182). We apologize for the ambiguity and added this in the text as “Using rotational qpAdm strategy with to further parse the Xiaojingshan admixture, Xiaojingshan can only be modeled by a 3-way model with 74.2% ancestry related to Early Neolithic Shandong ancestry related to Bianbian, Xiaogao, Boshan (SD9K), 9.8% ancestry related to Early Neolithic populations from Fujian (Fujian_EN), and 16.0% ancestry related to Amur River populations younger than 14,000 years ago (ARpost14K) (Table S5), confirming that Xiaojingshan shows additional genetic influences from northern and southern East Asian ancestries outside of the Shandong region” in Lines 190-197.

Regarding the data quality of Xiaojingshan, in this study, we included 3 Xiaojingshan individuals in addition to 3 previously published high-quality Xiaojingshan individuals, resulting in 6 individuals in total (~24,000 - 390,000 SNPs). As the reviewer suggested, the data yield of these three individuals are low. As all the Xiaojingshan individuals are genetically similar to each other, based on the PCA (Fig. 1C-D, 6 individuals cluster closely to each other) and ADMIXTURE (Fig. 2C, 6 individuals exhibited highly similar genetic structure) analyses, we combined the data of all 6 individuals as a population subgroup. By doing this, we increased the number of available SNPs (more than 75,000) for Xiaojingshan in both F4 analysis and qpAdm, without compromising the genetic characteristics of this population.

12. Regarding the genetic connection between Shandong and Ryukyu islands, are there any related linguistic evidence around this (e.g. Ryukyuan languages mentioned in Robbeets et al 2021)? Could the authors find or add related information about that regarding the newly discovered genetic connections versus linguistic connections, and explain if this gene flow has accompanied with the dispersal of agriculture as well?

Thanks for your comments. The ancestor of the Mongolic languages, according to Robbeets et. al 2021, occurred over Proto-Tungusic to the Amur–Ussuri–Khanka region, Proto-Koreanic to the Korean Peninsula and Proto-Japonic over Korea to the Japanese islands around ~5,485 BP, which is unlikely related to the gene flow between Shandong and Ryukyu islands identified in this study (~1,600 - 1,400 BP). In addition, it has been suggested the spread of agriculture to the Ryukyu islands occurred in the 10th and 11th centuries (Pellard 2021). While the timing is close to the gene flow dated here (~1,600 - 1,400 BP), we currently lack of archaeological evidence directly supporting cultural interactions (especially agricultural) between ancient Shandong and Ryukyu islanders. Therefore, we limited our discussion here to the trades and communications between East Asians and Ryukyu islanders, as historical records suggest political interactions between he Sui Dynasty and the Ryukyu Kingdom (such as “Sui Shu” from Sui Dynasty, “Chuzan-sefu” and “Chuzan-sekan” from the Ryukyu Kingdom).

Reviewer #3 (Remarks to the Author):

Lie et al. generated genome-wide data from 85 ancient individuals excavated from 11 sites across the Shandong region. These genomes were co-analyzed with other ancient and present-day datasets. The study identifies population interactions between inner and coastal East Asia and suggests a potential impact of coastal East Asians on the insular population of the Ryukyu Islands in Japan. The dataset has significant cross-disciplinary value for the broader research community, particularly for those working on East Asian genomics. However, I have several concerns regarding their analysis and interpretations.

Thank you for your acknowledgement of the research significance of this study and for your important suggestions on statistical significance, we have improved our analytical methodology and the chain of evidence for arguing our conclusions based on your comments to ensure more rigor and clarity in proving and presenting our conclusions.

1. My major concern is the over-interpretation of results without sufficient statistical confidence. The authors applied f4-tests to identify extra genetic affinities between a Shandong population (e.g. one of the DWK populations) and a Yellow River population relative to an earlier population from the Shandong region (e.g. one of the Early Neolithic populations). There are various combinations of Yellow River (4 populations) and earlier Shandong populations (3 for Shandong_EN; 3 for DaWenKou or DWK; 2 for LongShan or LS; 6 for Chinese dynasty or CD) used in the tests. However, the statistically significant signals are often sporadic; genetic affinity is evident only when a specific set of Yellow River and Shandong populations is used. This indicates either variability in the genetic affinity with Yellow River populations across contemporaneous Shandong populations or variation in the earlier populations. The only results that are convincing and correctly interpreted are the f4-tests presented in Table S6, which clearly demonstrate extra genetic affinities between post-agricultural ancient Japanese and ancient Shandong populations compared to pre-agricultural Japanese (i.e., hunter-gatherer Jomon), as most of the Shandong populations tested as P3 exhibit $Z < -3$. In this case, I fully agree with the author's assertion that these ancient Japanese could have some affinity with the Shandong populations.

Thanks for your comments. According to the reviewer's suggestion, we have now combined all the related evidence (including qpAdm, f4, Admixture, etc.) and re-examined the genetic history of Shandong population, instead of using F4 only. In the updated version, we have added supporting evidence to the main conclusions, especially those concerned by the reviewers (i.e., connections between (1)Shandong_EN and NEA(aAR/FE/aLR)/SEA(aSC); (2)DaWenKou and YR compared with Shandong_EN; (3)LongShan and YR; (4)early Chinese dynastic and YR; differences among (1)Shandong_EN; (2)DaWenKou; (3)LongShan; (4)early Chinese dynastic).

Overall, we maintain our main conclusions with additional evidence, and we combined section 2, 3, and 4 to provide a better overview of the genetic history of Shandong

populations, with a focus on population interactions between Shandong and inland populations in Lines 201-313.

Specifically, in section “**Two pulses of gene flow from inland to coastal populations in northern East Asia**”, we examined the connections between Shandong and YR populations using multiple analyses. As the reviewer suggested, f4 analysis is heavily depended on the selection of P1 and P3 populations, which may generate sporadic results and have limited resolution on closely related populations. Here, we instead employed a rotational qpAdm strategy, which can test the fitness of multiple ancestral candidates and identify the one that fit the best. In this study, we selected 11 representative East Asian populations as potential source ancestries to model the ancient Shandong populations to investigate the ancestries of different Shandong populations (tail_prob > 0.05, each ancestral proportion > standard error, pnest < 0.05) and their potential connections with YR populations (Table S5 and cite below here as Table R1).

Regarding the **DaWenKou populations (GSGroup, BQGroup, FJGroup)**, the rotational qpAdm results show that a proportion of the DaWenKou populations (BQGroup and GSGroup) can be modelled as a mixture ancestry related to Early Neolithic Shandong populations (Shandong_EN, ~29 - 87%) and YR populations (YR, ~13 - 71%), while the other DaWenKou population (FJGroup) can be “1-way” modelled as Shandong_EN (Tail_prob = 0.08) or BQGroup (Tail_prob = 0.47) (Fig. 4A, Table S5), suggesting varied YR components among the Shandong populations during DaWenKou period (6,000 - 4,600 BP). In addition, we observe that the population sampled toward the coastal line show a decrease in YR components compared to populations sampled away from the coastal line – the populations located close to coastal line possess ~0 - 13% of YR components (BQGroup and FJGroup), while the population located away from coastal line possess ~50 - 71% of YR components (Fig. 4A, Table S5). This observation likely reflects a decreased impacts of Yellow River populations toward costal line in Shandong region. We revised the related content in the main text Lines 202-238.

Regarding the **LongShan populations (CZYGroup, YJCGroup)**, the LongShan populations cannot be modelled directly by a mixture of Shandong and YR ancestries (no 2-way model can be passed using rotational qpAdm, Fig. 4A, Table S5), while can be instead modeled by a DaWenKou population (GSGroup) possess high level of YR component (~68 - 79%) and another Shandong population with low level (i.e., BQGroup) or without (i.e., Xiaojingshan) YR ancestry. Thus, the presence of YR components is a result of admixture of Shandong populations with YR components rather than direct introgression from YR popualtions. This observation is also consistent with F4 statistics, with $f_4(\text{Shandong_DWK}, \text{Shandong_LS}; \text{YR}, \text{Mbuti}) \sim 0$ ($-2.3 < Z < 3.3$, only one “Z-value” > 3 when the Shandong_DWK was GSGroup, which is likely due to the high level of YR conmpnents in GSGroup, Table S6). We revised the related content in the main text Lines 240-272.

Regarding **dynastic Shandong populations**, while all the sampled population possess a local Shandong component and a YR-related component (as can be observed in ADMIXTURE), the qpAdm modelling suggests three different population history among the sampled dynastic Shandong populations (Fig. 4A, Table S5). **First**, the dynastic HLGroup, LJZGroup, XCGroup can be 1-way modelled by LongShan CZYGroup (this populations contains YR component), likely represent populations descend directly from the earlier LongShan population. **Second**, the TLGroup and XZGroup can only be 1-way modelled by DaWenKou GSGroup, which contains 68 - 79% YR ancestry. As both the sampled LongShan populations (CZYGroup and YJCGroup) are distinct from the earlier DaWenKou populations, the 1-way modelling result can be from the genetic continuity of an unsampled LongShan population who genetically similar to the DaWenKou GSGroup, or a later introgression from YR that lead to incorporation of YR ancestry in these dynastic groups in a manner similar to the DaWenKou GSGroup. To explore which scenario shaped the genetics of these two dynastic groups (the TLGroup and XZGroup), we estimated the timing of introgression using DATES. We found that these two populations could be modeled as a mixture of Shandong populations older than 3,500 BP and YR population (YR_MN/YR_LN), with an estimated date of admixture around 4.6 to 18.9 generations prior to the dynastic period, or ~2,880 - 2,030 BP (Table S7), thus confirmed the YR components observed here is introduced recently instead of during or before the DaWenKou period (the first wave of gene flow from YR to Shandong populations before 6,000 BP, as discussed in the main text lines 205-243). **Third**, the YXGroup can be only modeled by a mixture of YR populations (~75 - 92%) and another population (e.g., ~25% CZYGroup, or ~9% SD9K) (Table S5), suggesting additional genetic influence from YR populations. Interestingly, the estimated dates of introgression of YR (YR_MN) components of this group is also around ~2,450 - 2,300 BP (~5.1 generations) (Table S7), which is contemporary to the introgression of YR components in the second dynastic groups (the TLGroup and XZGroup). Thus, we conclude that a second wave of gene flow from YR population occurred around ~2,880 - 2,030 BP, which impact the genetics of a proportion of dynastic Shandong population.

These qpAdm results are also consistent with observation in shifts in affinity to YR populations (PCA, Fig. 1C-1D) and proportions of YR components (ADMIXTURE, Fig. 2C) in Shandong populations over time. Similarly, Treemix (with 1,000 bootstrap trees) show that the Shandong and YR populations are located under the same branch, and that the Shandong population has gradually moved closer to the YR branch over time (Fig. 2B). We revised the related content in the main text Lines 274-306.

The related content is also revised in the main text. We reorganized the chain of evidence needed to argue for a conclusion that can be corroborated with each other and have overhauled this section in Lines 201-313.

	Target	Source1		Source2		Tail_prob	STD.ERR		
		Pop Name	pro	Pop Name	pro		err1	err2	pnest
DWK	BQGroup	Xiaojingshan	0.87	YR_LN	0.13	0.09	0.05	0.05	0.01
	GSGroup	Xiaojingshan	0.38	YR_MN	0.62	0.44	0.06	0.06	0.00
	GSGroup	Xiaojingshan	0.29	YR_LN	0.71	0.34	0.07	0.07	0.00
	GSGroup	SD9K	0.40	YR_LN	0.60	0.14	0.09	0.09	0.00
	GSGroup	SD9K	0.50	YR_MN	0.50	0.09	0.08	0.08	0.00
	FJGroup	BQGroup	1.00			0.47			
	FJGroup	SD9K	1.00			0.08			
LS	CZYGroup	GSGroup	0.87	ARpost14K	0.14	0.87	0.08	0.26	0.13
	CZYGroup	GSGroup	0.74	BQGroup	0.26	0.06	0.10	0.10	0.02
	YJCGroup	GSGroup	0.68	BQGroup	0.32	0.68	0.09	0.09	0.00
	YJCGroup	GSGroup	0.79	Xiaojingshan	0.21	0.56	0.08	0.08	0.02
	YJCGroup	GSGroup	0.79	SD9K	0.22	0.13	0.14	0.14	0.14
early dynastic period	HLGroup	CZYGroup	1.00			0.09			
	LJZGroup	CZYGroup	1.00			0.47			
	TLGroup	GSGroup	1.00			0.17			
	XCGroup	GSGroup	1.00			0.72			
	XCGroup	CZYGroup	1.00			0.15			
	XZGroup	GSGroup	1.00			0.24			
	YXGroup	YR_MN	0.87	ARpost14K	0.13	0.62	0.05	0.05	0.01
	YXGroup	YR_MN	0.75	CZYGroup	0.25	0.28	0.06	0.06	0.00
	YXGroup	YR_MN	0.84	WLR_BA	0.16	0.22	0.04	0.04	0.00
	YXGroup	YR_LN	0.82	CZYGroup	0.18	0.17	0.06	0.06	0.00
	YXGroup	YR_LN	0.90	ARpost14K	0.10	0.15	0.05	0.05	0.05
	YXGroup	YR_MN	0.80	YJCGroup	0.20	0.15	0.06	0.06	0.00
	YXGroup	YR_MN	0.87	Yumin	0.13	0.11	0.04	0.04	0.00
	YXGroup	YR_MN	0.85	SD9K	0.15	0.10	0.05	0.05	0.01
	YXGroup	YR_LN	0.91	SD9K	0.09	0.09	0.05	0.05	0.11
	YXGroup	YR_LN	0.92	aFujian_EN	0.08	0.07	0.04	0.04	0.04
	YXGroup	YR_LN	0.90	WLR_BA	0.10	0.07	0.04	0.04	0.02
	YXGroup	YR_LN	0.85	YJCGroup	0.15	0.07	0.06	0.06	0.01
	YXGroup	YR_MN	0.87	BQGroup	0.13	0.05	0.04	0.04	0.00
aJP	Nagabaka_2800BP	EarlyJomon	1.00			0.17			
	Nagabaka_2800BP	InitialJomon	1.00			0.42			
	Nagabaka_2800BP	LateJomon	1.00			0.62			
	Nagabaka_historic	YJCGroup	0.75	LateJomon	0.25	0.06	0.01	0.01	0.00
	Nagabaka_historic	CZYGroup	0.75	LateJomon	0.25	0.37	0.01	0.01	0.00

Table R1. Rotational qpAdm strategy

Additional comments that are linked to this first major concern:

1.1 L175-178: The results from the f4-tests do not seem to robustly support the connection between Xiaojingshan and non-Shandong populations. Firstly, the Z-scores are not consistently significant. Secondly, in most cases, statistical significance is only supported by either one of the P1 populations (Xiaogao or Boshan), rather than both. I recommend using a more stringent cut-off of $Z < -3$ or $Z > 3$ for statistical significance. If this stricter threshold is applied, the signals become even more sporadic. Overall, the results do not appear robust enough to support the proposed connection. Additionally, why are the signals of genetic affinities so different between the P1 populations? Please clarify the source of this variability, e.g. differences in their genetic ancestry between Xiaogao and Boshan.

Thanks for your comments. As mentioned above (see details in responses to comment #1 by this reviewer, L391-507), we have now updated the results with additional supporting evidence to investigate the connections between Xiaojingshan and non-Shandong populations. Specifically, we grouped Bianbian, Boshan, and Xiaogao as one group (SD9K) to represent early Neolithic Shandong population. This is because all three populations are genetically similar to each other and grouping them can compensate potential biases introduced by insufficient data (discussed in detail section regarding “variability among early Neolithic Shandong populations” below). Further,

we included additional East Asian populations in P1 of the f4-test to thoroughly explore the gene flow between Xiaojingshan and other East Asian populations in P3. As a result, significant negative Z values are obtained for the majority of the tested populations in the form of $f_4(\text{Bianbian/Boshan/Xiaogao/SD9K/aYR/aLR/aSC}, \text{Xiaojingshan}; \text{aAR/FE}, \text{Mbuti})$ ($-16.1 < Z < 0.4$) (Table S4, Table R2A). Similarly, additionally shared alleles between Xiaojingshan and SEA can be observed in $(\text{Bianbian/Boshan/Xiaogao/SD9K/aYR/aLR}, \text{Xiaojingshan}; \text{aSC}, \text{Mbuti}) < 0$ ($-7.9 < Z < 1.6$) (Table S4, Table R2B).

While the above F4 results may suggest genetic connections between Xiaojingshan and NEA and SEA populations, we further validated this hypothesis using rotational qpAdm. Specifically, we used rotational qpAdm to screen for the most appropriate ancestral source populations of Xiaojingshan. We found that Xiaojingshan can only be modeled by a 3-way model with 74.2% ancestry related to Early Neolithic Shandong ancestry related to Bianbian, Xiaogao, Boshan (SD9K), 9.8% ancestry related to Early Neolithic populations from Fujian (Fujian_EN), and 16.0% ancestry related to Amur River populations younger than 14,000 years ago (ARpost14K), confirming the connections between Xiaojingshan and NEA and SEA populations.

We have now revised this section as “In an f4-analysis assessing whether any Early Neolithic Shandong individuals share excess ancestry with other ancient East Asians, we observed that the 7,700 BP Xiaojingshan individuals share additional alleles with northern East Asians (aAR), i.e. most $f_4(\text{Bianbian/Boshan/Xiaogao/SD9K/aYR/aLR/aSC}, \text{Xiaojingshan}; \text{aAR/FE}, \text{Mbuti}) < 0$ ($-16.1 < Z < 0.4$, Table S4a), and southern East Asians (aSC), i.e. most $f_4(\text{Bianbian/Boshan/Xiaogao/SD9K/aYR/aLR}, \text{Xiaojingshan}; \text{aSC}, \text{Mbuti}) < 0$ ($-7.9 < Z < 1.6$, Table S4b) relative to older Shandong individuals dating to ~9,000 BP and other northern East Asians (except for AR9.2K_o, the individual shares some genetic affinity with ancient Shandong populations). Using rotational qpAdm strategy with to further parse the Xiaojingshan admixture, Xiaojingshan can only be modeled by a 3-way model with 74.2% ancestry related to Early Neolithic Shandong ancestry related to Bianbian, Xiaogao, Boshan (SD9K), 9.8% ancestry related to Early Neolithic populations from Fujian (Fujian_EN), and 16.0% ancestry related to Amur River populations younger than 14,000 years ago (ARpost14K) (Table S5), confirming that Xiaojingshan shows additional genetic influences from northern and southern East Asian ancestries outside of the Shandong region. This suggests that as early as the Early Neolithic, there was already some interaction with northern and southern East Asian populations from other regions of East Asia.” in Lines 181-199.

		aAmurRiver					
		AR19K	AR14K	AR13-10K	ARpost9K	AR9.2K_o	Yankovsky_IA
YellowRiver	YR_MN_W	-3.2	-7.8	-7.9	-8.4	-5.1	-3.7
	YR_MN_XV	-1.0	-7.4	-6.6	-7.8	-4.4	-3.3
	YR_LN	-3.6	-8.6	-9.9	-10.1	-5.4	-4.1
	YR_LBIA	-2.6	-9.3	-10.4	-10.9	-6.0	-4.3
aShandong	Bianbian	0.8	-2.0	-0.2	-0.8	0.2	0.3
	Xiaogao	-0.4	-3.3	-3.3	-3.7	-3.9	-0.9
	Boshan	1.7	-2.2	-3.0	-1.8	-2.6	0.4
	SD9K	0.6	-3.8	-3.8	-3.3	-3.4	-0.4
aLiaoRiver	WLR_MN	-1.5	-3.5	-3.5	-3.8	-4.2	-0.8
	WLR_LN	-2.6	-7.9	-8.4	-8.1	-4.8	-2.8
	WLR_BA	-1.5	-4.7	-3.9	-3.6	-2.7	-0.3
	WLR_BA_o	0.7	-0.2	1.5	3.6	-2.3	3.5
SouthChina	HMMH_MN	-2.5	-3.6	-3.2	-2.4	-3.0	-0.8
	Qihe	-4.1	-6.3	-8.3	-8.5	-2.3	-6.1
	Qihe3	-4.4	-10.9	-9.6	-11.5	-5.0	-7.9
	Liangdao1	-2.2	-7.7	-8.8	-10.3	-3.8	-6.3
	Liangdao2	-3.2	-10.0	-10.5	-12.2	-7.3	-7.4
	Suogang	-1.5	-2.1	-3.4	-3.4	-0.7	-2.5
	Xitoucun	-1.1	-4.0	-5.8	-6.4	-2.6	-4.0
	Tanshishan	-1.0	-5.0	-4.4	-6.5	-3.3	-5.5
	Chuanynun	0.5	-0.8	-1.6	-1.6	-0.9	-0.3
	Taiwan_Ha	-3.6	-11.5	-13.2	-14.8	-5.6	-6.7
	Taiwan_Go	-1.3	-5.7	-5.9	-6.3	-3.0	-4.3
	Longlin	-6.3	-8.4	-10.5	-12.5	-3.4	-7.8
	Baojianshan	-8.2	-12.5	-14.7	-16.1	-7.8	-10.7
	Dushan	-3.9	-9.9	-11.3	-11.7	-3.9	-8.5
	LaCen	-1.3	-7.1	-7.9	-7.9	-4.3	-5.7
	GaoHuaHu	-2.8	-6.9	-7.0	-7.3	-2.6	-3.9
	BaBanQinC	-2.5	-6.2	-9.8	-8.8	-2.9	-4.0
	Shenxian	-1.6	-6.9	-6.5	-8.7	-3.9	-4.8
	Layi	-1.8	-4.9	-6.5	-6.0	-3.6	-3.5
	Yiyang	-1.1	-3.7	-3.1	-2.7	-0.7	-1.9

Table R2A. f4 (Bianbian/Boshan/Xiaogao/SD9K/aYR/aLR/aSC, Xiaojingshan; aAR/FE, Mbuti)

		aSouthChina																		
		Qihc	Qihc3	Liangdao1	Liangdao2	Suogang	Xitoucun	Tanshishan	Chuanyun	Taiwan_Hanben	Taiwan_Gongguan	Longlin	Baojianshan	Dushan	LaCen	GaoHuaHua	BaBanQinCen	Shenxian	Layi	Yiyang
YellowRiver	YR_MN_WGM	-3.6	-2.7	-3.5	-2.1	-2.7	-4.3	-3.4	-1.3	-2.5	0.4	-1.8	-2.1	-2.9	-1.9	-3.1	-5.0	-2.5	-1.1	-1.7
	YR_MN_XW	-3.2	-1.4	-2.1	-2.0	-2.6	-3.0	-1.8	-1.9	-0.7	0.4	-0.5	-3.0	-2.5	-1.3	-2.3	-3.2	-1.5	-0.9	-3.4
	YR_LN	-4.5	-3.2	-3.3	-2.6	-2.8	-4.3	-2.6	-0.3	0.3	1.4	-1.4	-2.3	-3.8	-0.9	-3.0	-3.4	-0.9	-0.8	-1.7
	YR_LBIA	-4.6	-2.5	-3.8	-2.1	-2.5	-4.0	-3.2	-0.3	-0.7	1.6	-1.1	-2.6	-3.8	-0.5	-2.1	-3.2	-1.0	-0.7	-1.6
aShandong	Bianbian	-0.2	0.5	0.6	-0.5	0.1	1.1	0.9	-0.2	0.3	1.3	1.5	-0.8	-0.7	0.7	-1.7	-0.5	1.2	-0.7	-1.5
	Xiaogao	-2.5	-1.2	-1.3	-2.1	-2.5	-2.1	-1.9	-1.0	-2.5	-1.1	-0.6	-2.6	-1.7	-2.5	-3.0	-3.4	-2.6	-1.9	-0.5
	Boshan	-3.2	-1.8	-0.9	-2.0	-1.9	-3.7	-1.8	-0.6	-0.7	0.2	-1.0	-0.7	-1.2	-0.5	-2.8	-2.1	-0.9	-0.9	0.3
	SD9K	-3.6	-1.7	-1.4	-2.4	-2.1	-3.2	-1.7	-0.8	-1.8	-0.4	-0.6	-2.2	-1.8	-1.5	-3.5	-3.4	-1.8	-1.8	-0.5
aLiaoRiver	WLR_MN	-4.4	-4.1	-3.6	-3.6	-3.4	-5.5	-3.8	-1.6	-3.5	-0.5	-1.8	-2.6	-3.1	-3.3	-5.2	-5.1	-3.7	-3.8	-2.8
	WLR_LN	-4.6	-2.9	-3.2	-2.5	-2.2	-4.2	-3.3	-1.2	-1.7	0.3	-1.1	-2.4	-2.4	-1.5	-3.1	-2.3	-1.8	-1.3	-1.5
	WLR_BA	-3.9	-1.7	-2.8	-2.5	-1.8	-3.1	-3.0	-0.7	-1.4	-0.4	-2.7	-2.5	-3.2	-1.0	-2.0	-2.4	-1.1	-0.2	0.6
	WLR_BA_o	-4.0	-2.7	-4.1	-2.8	-3.0	-6.2	-5.1	-1.6	-4.6	-1.7	-1.2	-1.5	-2.2	-4.0	-4.2	-3.6	-2.1	-2.1	-2.8
aAmurRive	HMMH_MN	-4.4	-3.7	-3.9	-4.2	-3.5	-6.9	-5.9	-4.1	-7.2	-4.2	-2.6	-3.9	-4.0	-3.6	-6.2	-8.1	-3.8	-2.7	-4.3
	AR19K	-3.6	-1.7	-1.3	-0.8	-2.5	-3.1	-0.4	-2.6	-3.8	-1.4	-0.8	-2.1	-1.6	-1.4	-4.6	-4.0	-2.3	-2.0	-3.2
	Boisman_MN	-4.1	-4.2	-3.8	-2.8	-3.2	-5.4	-4.5	-3.0	-4.9	-1.7	-1.9	-2.4	-4.3	-4.1	-5.8	-5.9	-3.5	-1.8	-3.2
	DevilsCave_N	-4.4	-4.6	-5.5	-3.6	-2.3	-7.2	-5.5	-3.0	-5.8	-2.4	-0.7	-3.9	-5.0	-3.5	-6.8	-7.9	-3.5	-4.0	-4.1
	AR14K	-1.4	-2.9	-2.5	-2.4	-0.3	-0.3	-1.0	-1.0	-4.0	-0.8	0.1	0.2	-1.8	-2.1	-3.4	-2.5	-2.6	-1.5	-1.5
	AR13-10K	-2.0	-1.1	-2.5	-1.5	-3.0	-1.5	-0.1	-1.5	-4.6	0.0	0.9	-0.1	-2.0	-1.0	-3.3	-4.5	-0.9	-2.4	-0.2
	ARpost9K	-1.6	-1.7	-2.9	-2.1	-1.1	-1.7	-1.2	-1.4	-5.5	-0.7	-0.6	-0.9	-1.2	-1.6	-3.7	-3.8	-2.1	-1.7	-1.3
	AR9.2K_o	0.3	0.2	0.1	-2.3	0.2	0.0	-1.4	-1.7	-1.8	-0.4	2.8	-1.4	-0.4	-0.8	-1.7	-1.0	-2.0	-2.0	0.8
	Yankovskv_IA	-1.7	-1.9	-1.7	-0.6	-1.8	-1.1	-2.5	-0.6	-0.4	-0.4	0.0	0.1	-1.4	-1.0	-1.1	-1.2	-1.0	-1.0	-0.5

Table R2B. f4 (Bianbian/Boshan/Xiaogao/SD9K/aYR/aLR, Xiaojingshan; aSC, Mbuti)

According to the reviewer's suggestion, we also explored the source of the variability among early Neolithic Shandong populations (SD9K) (Table R3). We found that Boshan shares more alleles with populations from Yellow River than Xiaogao in f4(Boshan, Xiaogao; YR/WLR_LN, Mbuti) >0, 1.5 > Z > 3.0, which may result from (A) additional genetic connections between Boshan and YR population comparing to

Xiaogao, (B) less genetic connections between Xiaogao and YR population comparing to Boshan, or (C) individual-level variability. We then tests these scenarios using F4. We found neither additional genetic connection between the Boshan population and the Yellow River Basin population (f4(Bianbian/Xiaojingshan, Boshan; YR/WLR_LN, Mbuti) ~0), nor additional genetic connections between Early Shandong populations and YR populations comparing to Xiaogao (f4(Bianbian/Xiaojingshan, Xiaogao; YR/WLR_LN, Mbuti) ~0). Therefore, we reason the observed genetic variability among early Neolithic Shandong populations are resulted from individual-level difference, as all three populations (Bianbian, Boshan, and Xiaogao) only include 1 individual and cannot capture the within population variability. Therefore, we combined these three populations into one group, SD9K, and included in related analyses.

		aYellowRiver					aLiaoRiver					aAmurRiver/FE										
		YR_MN_WGM	YR_MN_XIW	YR_LN	YR_LBIA	WLR_MN	WLR_LN	WLR_BA	WLR_BA_o	HMMH_MN	AR19K	AR_EN	Boisman_MN	DevilsCave_N	AR14K	AR13-10K	ARpost9K	AR9_2K_o	Yankovsky_IA	AR_Xianbei_IA	Heshui_Mohe	
P2: Bianbian	Xiaogao	-0.7	-0.5	-0.3	-0.7	0.2	-0.8	-0.8	0.0	-0.7	-0.3	-0.5	0.2	-0.5	0.0	-1.6	-1.3	-3.0	0.2	1.8	-0.3	
	Boshan	0.8	0.1	1.0	0.9	0.3	1.4	-1.4	-0.2	0.6	0.4	-0.8	1.5	1.6	0.6	-1.1	-0.7	-1.6	0.8	1.0	0.7	
	Xiaojingshan	-1.2	-1.5	-0.8	-0.9	-1.3	-0.1	-1.4	-0.7	0.0	-0.8	-2.0	-0.4	-0.7	2.0	0.2	0.8	-0.2	-0.3	0.5	1.1	
P2: Xiaogao	Bianbian	0.7	0.5	0.3	0.7	-0.2	0.8	0.8	0.0	0.7	0.3	0.5	-0.2	0.5	0.0	1.6	1.3	3.0	-0.2	-1.8	0.3	
	Boshan	2.7	1.5	2.6	3.0	0.4	3.1	0.3	0.8	1.8	1.9	1.5	2.3	2.3	1.2	1.3	1.6	0.9	1.9	0.7	0.6	
	Xiaojingshan	0.7	0.6	0.3	0.9	-0.5	1.7	0.9	0.4	1.8	0.4	-0.9	0.5	0.0	3.3	3.3	3.7	3.9	0.9	-0.6	1.6	
P2: Boshan	Bianbian	-0.8	-0.1	-1.0	-0.9	-0.3	-1.4	1.4	0.2	-0.6	-0.4	0.8	-1.5	-1.6	-0.6	1.1	0.7	1.6	-0.8	-1.0	-0.7	
	Xiaogao	-2.7	-1.5	-2.6	-3.0	-0.4	-3.1	-0.3	-0.8	-1.8	-1.9	-1.5	-2.3	-2.3	-1.2	-1.3	-1.6	-0.9	-1.9	-0.7	-0.6	
	Xiaojingshan	-1.5	-0.8	-2.0	-1.6	-0.6	-1.1	0.5	0.2	0.2	-1.7	-2.5	-1.9	-2.7	2.2	3.0	1.8	2.6	-0.4	-1.2	0.4	
		aSouthChina																				
		Qihc	Qihc3	Liangdao1	Liangdao2	Suogang	Xitoucun	Tanshishan	Chunayun	Taiwan_Hanben	Taiwan_Gongguan	Longlin	Baojianshan	Dushan	LaCen	GaoHuaHua	BaBanQinCen	Shenxian	Layi	Yiyang		
P2: Bianbian	Xiaogao	0.5	-0.4	-1.2	-0.7	-2.3	-0.8	-1.0	-0.5	-1.2	-1.1	-0.8	-1.2	-0.9	-2.2	-1.1	-0.9	-1.7	-0.5	1.3		
	Boshan	-1.3	-1.7	-0.6	-0.4	-1.1	-2.5	-2.3	-0.1	-0.1	-0.1	-1.2	0.1	-0.1	-0.3	-1.0	-0.2	-0.7	-0.3	1.3		
	Xiaojingshan	0.2	-0.5	-0.6	0.5	-0.1	-1.1	-0.9	0.2	-0.3	-1.3	-1.5	0.8	0.7	-0.7	1.7	0.5	-1.2	0.7	1.5		
P2: Xiaogao	Bianbian	-0.5	0.4	1.2	0.7	2.3	0.8	1.0	0.5	1.2	1.1	0.8	1.2	0.9	2.2	1.1	0.9	1.7	0.5	-1.3		
	Boshan	-0.3	0.1	1.0	0.1	0.7	-1.5	0.0	0.1	2.0	1.2	-0.7	1.4	0.4	1.5	0.4	1.5	1.8	0.9	0.5		
	Xiaojingshan	2.5	1.2	1.3	2.1	2.5	2.1	1.9	1.0	2.5	1.1	0.6	2.6	1.7	2.5	3.0	3.4	2.6	1.9	0.5		
P2: Boshan	Bianbian	1.3	1.7	0.6	0.4	1.1	2.5	2.3	0.1	0.1	0.1	1.2	-0.1	0.1	0.3	1.0	0.2	0.7	0.3	-1.3		
	Xiaogao	0.3	-0.1	-1.0	-0.1	-0.7	1.5	0.0	-0.1	-2.0	-1.2	0.7	-1.4	-0.4	-1.5	-0.4	-1.5	-1.8	-0.9	-0.5		
	Xiaojingshan	3.2	1.8	0.9	2.0	1.9	3.7	1.8	0.6	0.7	-0.2	1.0	0.7	1.2	0.5	2.8	2.1	0.9	0.9	-0.3		

Table R3. f4(Bianbian/Boshan/Xiaogao/Xiaojingshan, Bianbian/Boshan/Xiaogao/Xiaojingshan; aYR/aLR/aAR/FE/aSC, Mbuti)

1.2 L179-183: In Table S11, Xiaojingshan and BQ should be included in the form of f4-tests in the title of this table. Additionally, why is Bianbian not included in this analysis despite being included in other f4 tests (e.g., Table S8)? The genetic affinity of DWK with SEA varies significantly among the three DWK populations. For each DWK population, 3 Shandong_EN and 28 SEA and NEA populations were tested as P1 and P3 in the form of f4(P1, DWK; P3, Mbuti), resulting in a total of 84 f4-tests. Only two tests show significant affinities between FJ and SEA, in particular when Xiaogao was used as P1. There are more significant signals from the other two DWK populations, GS and BQ; however, these signals are not consistent across the three Shandong_EN. In the case of the BQGroup, there are significant signals from nearly half of the Amur River populations (NEA) tested in f4(Boshan/Xiaogao, BQGroup; Y,

Mbuti). The connection between DWK and SEA suggested by the authors strongly depends on the choices of P1 and P3, which raises concerns about its robustness.

Thanks for your comments. We have now updated F4 results and included related information in Table R3, as discussed in detail above (in comments #1.1 by this reviewer, L510-591). We have also now included Bianbian in the analyses (Table S4). As the reviewer suggested, the genetic affinity of DWK with SEA are vary and inconsistent, and thus cannot support this conclusion. Therefore, we removed related discussion and instead only discuss the connections between Xiaojingshan and NEA and SEA.

1.3 L186-191: Before testing any differences in the genetic affinity between Shandong_EN and DWK, please run f4 analyses using the forms (Boshan/Xiaogao/Xiaojingshan, Boshan/Xiaogao/Xiaojingshan; SEA/NEA, Mbuti) and (GSGGroup/FJGroup/BQGroup, GSGGroup/FJGroup/BQGroup; NEA/SEA, Mbuti) to examine intra-population variation in their affinity with SEA and NEA.

Thanks for your comments. In the previous response of comments 1.1, we discussed the differences among the Shandong_EN (Bianbian, Boshan, Xiaogao, Xiaojingshan). Here we added the F4 analysis (f4 (GSGGroup/FJGroup/BQGroup, GSGGroup/FJGroup/BQGroup; aYR/aLR/aAR/FE/aSC, Mbuti), in Table R4) to test differences among the DWK populations and found that BQ shared more alleles with AR populations than GS in f4 (BQ, GS; aAR, Mbuti) > 0, -0.1 < Z < 5.2, and GS shared more alleles with YR populations than BQ in f4 (BQ, GS; aYR, Mbuti) < 0, -3.0 < Z < -1.0.

		aYellowRiver										aLiaoRiver										aAmurRiver/FE										aSouthChina									
		YR_MN_WGM	YR_MN_XW	YR_LN	YR_LHA	WLR_MN	WLR_LN	WLR_BA	WLR_BA_o	HMMH_MN	AR19K	AR_EN	Boisman_MN	DevilsCave_N	AR14K	AR13_10K	ARpost9K	AR20_2K_o	Yankovsky_LA	AR_Xianbei_LA	Heshui_Mote	Qibei	Qibei3	Liangdao1	Liangdao2	Shogang	Xitoutan	Tanshishan	Chunyun	Taiwan_Hanlun	Taiwan_Gongguan	Longlin	Dushan	LaCen	GaohuHua	BaBanQuCen	Shexian	Layi	Yuyang		
P2: BQ	FJGroup	-0.1	-0.7	0.4	0.5	0.2	0.1	0.5	-1.1	0.4	0.5	-0.3	-1.5	-0.3	-0.2	-1.3	-1.4	0.1	-1.5	-0.6	0.6	-1.4	0.3	0.3	-1.0	0.1	-0.6	0.1	-0.1	-0.4	-0.9	0.7	-1.1	-1.3	0.1	-1.6	-0.5	0.1	-0.8	0.0	
	GSGroup	3.0	1.0	1.1	1.3	-0.8	1.7	-0.2	-2.0	-0.1	0.1	-1.3	-3.2	-0.9	-4.2	-5.2	-4.4	-1.8	-3.1	-0.9	-0.6	0.7	2.4	-1.2	-0.3	1.0	0.4	1.7	0.1	-0.5	-0.3	-1.3	-0.2	1.1	0.1	0.8	-1.2	2.7	2.1	0.2	
P2: GS	FJGroup	0.7	-0.7	1.7	0.7	1.0	0.7	0.6	0.9	1.6	0.0	0.8	1.1	1.8	2.0	1.5	2.7	1.0	1.5	-1.0	1.3	-0.4	0.0	1.4	-0.2	-0.7	0.5	0.9	1.0	1.1	1.2	2.2	0.1	-0.1	1.4	-0.7	1.2	-0.7	0.7	1.3	
	BQGroup	-3.0	-1.0	-1.1	-1.3	0.8	-1.7	0.2	2.0	0.1	-0.1	1.3	3.2	0.9	4.2	5.2	4.4	1.8	3.1	0.9	0.6	-0.7	-2.4	1.2	0.3	-1.0	-0.4	-1.7	-0.1	0.5	0.3	1.3	0.2	-1.1	-0.1	-0.8	1.2	-2.7	-2.1	-0.2	
P2: FJ	BQGroup	0.1	0.7	-0.4	-0.5	-0.2	-0.2	-0.5	1.1	-0.4	-0.5	0.3	1.5	0.3	0.2	1.3	1.4	-0.1	1.5	0.6	-0.6	1.4	-0.3	-0.3	1.0	-0.1	0.6	-0.1	0.1	0.4	0.9	-0.7	1.1	1.3	-0.1	1.6	0.5	-0.1	0.8	0.0	
	GSGroup	-0.7	0.7	-1.7	-0.7	-1.0	-0.7	-0.6	-0.9	-1.6	0.0	-0.8	-1.1	-1.8	-2.0	-1.5	-2.7	-1.0	-1.5	1.0	-1.3	0.4	0.0	-1.4	0.2	0.7	-0.5	-0.9	-1.0	-1.1	-1.2	-2.2	-0.1	0.1	-1.4	0.7	-1.2	0.7	0.7	-1.3	

Table R4. f4(GSGGroup/FJGroup/BQGroup, GSGGroup/FJGroup/BQGroup; aYR/aLR/aAR/FE/aSC, Mbuti)

However, not all of the results from the F4 analysis were significant, so we instead employed additional analyses to explore the differences among the DWK populations. Using the rotational qpAdm strategy, we found that the DaWenKou populations (BQGroup and GSGGroup) can be modelled as a mixture ancestry related to Early Neolithic Shandong populations (Shandong_EN, ~29 - 87%) and YR populations (YR, ~13 - 71%), while FJGroup can be “1-way” modelled as Shandong_EN (Tail_prob = 0.08) or BQGroup (Tail_prob = 0.47) (Table S5). Consistent with previous observation that the three sampled DaWenKou population shows different affinity to the YR populations, the proportion calculated by qpAdm of YR ancestry in 6,000-4,600 BP Shandong populations vary, with the GSGGroup showing the highest levels of YR ancestry (~50 - 71%, Fig. 4A, Table S5), followed by BQGroup (~13%, Fig. 4A, Table

S5) and FJGroup (~0 - 13%, Fig. 4A, Table S5). Similar patterns can be also observed in ADMIXTURE analysis, with the GSGroup shows a higher proportion of YR component (orange) compared to other DaWenKou populations (Fig. 2C). We mainly discussed these differences among DWK populations in Lines 245-277.

1.4 L191-195: Given the differential genetic affinities observed between the DWK and Shandong_EN populations, there may be variations in genetic ancestry within each of these groups. This needs to be thoroughly clarified, as the authors use an earlier population to assess the impacts of non-Shandong populations on a later population.

Thanks for your comments. We have discussed the variations in genetic ancestry within each of Shandong populations (Shandong_EN, Shandong_DWK populations) in the comments of #1.1, L510-591, and #1.3, L615-646, by this reviewer.

1.5 L209-212: I appreciate the authors providing detailed results from their f4-tests. However, the significant genetic affinities with the Yellow River populations appear sporadic and seem to depend heavily on the specific Shandong_EN and Yellow River populations chosen. Thus, the results do not convincingly demonstrate that the DWK populations have additional genetic affinity with the Yellow River populations compared to the Early Neolithic populations. There may be variation in genetic ancestry among the four Early Neolithic populations, among the Yellow River populations, and among the three DWK populations. Additionally, the variation could be due to low-coverage of data. The authors need to clarify these key questions before drawing any conclusions from this analysis.

Thanks for your comments. As you suggested, we do not use the f4-test as the significant genetic affinities. As we reclarified this conclusion in comments #1 by this reviewer, L391-507, these connections were supported by the qpAdm/PCA/ADMIXTURE analysis as all three DWK populations can be modeled as a mixture of Yellow River ancestry and ancestry related to Early Neolithic Shandong populations. We then revised to text to clarify this finding:

“We next investigated genetic relationships during the Middle and Late Neolithic between coastal Shandong populations (6,000-4,600 BP) associated with the DaWenKou culture and inland Yellow River populations (7,000-5,000 BP) associated with the Yangshao culture. In the PCA (Fig. 1C-1D), we observed that the three DaWenKou Shandong populations, from the more inland GangShang site (GSGroup) to the more coastal BeiQian (BQGroup) and FuJia (FJGroup) sites, are shifted to the left of Early Neolithic Shandong populations (Shandong_EN), and are closer to Yangshao-related Yellow River (YR) populations. It can be observed that the three populations from the DaWenKou period distributed along this axis (from Shandong_EN to YR) show different affinities to YR populations. Specifically, the inland GSGroup cluster with YR populations, whereas the coastal BQGroup and FJGroup fall between the YR and Early Neolithic Shandong populations (Fig. 1C-1D).

To further determine whether DaWenKou populations show additional YR ancestry relative to Early Neolithic Shandong populations, we employed a rotational qpAdm

strategy to estimate ancestral components found in the DaWenKou populations. A total of 11 key East Asian populations (e.g. ARpost14K, aFujian_EN) were rotated as potential source ancestries for the three DaWenKou groups (Table S5). Using this strategy, only the population shows highest fitness to the model will be considered as an ancestral source population (tail_prob > 0.05, each ancestral proportion > standard error, pnest < 0.05). With the rotational qpAdm strategy, we found that the DaWenKou populations (BQGroup and GSGroup) can be modelled as a mixture ancestry related to Early Neolithic Shandong populations (Shandong_EN, ~29 - 87%) and YR populations (YR, ~13 - 71%), while FJGroup can be “1-way” modelled as Shandong_EN (Tail_prob = 0.08) or BQGroup (Tail_prob = 0.47) (Fig. 4A, Table S5).

Consistent with previous observation that the three sampled DaWenKou population shows different affinity to the YR populations, the proportion calculated by qpAdm of YR ancestry in 6,000-4,600 BP Shandong populations vary, with the GSGroup showing the highest levels of YR ancestry (~50 - 71%, Fig. 4A, Table S5), followed by BQGroup (~13%, Fig. 4A, Table S5) and FJGroup (~0 - 13%, Fig. 4A, Table S5). In an ADMIXTURE analysis, the GSGroup shows a higher proportion of YR component (orange) compared to other DaWenKou populations (Fig. 2C). Collectively, these patterns suggest that ancestry related to YR populations impacted populations in the Shandong region during the DaWenKou cultural period. The inland geography of the GSGroup relative to the coastal location of the BQGroup and FJGroup suggest that such impacts decrease with proximity to the coast.” in Lines 202-238.

1.6 L216-224: Why did the authors fix the source of the Early Neolithic to Xiaojingshan? What rationale supports this choice? What results would be obtained if other Early Neolithic populations were used in modelling? Additionally, could you model the FJGroup using a two-way admixture of the Early Neolithic and YR_LN, similar to the model used for the BQGroup? It is unclear why one of the DWK populations was modelled with another DWK population. I cannot see any evidence in Table S11 or the other results supporting a close genetic relationship between FJ and BQ.

Thanks for your comments. Sorry for the confusion, we did not fix the source of EN to Xiaojingshan, instead, we used a total of 11 key East Asian populations (e.g. SD9K, Xiaojingshan, ARpost14K, aFujian_EN) and these populations were rotated as potential source ancestries in a rotational qpAdm strategy, just as we now noted in main text as “To further determine whether DaWenKou populations show additional YR ancestry relative to Early Neolithic Shandong populations, we employed a rotational qpAdm strategy to estimate ancestral components found in the DaWenKou populations. A total of 11 representative East Asian populations (e.g. ARpost14K, aFujian_EN) were rotated as potential source ancestries for the three DaWenKou groups (Table S5). Using this strategy, only the population shows highest fitness to the model will be considered as an ancestral source population (Tail_prob > 0.05, each ancestral proportion > standard error, pnest < 0.05).” in Lines 215-222. And in a rotational qpAdm strategy, BQGroup and GSGroup can be modelled as a mixture ancestry related to Early Neolithic Shandong populations (SD9K/Xiaojingshan, ~29 - 87%) and YR

populations (YR_MN/YR_LN, ~13 - 71%), while FJGroup can be “1-way” modelled as Shandong_EN (Tail_prob = 0.08) or BQGroup (Tail_prob = 0.47).

1.7 L240-243: This extra genetic affinity is observable in the CZYGroup only when Xiaojingshan and YR_LN are used as P1 and P3. The other 15 combinations of P1 and P3 do not support a stronger genetic affinity between the CZYGroup and the Yellow River populations compared to Shandong_EN. It is unclear if this is due to low data coverage in the CZYGroup or if the CZYGroup is more genetically similar to Shandong_EN than to another LongShan population, YJC. In either case, the f4-tests do not seem to support this statement. There should be differences in genetic ancestry between CZY and YJC, although the admixture modelling presented in Figure 4 does not support this idea. All of these results are confusing, and the authors need to provide a clear explanation for these discrepancies.

Thanks for your comments, and we have now carefully examined the genetics of CZYGroup and YJCGroup. As the reviewer suggested, while these two groups are overall similar to each other, there is slight difference in their genetic ancestry. Specially, these two groups are genetically similar, as CZY and YJC always share more alleles compared to other East Asians sampled to date ($f_4(X, CZY; YJC, Mbuti) < 0$ ($-28 < Z < 1.6$), $f_4(X, YJC; CZY, Mbuti) < 0$ ($-30 < Z < 0.5$), $f_4(CZY, YJC; X, Mbuti) \sim 0$ ($-4 < Z < 1.5$)) (Table R5A-C). However, we also observe a difference between YJC and CZY when comparing them to certain populations, as $f_4(CZY, YJC; X, Mbuti) < 0$ when X are some of Shandong populations, a Southern East Asian population (Qihe3), and Nagabaka_historic while no Z-Value > 0 (see Table R5C below). No specific population can be identified; thus it is likely introduced by unsampled populations. Such genetic difference led to the different values between $f_4(SD9K/Xiaojingshan, YJCGroup; aYR, Mbuti)$ and $f_4(SD9K/Xiaojingshan, CZYGroup; aYR, Mbuti)$ (Table R5D). Combining with the qpAdm results that show no difference in ancestry in modeling CZY and YJC, we found that both LongShan YJCGroup and CZYGroup can only be modeled as a mixture of ancestry related to the GSGroup (68 - 79% YR) and another DaWenKou population (the BQGroup) or an Early Neolithic Xiaojingshan population (21-32%, Fig. 4A, Table S5). The genetic difference introduced by the unsampled populations is likely minimum and not directly related to YR populations with $f_4(CZY, YJC; YR, Mbuti) \sim 0$, $-2.3 < Z < -0.7$.

We also confirmed that such difference is not due to low data coverage, as sufficient data are obtained for CYZ populations (SNPs = ~ 41,000 - 65,000).

We have now explored this observation in the main text Lines 240-272.

		YJCGroup			CZYGroup
aYellowRiver	YR_MN_WGM	-8.0	aYellowRiver	YR_MN_WGM	-10.2
	YR_MN_XW	-8.9		YR_MN_XW	-8.6
	YR_LN	-10.2		YR_LN	-12.3
	YR_LBIA	-10.2		YR_LBIA	-12.0
aShandong	Bianbian	-1.1	aShandong	Bianbian	-3.9
	Xiaogao	-6.5		Xiaogao	-6.4
	Boshan	-1.6		Boshan	-5.3
	SDEN	-5.1		SDEN	-7.6
	Xiaojingshan	-0.2		Xiaojingshan	-3.1
	FJGroup	0.2		FJGroup	0.5
	BQGroup	1.6		BQGroup	-1.1
	GSGroup	-2.6		GSGroup	-3.4
	YJCGroup	0.0		YJCGroup	0.0
	CZYGroup	0.0		CZYGroup	0.0
	TLGroup	-6.7		TLGroup	-7.6
	HLGroup	0.1		HLGroup	0.4
	LJZGroup	-2.5		LJZGroup	-3.4
	YXGroup	-7.0		YXGroup	-8.3
aLiaoRiver	XZGroup	-3.4	aLiaoRiver	XZGroup	-4.4
	XCGGroup	-3.0		XCGGroup	-5.6
	WLR_MN	-8.6		WLR_MN	-9.7
	WLR_LN	-8.7		WLR_LN	-9.4
	WLR_BA	-9.4		WLR_BA	-9.0
	WLR_BA_o	-9.1		WLR_BA_o	-10.5
aAmurRiver	HMMH_MN	-10.2	aAmurRiver	HMMH_MN	-11.2
	AR19K	-9.8		AR19K	-10.4
	AR_EN	-9.2		AR_EN	-10.8
	Boisman_MN	-12.2		Boisman_MN	-13.1
	DevilsCave_N	-13.0		DevilsCave_N	-14.4
	AR14K	-5.3		AR14K	-7.4
	AR13-10K	-5.4		AR13-10K	-6.2
	ARpost9K	-6.6		ARpost9K	-8.1
	AR9.2K_o	-1.1		AR9.2K_o	-0.5
	Yankovsky_IA	-3.3		Yankovsky_IA	-4.8
	AR_Xianbei_IA	-9.6		AR_Xianbei_IA	-9.8
	Heshui_Mohe	-7.1		Heshui_Mohe	-7.5
aSouthChina	Qihe	-10.0	aSouthChina	Qihe	-10.4
	Qihe3	-13.1		Qihe3	-15.5
	Liangdao1	-9.0		Liangdao1	-9.0
	Liangdao2	-13.3		Liangdao2	-14.9
	Suogang	-2.3		Suogang	-2.4
	Xitoucun	-5.5		Xitoucun	-7.3
	Tanshishan	-7.0		Tanshishan	-7.5
	Chuanyun	-3.0		Chuanyun	-1.6
	Taiwan_Hanben	-15.2		Taiwan_Hanben	-14.9
	Taiwan_Gongguan	-4.9		Taiwan_Gongguan	-6.3
	Longlin	-16.0		Longlin	-15.4
	Baojianshan	-19.7		Baojianshan	-20.6
	Dushan	-12.4		Dushan	-14.2
	LaCen	-9.3		LaCen	-9.9
	GaoHuaHua	-8.0		GaoHuaHua	-7.3
	BaBanQinCen	-9.1		BaBanQinCen	-8.3
	Shenxian	-8.5		Shenxian	-8.8
	Layi	-7.9		Layi	-7.5
aJP	Yiyang	-3.6	aJP	Yiyang	-4.5
	EarlyJomon	-28.4		EarlyJomon	-30.1
	Kofun	-12.4		Kofun	-13.7
	MidLateJomon	-26.9		MidLateJomon	-28.4
	Jomon	-22.5		Jomon	-23.3
	Yayoi	-1.6		Yayoi	-1.8
	Nagabaka_4000BP	-9.7		Nagabaka_4000BP	-10.6
	Nagabaka_2800BP	-22.5		Nagabaka_2800BP	-23.7
aKr	Nagabaka_historic	-12.4	aKr	Nagabaka_historic	-14.9
	InitialJomon	-22.9		InitialJomon	-24.4
	LateJomon	-16.5		LateJomon	-19.1
	Korea_Ando	-3.9		Korea_Ando	-3.9
	Korea_Changhang	-14.8		Korea_Changhang	-15.6
	Korea_Taejungni	-9.2		Korea_Taejungni	-10.5
	Korea_Yokchido	-14.5		Korea_Yokchido	-16.4
	Korea_Yondaedo_1	-13.5		Korea_Yondaedo_1	-14.1
	Korea_Yondaedo_2	-8.5		Korea_Yondaedo_2	-8.6
	DD_ThreeKingdoms	-18.5		DD_ThreeKingdoms	-19.4
	GY_ThreeKingdoms	-9.3		GY_ThreeKingdoms	-10.1

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Table R5A. $f_4(X, CZY; YJC, Mbuti)$ and Table R5B. $f_4(X, YJC; CZY, Mbuti)$

	aYellowRiver										aShandong										aLiaoRiver										aAmurRiver									
	YR_MN_WGM	YR_MN_XW	YR_LN	YR_LBA	Baishan	Xiangao	Baishan	SDEN	XiaoJingshan	PEGroup	BOGroup	GSGroup	YJGroup	CZYGroup	TJGroup	HJGroup	LEZGroup	YXGroup	XZGroup	XCGroup	WR_MN	WR_LN	WR_BA	WR_BA_o	HMMH_MN	AR19K	AR_EN	Bosman_MN	DanilCave_N	AR19K	AR13-10K	AR13-5K	AR13-2K_o	Yankeye_JA	AR_Xianbei_JA	Haihu_Mohe				
CZYGroup	-2.2	-0.7	-2.3	-1.7	-3.0	-0.3	-4.0	-3.0	-2.6	0.4	-2.6	-0.9	0.0	0.0	0.3	-1.0	-1.0	-1.8	-1.3	-2.6	-1.6	-1.0	0.3	-0.9	-1.4	-0.7	-1.5	-1.0	-1.7	-2.2	-0.9	-1.7	0.6	-1.6	0.1	-0.4				
	aSouthChina										aJP										aKr																			
	QJhe	QJhe3	Lengdao1	Lengdao2	Kixicun	Tamshihai	Chuangyin	Taiwan_Haibin	Taiwan_Gongguan	Longlin	Bioglanhai	Duhen	Baishan	LaCin	GaoHuahua	BaBeiQinCen	Shenshan	Laji	Yiyang	EarlyLemon	Kofun	MedanLemon	Jomon	Yayoi	Nagabale_4000BP	Nagabale_2000BP	Nagabale_historic	InitialLemon	LateLemon	Korea_Ando	Korea_Chengchang	Korea_Taeungni	Korea_Yochido	Korea_Yondado_1	Korea_Yondado_2	DD_ThreeKingdoms	GY_ThreeKingdoms			
CZYGroup	-0.7	-3.3	0.3	-2.2	0.0	-1.6	-0.7	1.5	-0.9	-1.6	-0.4	-1.1	-1.6	-0.7	0.8	0.2	-0.1	-0.2	-0.7	-1.7	-2.0	-1.5	-1.0	-0.3	-0.6	-1.7	-2.8	-2.0	-2.4	0.0	-0.5	-1.5	-1.8	0.1	0.1	-1.7	-0.6			

Table R5C. $f_4(\text{CZY}, \text{YJC}; X, \text{Mbuti})$

		P2:CZY				P2:YJC			
		aYellowRiver				aYellowRiver			
		YR_MN_WGM	YR_MN_XW	YR_LN	YR_LBIA	YR_MN_WGM	YR_MN_XW	YR_LN	YR_LBIA
		YR_MN_WGM	YR_MN_XW	YR_LN	YR_LBIA	YR_MN_WGM	YR_MN_XW	YR_LN	YR_LBIA
aShandong	SD9K	-0.5	-0.4	-1.1	-0.8	-2.1	-1.3	-2.9	-2.6
	XiaoJingshan	-1.5	-1.1	-2.7	-1.6	-3.2	-2.0	-4.9	-4.1

Table R5D. $f_4(\text{SD9K/XiaoJingshan}, \text{CZYGroup/YJCGGroup}; \text{aYR}, \text{Mbuti})$

1.8 L256-L261: If this was the authors' interpretation, I would consider that the Yellow River populations share a similar number of alleles with both Shandong_EN and the CZYGroup, as 15 out of the 16 f_4 -tests do not support any statistically significant extra affinity between the Yellow River populations and the CZYGroup (see my previous comment). This lack of confidence in the interpretation arises from the weak statistical significance of the genetic link between the CZY and Yellow River populations. Even for the other LongShan population, YJC, how do the authors explain that their extra genetic affinity with the Yellow River populations is not observable when Boshan is used as P1?

Thanks for your comments. We have now employed additional modelling to investigate the genetics of these ancient Shandong populations instead of using F4 only. Please see details in the response of comments #1.7 by this reviewer, L734-775. With updated evidence, the connection between YJCGGroup/CZYGroup and YR is likely from the mixture between different DaWenKou ancestors, and LongShan populations were shown no more additional influence from the YR populations.

1.9 L261-267: The results from qpAdm contradict those from the f_4 -tests, as the genetic affinity with the Yellow River populations differs significantly between the CZYGroup and YJCGGroup (Table S8).

Thanks for your comments. We now noted CZYGroup is very similar to the YJCGGroup and both CZYGroup and YJCGGroup share a similar connection with the YR populations by f_4 -test as the response of comments #1.7 by this reviewer, L734-775. However, we also note that the two populations during the LongShan culture shared similar patterns with more clearly analysis (such as PCA/ADMIXTURE/qpAdm analysis) as the response of comments #1.7, L734-775. In particular, in the rotational qpAdm analysis, both the two LongShan admixtures cannot be modeled using the "Shandong-related ancestors" and the "YR population", but only as mixtures of the two Shandong

ancestries (GS+BQ/Xiaojingshan), further suggesting a similar patterns of genetics between the both two LongShan populations.

1.10 L285-288: I don't think the results support this conclusion. With 2 LongShan populations and 4 different Yellow River populations tested, there are 8 f4-tests for each of XZ and YX. However, only two tests for XZ and one for YX show significant affinity with specific Yellow River populations. There are more tests without statistical support for this relationship than those with support. The authors need to identify the sources of this variability before drawing any conclusions.

Thanks for your comments. As you point out, we have now removed the inconsistent F4 and related conclusion, and instead focused on the shift in YR components and related introgression events in these populations, as discussed in comments #1 by this reviewer, L391-507.

We reorganized the chain of evidence (qpAdm/PCA/ADMIXTURE/DATES) now for the connections between early dynastic Shandong and YR populations, as we noted in the comment #1 by this reviewer, L391-507. We now categorized the Shandong populations during the early dynastic period into three different patterns mainly with rotational qpAdm strategy: (1)The HLGroup, LJZGroup, XCGroup can be modelled by CZYGroup of LongShan in a "1-way" pattern, showed no additional connections from YR ancestries between these 3 populations and LongShan populations; (2)The TLGroup and XZGroup can only be modelled by "GSGroup", the DaWenKou population with elevated YR ancestry (68 - 79%), in a "1-way" pattern, suggested the 2 populations may share more YR ancestries as the same pattern as the DaWenKou populations rather than the LongShan populations; (3)And the YXGroup is only modeled by a mixture of YR populations (~75 - 92%) and another populations (e.g., ~25% CZYGroup, or ~9% SD9K) (Table S5).

In addition, to explore what happened to these three early dynastic Shandong populations (XZGroup, YXGroup and TLGroup), we estimated the timing of introgression using DATES, and found that these three populations could be modeled as a mixture of Shandong populations older than 3,500 BP and YR population (YR_MN/YR_LN), with an estimated date of admixture around 4.6 to 11.5 generations prior to the dynastic period, or ~2,880 - 2,030 BP (Table S7), supporting that these three populations may have been genetically influenced by a second YR population at least after the LongShan culture period as the second wave of introgression, distinguished from the mixed events that occurred during the DaWenKou period (at least ~6,000 - 4,600 BP). We revised this part as:

"Using the rotational qpAdm strategy (Fig. 4A, Table S5) for early dynastic Shandong populations, we found that they can be modeled in three different admixture patterns: (1)The Houli (HLGroup), Liujiashuang (LJZGroup), Xichen (XCGroup) populations can be modelled by "CZYGroup" of Longshan in a "1-way" pattern, showed no additional connections to YR ancestry between these 3 populations and Longshan

populations; (2)The Tonglin (TLGroup) and Xinzhi (XZGroup) populations can only be modelled by “GSGroup”, the Dawenkou population shows an elevated YR ancestry (68 - 79%), in a “1-way” model, suggested the 2 populations may share more YR ancestries as the same pattern as the Dawenkou populations rather than the Longshan populations; (3)And the Yixi population (YXGroup) is only modeled by a mixture of YR populations (~75 - 92%) and another populations (e.g., ~25% CZYGroup, or ~9% SD9K) (Table S5).

We further noted that the TLGroup, XZGroup, and YXGroup admixtures (were all younger than 3,000 BP) have different modeling patterns compared with the Longshan admixture plainly. To explore what happened to these three early dynastic Shandong populations (XZGroup, YXGroup and TLGroup), we estimated the timing of introgression using DATES, and found that these three populations could be modeled as a mixture of Shandong populations older than 3,500 BP and YR population (YR_MN/YR_LN), with an estimated date of admixture around 4.6 to 11.5 generations prior to the dynastic period, or ~2,880 - 2,030 BP (Table S7), supporting that these three populations may have been genetically influenced by a second YR population at least after the Longshan culture period as the second wave of introgression, distinguished from the mixed events that occurred during the DaWenKou period (at least ~6,000 - 4,600 BP). Also in an ADMIXTURE analysis, we observe that individuals from the TLGroup, XZGroup, and YXGroup show a higher proportion of a component found in Yellow River populations (orange) compared with the other Shandong populations (Fig. 2C)” in Page 277 - 304.

1.11 L338-340: To determine if this genetic affinity is truly unique to Nagabaka_historic, please test f4 with the forms (Kofun, 6000-1500 BP Shandong populations; Nagabaka_historic, Mbuti) and (Kofun, Nagabaka_historic; 6000-1500 BP Shandong populations, Mbuti). If any Shandong populations still show significant differences, the authors should model Nagabaka_historic as an admixture between Kofun and additional ancestry that exhibits significant affinities with Nagabaka_historic compared to Kofun in the f4-test.

Thanks for your comments. Based on your suggestions, we now added the suggested two f4-analyses to test if this genetic affinity is truly unique to Nagabaka_historic:

P1	P2	P3	P4	F4	Z
Kofun	GSGroup	Nagabaka_historic	Mbuti	0.0091	2.159
Kofun	FJGroup	Nagabaka_historic	Mbuti	0.0068	1.245
Kofun	BQGroup	Nagabaka_historic	Mbuti	0.003	1.18
Kofun	YJCGroup	Nagabaka_historic	Mbuti	0.0055	1.948
Kofun	CZYGroup	Nagabaka_historic	Mbuti	0.0117	4.269
Kofun	TLGroup	Nagabaka_historic	Mbuti	0.0078	3.031
Kofun	HLGroup	Nagabaka_historic	Mbuti	0.0098	2.239
Kofun	LJZGroup	Nagabaka_historic	Mbuti	0.0101	4.057
Kofun	YXGroup	Nagabaka_historic	Mbuti	0.0065	2.319
Kofun	XZGroup	Nagabaka_historic	Mbuti	0.007	2.495
Kofun	XCGroup	Nagabaka_historic	Mbuti	0.0084	2.196
P1	P2	P3	P4	F4	Z
Kofun	Nagabaka_historic	GSGroup	Mbuti	0.0102	2.486
Kofun	Nagabaka_historic	FJGroup	Mbuti	0.0112	2.023
Kofun	Nagabaka_historic	BQGroup	Mbuti	0.0044	1.703
Kofun	Nagabaka_historic	YJCGroup	Mbuti	0.0061	2.144
Kofun	Nagabaka_historic	CZYGroup	Mbuti	0.008	2.948
Kofun	Nagabaka_historic	TLGroup	Mbuti	0.0113	4.237
Kofun	Nagabaka_historic	HLGroup	Mbuti	0.0058	1.355
Kofun	Nagabaka_historic	LJZGroup	Mbuti	0.0077	2.959
Kofun	Nagabaka_historic	YXGroup	Mbuti	0.0093	3.293
Kofun	Nagabaka_historic	XZGroup	Mbuti	0.0116	4.23
Kofun	Nagabaka_historic	XCGroup	Mbuti	0.0081	2.135

Table R6. $f_4(\text{Kofun}, 6000\text{-}1500 \text{ BP Shandong populations}; \text{Nagabaka_historic}, \text{Mbuti})$ and $f_4(\text{Kofun}, \text{Nagabaka_historic}; 6000\text{-}1500 \text{ BP Shandong populations}, \text{Mbuti})$

In the first F_4 analysis ($\text{Kofun}, 6000\text{-}1500 \text{ BP Shandong populations}; \text{Nagabaka_historic}, \text{Mbuti}$) (Table R6), the Nagabaka_historic population shared more alleles with the Kofun population, which indicated that genetic affinity was still higher among the Japanese population. In the second F_4 analysis ($\text{Kofun}, \text{Nagabaka_historic}; 6000\text{-}1500 \text{ BP Shandong populations}, \text{Mbuti}$) (Table R6), we can observe that most of the Shandong population shares more alleles with Kofun with $Z > 1.35$, and we can also observe that the Shandong population shares more alleles with the Kofun/Nagabaka_historic population in $f_4(\text{aJapanese} > 3,000 \text{ BP}, \text{Kofun}/\text{Nagabaka_historic}; 6,000\text{-}1,500 \text{ BP Shandong populations}, \text{Mbuti}) < 0, -21.7 < Z < -2.5$ (Table S9).

The reason we observed higher allele sharing between the Kofun and Shandong populations in the second F_4 analysis is because of the higher presence of Yellow River ancestry in the Kofun population located on the main island of Japan compared to the Nagabaka population located in the Ryukyu Islands (Table S5). In contrast, the Nagabaka population contained a higher Jomon population component (Table S5).

Both F_4 analyses suggest that genetic links exist between both the Shandong and Kofun/Nagabaka populations, so we then explored whether this connection is a general connection between Japanese populations and East Asian populations or a specific connection between the Japanese populations and Shandong populations. We found

that $f_4(\text{AR/FE/WLR/YR, Shandong; Kofun, Mbuti}) \sim 0$, thus the above observed affinity between Shandong and Kofun is a general connection between Kofun and East Asian. However, $f_4(\text{AR/FE/WLR/YR, Shandong; Nakabaka_historic, Mbuti}) < 0$ ($Z < -2.5$, Fig. 3, Table S10), confirming a unique connection between Shandong and Nakabaka_historic.

2. When performing admixture modelling using qpAdm, please provide not only tail probabilities, which represent the fit of the tested model, but also nested p-values for competing models (e.g., between three-way and two-way admixture models or between two-way and single-ancestry models). These nested p-values are critical for quantitatively assessing model fit, helping to determine whether a two-way admixture sufficiently explains the genetic makeup of a target population or if a three-way admixture better fits the data. To calculate this p-value, you can use the following command in R: `1 - pchisq(chisq_model1 - chisq_model2, df = dof_model1 - dof_model2)`. Additionally, please include error bars in the bar plots presenting the qpAdm results, as this would make it easier to visualize the data compared to referencing the text and supplementary tables separately.

Thanks for your comments. We have now added the pnest values results in Table S5 and Table R7A-B. And we added error bars in the bar plots presenting the qpAdm results in Fig. 4A.

	Target	Source1		Source2		Tail_prob	STD.ERR		pnest
		Pop Name	pro	Pop Name	pro		err1	err2	
DWK	BQGroup	Xiaojingshan	0.873	YR_LN	0.127	0.090	0.045	0.045	0.011
	GSGroup	Xiaojingshan	0.380	YR_MN	0.620	0.443	0.064	0.064	0.000
	GSGroup	Xiaojingshan	0.288	YR_LN	0.712	0.338	0.074	0.074	0.000
	GSGroup	SD9K	0.400	YR_LN	0.600	0.142	0.091	0.091	0.000
	GSGroup	SD9K	0.502	YR_MN	0.498	0.085	0.084	0.084	0.000
	FJGroup	BQGroup	1.000			0.474			
LS	FJGroup	SD9K	1.000			0.082			
	CZYGroup	GSGroup	0.865	ARpost14K	0.135	0.865	0.081	0.261	0.131
	CZYGroup	GSGroup	0.744	BQGroup	0.256	0.060	0.099	0.099	0.019
	YJCGroup	GSGroup	0.678	BQGroup	0.322	0.684	0.087	0.087	0.003
	YJCGroup	GSGroup	0.793	Xiaojingshan	0.207	0.560	0.080	0.080	0.022
	YJCGroup	GSGroup	0.785	SD9K	0.215	0.130	0.144	0.144	0.136
early dynastic period	HLGroup	CZYGroup	1.000			0.088			
	LJZGroup	CZYGroup	1.000			0.470			
	TLGroup	GSGroup	1.000			0.171			
	XCGroup	GSGroup	1.000			0.716			
	XCGroup	CZYGroup	1.000			0.147			
	XZGroup	GSGroup	1.000			0.244			
	YXGroup	YR_MN	0.872	ARpost14K	0.128	0.624	0.048	0.048	0.013
	YXGroup	YR_MN	0.754	CZYGroup	0.246	0.285	0.059	0.059	0.000
	YXGroup	YR_MN	0.839	WLR_BA	0.161	0.218	0.042	0.042	0.001
	YXGroup	YR_LN	0.818	CZYGroup	0.182	0.169	0.059	0.059	0.003
	YXGroup	YR_LN	0.898	ARpost14K	0.102	0.154	0.050	0.050	0.046
	YXGroup	YR_MN	0.803	YJCGroup	0.197	0.147	0.061	0.061	0.003
	YXGroup	YR_MN	0.870	Yumin	0.130	0.112	0.039	0.039	0.002
	YXGroup	YR_MN	0.849	SD9K	0.151	0.098	0.053	0.053	0.007
	YXGroup	YR_LN	0.912	SD9K	0.088	0.094	0.054	0.054	0.112
	YXGroup	YR_LN	0.917	aFujian_EN	0.083	0.071	0.040	0.040	0.043
	YXGroup	YR_LN	0.898	WLR_BA	0.102	0.069	0.044	0.044	0.024
	YXGroup	YR_LN	0.847	YJCGroup	0.153	0.065	0.059	0.059	0.011
	YXGroup	YR_MN	0.869	BQGroup	0.131	0.055	0.043	0.043	0.005
aJP	Nagabaka_2800BP	EarlyJomon	1.000			0.173			
	Nagabaka_2800BP	InitialJomon	1.000			0.423			
	Nagabaka_2800BP	LateJomon	1.000			0.625			
	Nagabaka_historic	YJCGroup	0.750	LateJomon	0.250	0.059	0.010	0.010	0.000
	Nagabaka_historic	CZYGroup	0.752	LateJomon	0.248	0.371	0.010	0.010	0.000

Table R7A. 2-way qpAdm analysis.

Target	Source1		Source2		Source3		Tail prob	STD.ERR			pnest	
	Pop Name	pro	Pop Name	pro	Pop Name	pro		err1	err2	err3	pnest 2	pnest 1
Xiaojingshan	SD9K	0.742	aFujian_EN	0.098	ARpost14K	0.160	0.992	0.096	0.050	0.082	0.069	0.104
Nagabaka_historic	YR_LN	0.199	YJCGGroup	0.556	LateJomon	0.245	0.119	0.095	0.094	0.010	0.036	0.000
Nagabaka_historic	YR_LN	0.172	CZYGroup	0.583	LateJomon	0.244	0.418	0.100	0.099	0.010	0.112	0.000
Nagabaka_historic	WLR_MN	0.296	YJCGGroup	0.457	LateJomon	0.247	0.103	0.160	0.159	0.010	0.053	0.000
Nagabaka_historic	WLR_BA	0.128	YJCGGroup	0.626	LateJomon	0.246	0.306	0.088	0.086	0.011	0.201	0.000
Nagabaka_historic	WLR_BA	0.107	CZYGroup	0.649	EarlyJomon	0.243	0.056	0.072	0.071	0.011	0.090	0.000
Nagabaka_historic	WLR_BA	0.116	CZYGroup	0.629	InitialJomon	0.255	0.168	0.072	0.071	0.010	0.087	0.000
Kofun	YR_MN	0.544	WLR_BA	0.342	EarlyJomon	0.113	0.141	0.044	0.044	0.010	0.000	0.000
Kofun	YR_MN	0.536	WLR_BA	0.339	InitialJomon	0.125	0.114	0.044	0.044	0.008	0.000	0.000
Kofun	YR_MN	0.555	WLR_BA	0.324	LateJomon	0.121	0.377	0.056	0.056	0.009	0.000	0.000
Kofun	YR_MN	0.695	ARpost14K	0.183	EarlyJomon	0.121	0.282	0.050	0.049	0.010	0.001	0.000
Kofun	YR_MN	0.694	ARpost14K	0.176	InitialJomon	0.130	0.354	0.050	0.050	0.008	0.001	0.000
Kofun	YR_MN	0.720	ARpost14K	0.153	LateJomon	0.128	0.435	0.065	0.064	0.009	0.031	0.000
Kofun	YR_LN	0.408	WLR_MN	0.461	LateJomon	0.131	0.116	0.129	0.126	0.009	0.002	0.000
Kofun	YR_LN	0.586	WLR_BA	0.289	LateJomon	0.126	0.421	0.057	0.057	0.009	0.000	0.000
Kofun	YR_LN	0.489	Yumin	0.384	EarlyJomon	0.127	0.080	0.054	0.052	0.010	0.000	0.000
Kofun	YR_LN	0.479	Yumin	0.384	InitialJomon	0.137	0.068	0.055	0.053	0.008	0.000	0.000
Kofun	YR_LN	0.564	Yumin	0.299	LateJomon	0.136	0.139	0.071	0.069	0.009	0.000	0.000
Kofun	YR_LN	0.620	ARpost14K	0.253	EarlyJomon	0.126	0.123	0.053	0.052	0.009	0.000	0.000
Kofun	YR_LN	0.616	ARpost14K	0.251	InitialJomon	0.133	0.181	0.052	0.052	0.008	0.000	0.000
Kofun	YR_LN	0.664	ARpost14K	0.202	LateJomon	0.134	0.653	0.066	0.065	0.008	0.003	0.000
Kofun	GSGGroup	0.707	WLR_BA	0.159	LateJomon	0.134	0.079	0.118	0.121	0.012	0.369	0.000

Table R7B. 2-way qpAdm analysis.

Additional comments that are linked to this second major concern:

2.1 L292-294: Please replace CZYGroup with BQGroup and GSGGroup or with Xiaojingshan and Yellow River populations, and model the admixture in each of the CD populations. The authors modelled the genetic ancestry of a target population using an earlier population and additional ancestry, but all these sources trace their origins back to Shandong_EN and Yellow River ancestry, with varying proportions of each. This approach will clearly illustrate how Yellow River ancestry evolved over time.

Thanks for your comments. Based on your suggestion, we checked the results of modeling each Shandong populations with the unified “SD9K/Xiaojingshan/BQGroup/GSGGroup+YR” model, but in the most of the models with “SD9K/Xiaojingshan/BQGroup/GSGGroup+YR” were not passed (Tail_prob < 0.05) with rotational qpAdm strategy result (shown in the comments 1.) suggested SD9K/Xiaojingshan/BQGroup/GSGGroup were not always the suitable Ancestors of the younger Shandong populations. This may be due to genetic drift of Shandong populations over time, which makes it more difficult for older Shandong populations (SD9K/Xiaojingshan/BQGroup/GSGGroup) to pass the model when competing with Shandong populations closer to the age of the “target” population for a more suitable ancestor in the rotation strategy. In another hand, the layer-by-layer modeling pattern also suggests a higher genetic similarity shared between populations in Shandong that are closer in age (i.e., model populations use recent ancestral populations, as the signal of populations that are chronologically distant is more likely diluted by drift) (shown in the comments #1, L391-507, and #2 by this reviewer, L912-929).

2.2 L347: In the paper reporting the tri-ancestry structure of Japanese populations, WLR_BA_o and HMMH_MN were used to represent the northern inland population, as both show a stronger affinity with Amur River populations than with Yellow River populations. However, as demonstrated by Ning et al. (2020), the West Liao River

populations exhibit heterogeneous genetic ancestry over time. Please use WLR_BA_o and HMMH_MN as the northern inland population in your modeling.

Thanks for your comments. We added WLR_BA_o and HMMH_MN into the alternative NE ancestor list as northern inland populations for the rotational qpAdm strategy. But Shandong populations were still the better ancestors than WLR_BA_o/HMMH_MN, because no WLR_BA_o/HMMH_MN can model the Nagabaka_historic in the rotational qpAdm strategy (with Tail_Prob < 0.05) (Table R8).

Target	Source A	pro_A	Source B	pro_B	Tail_prob	STD.ERR
Nagabaka_historic	YJCGroup	0.752	EarlyJomon	0.248	0.600	0.013
Nagabaka_historic	YJCGroup	0.747	InitialJomon	0.253	0.381	0.012
Nagabaka_historic	YJCGroup	0.750	LateJomon	0.250	0.584	0.012
Nagabaka_historic	CZYGroup	0.744	EarlyJomon	0.256	0.524	0.013
Nagabaka_historic	CZYGroup	0.745	InitialJomon	0.255	0.239	0.012
Nagabaka_historic	CZYGroup	0.751	LateJomon	0.249	0.638	0.011
Nagabaka_historic	TLGroup	0.757	EarlyJomon	0.243	0.235	0.012
Nagabaka_historic	TLGroup	0.759	InitialJomon	0.241	0.084	0.011
Nagabaka_historic	TLGroup	0.766	LateJomon	0.234	0.181	0.011
Nagabaka_historic	HLGroup	0.741	EarlyJomon	0.259	0.383	0.016
Nagabaka_historic	HLGroup	0.742	InitialJomon	0.258	0.285	0.015
Nagabaka_historic	HLGroup	0.746	LateJomon	0.254	0.281	0.015
Nagabaka_historic	LJZGroup	0.745	EarlyJomon	0.255	0.617	0.012
Nagabaka_historic	LJZGroup	0.745	InitialJomon	0.255	0.403	0.011
Nagabaka_historic	LJZGroup	0.753	LateJomon	0.247	0.750	0.011
Nagabaka_historic	XCGroup	0.750	EarlyJomon	0.250	0.374	0.015
Nagabaka_historic	XCGroup	0.745	InitialJomon	0.255	0.269	0.014
Nagabaka_historic	XCGroup	0.750	LateJomon	0.250	0.426	0.014

Table R8. Rotational qpAdm strategy with WLR_BA_o/HMMH_MN

2.3 L349-353: Surprisingly, the genetic affinity between Nagabaka_historic and the CZYGroup is weaker than that between Nagabaka_historic and the YJCGroup. Considering this and the other points I've commented on, the genetic ancestry could differ between these two LongShan populations. Please conduct a more thorough analysis of any genetic heterogeneity within the different cultural populations in the Shandong region. Additionally, the authors should use the results from the f4-tests to screen potential sources for testing in qpAdm, as the current results are quite confusing. Furthermore, the authors should provide nested p-values for three-way versus two-way admixture models for Nagabaka_historic. This will help determine whether a two-way admixture model sufficiently explains the genetic ancestry of Nagabaka_historic or if a three-way admixture model offers a better explanation. This approach should also be applied to the northern inland populations represented by WLR_BA_o and HMMH_MN.

Thanks for your comments. (1) Based on this comment and your other comments, we have discussed the genetic heterogeneity within the different cultural populations, such as the Shandong_EN in comment #1.1 and #1.2 by this reviewer, the DaWenKou populations in comments #1.3 and #1.5 by this reviewer, the LongShan populations in comments #1.7 by this reviewer, the early dynastic Shandong populations in comments #1.10 by this reviewer, L510-870. (2) As the f4-test cannot robustly support the conclusion, we instead screened potential ancestral sources using a rotational qpAdm strategy noted in comment #1 by this reviewer, L391-507, and added the pnest values into Table S4 which was noted in comment #2 by this reviewer L912-929. (3) We also checked the WLR_BA_o and HMMH_MN as alternative NE ancestors, but Shandong

populations were better than both the two inlandnorthern inland populations, while WLR_BA_o and HMMH_MN can not model the Nagabaka_historic in the rotational qpAdm strategy as we noted in comments #2.2 by this reviewer, L954-966.

3. Minor comments:

3.1 I found it strange that supplementary tables are presented in descending order. Can you please correct this?

Thanks for your comments. We have modified the order so that the supplemental table are sorted in ascending order.

3.2 Table S7: I believe there is a typo in the label for P2. It should be “CZY” instead of “GZY.”

Thanks for your comments. We fixed the error in Table S7 from “GZY” to “CZY (Table S10, now.)” and checked the full text to make sure there were no similar errors.

3.3 L69: Please specify which study you are referring to here. It seems like it could be reference 10, but clarifying this will help avoid any confusion.

Thanks for your comments. We apologize that the phrase “In this study” may have caused misunderstanding among readers and used “In previously published studies” in Line 70 instead of the original phrase. Modifications are marked in blue in the text.

3.4 L71: “main island” should be plural.

Thanks for your comments. We apologize for using the singular form, we changed the island to the plural form as “main islands” in Lines 74 and check the full text to correct similar errors. Modifications are marked in blue in the text.

3.5 L73: Reference 10 is not the only study that has demonstrated population stratification in the Japanese archipelago, particularly the differences between the Ryukyu and main islands. Please include additional references, such as Yamaguchi-Kabata et al. (2008; <https://doi.org/10.1016/j.ajhg.2008.08.019>), Okada et al. (2018; <https://doi.org/10.1038/s41467-018-03274-0>), and Sakaue et al. (2020; <https://doi.org/10.1038/s41467-020-15194-z>).

Thank you for pointing out the citations that should be added, we added these citations and correct the text accordingly in Line 73.

3.6 L78-80: Please cite a reference on the Neolithic Shandong data.

We apologize for missing a reference, but we will add these citations for the Shandong population data in Lines 79 and 149.

3.7 L162-165: In the ADMIXTURE plots (Fig. 2C), could you please add labels indicating which groups correspond to the different Shandong populations (i.e., Early Neolithic, DWK, LS, or CD)?

Thanks for your suggestion. We have now added those labels in Fig. 2C.

3.8 L168-170: Please clarify which results you are referring to that support the genetic affinity between the Early Neolithic and Amur River populations. Adding the appropriate citation to these results would provide clarity.

Thanks for your question. We apologize that we did not cite the results obtained in the previous analysis in this section, where we will restate and summarize the results from the F4 analysis and cite the corresponding Table as “Table S4”.

3.9 L360-363: Again, a simple way to prove this is to use f4(Kofun, 6000-1500 BP Shandong populations; Nagabaka_historic, Mbuti) and f4(Kofun, Nagabaka_historic; 6000-1500 BP Shandong populations, Mbuti).

Thanks for your comments. We have responded this in comments #1.11 by this reviewer, L872-910.

L367-368: This dating should be revisited once the authors have addressed the other comments on admixture modelling of Nagabaka_historic.

Thanks for your comment. We would like to clarify that the DATEs analysis is independent of the other analyses, and we tested the full range of possible mixture models (Table S7), so the estimated time should not be affected.

Reviewer #3

I would like to express my appreciation to the authors for their efforts in addressing the comments I provided on the original manuscript. While my primary concerns regarding the f4 analyses have been largely resolved, I have additional comments on some of the points I previously raised. In particular, I remain entirely unconvinced by the proposed connection between the ShanDong populations and Nagabaka_historic.

3.1 Firstly, I appreciate the authors' efforts in conducting the analyses I suggested in my original comments, specifically the f4-tests in the forms of: f4(Kofun, ShanDong populations; Nagabaka_historic, Mbuti) and f4(Kofun, Nagabaka_historic; ShanDong, Mbuti). However, based on the results of these tests, I do not find clear support for additional genetic affinities between the ShanDong populations and Nagabaka_historic when compared to the Kofun.

As demonstrated in a previous study cited in this manuscript (Cooke et al. 2023), the genetic structure of Nagabaka_historic is best explained by the tripartite ancestry model used for the Kofun, which includes Jomon, Northeast Asian (represented by WLR_BA_o and HMMH_MN), and East Asian (represented by Han) ancestry, albeit in different proportions. Therefore, if there were indeed a significant genetic connection between the ShanDong populations and Nagabaka_historic, I would expect the Kofun to serve as the most appropriate reference for identifying any additional connections between the ShanDong and Nagabaka_historic, which is absent in the Kofun.

Because of this, while the authors have presented evidence suggesting differential affinities to ShanDong populations between the Kofun and Nagabaka_historic, I find that the data presented do not convincingly establish a unique genetic connection between the ShanDong populations and Nagabaka_historic. Upon closer examination of Table S10, these signals of genetic affinities appear to be somewhat inconsistent, varying depending on the specific ShanDong populations used. This variability raises questions about the robustness of the results and the authors' interpretations.

Response: Thanks for your comments. You're correct that we did find that $f_4(\text{Kofun, Nagabaka_historic; 6000-1500 BP ShanDong populations, Mbuti}) \geq 0, 1.3 > Z > 4.2$ (Table #3.1) did not show clear support for a connection between Nagabaka_historic and ShanDong. This is because (1) the Kofun possess a high proportion of northern East Asian components, as mentioned in Cooke et al (2023), which the ShanDong population also has, and (2) Nagabaka_historic has more Jomon components than the Kofun (~25%, Table S5). Thus, the f4-test could be reflecting more shared northern EA ancestry in the Kofun and ShanDong populations, masking any genetic connection between Nagabaka_historic and the ShanDong populations. Thus, we used alternative analyses to further test the connection between ShanDong and Nagabaka_historic, described below.

In the previous study (Cooke et al. 2023), Nagabaka_historic and Kofun were modeled in similar 3-way models with the Han as a source. But when the present-day Han population was used as a source, it was not possible to distinguish between the differing levels and types of northern EA ancestry in the Nagabaka_historic and Kofun populations. This is because the present-day Han is a mixed population, possessing both coastal and inland northern East Asian components. Thus, instead of using f4 and the previously published qpAdm method with present-day populations, we

used a rotating qpAdm analysis to verify the additional connections between Nagabaka_historic and ShanDong. In our qpAdm analysis with rotation strategy, the Nagabaka_historic population can only be modeled by the ShanDong and Jomon populations in 2-way models, while the Kofun population cannot be modeled by the ShanDong population and instead can only be modeled by 3-way models (YR+WLR/AR+Jomon, Table S5). The rotating qpAdm results allows for a clearer distinction between the Nagabaka_historic and Kofun populations in terms of differences in the ancestral components of the northern coastal populations. Also, we tested whether Kofun could serve as an ancestral population for the Nagabaka population in the rotating qpAdm, but no n-way model passed.

As you noted, the f4-analysis in Table S10 did not show the strongest supporting evidence. To demonstrate more robust evidence, we added a simulation method (Loosdrecht et al. 2018, Bai et al. 2024) to further confirm this connection between Nagabaka_historic and ShanDong. We first verified the constructed model using f4 (X, Nagabaka_historic; SDEN/Xiaojingshan/BQGroup, Mbuti), in which X is a simulated population ((1-x)% Jomon+x% Longshan ShanDong populations (YJCGroup, CZYGroup), sample size = 30) (Fig S8 and #3.1). In the simulation, we can observe that f4 reaches 0 when the proportions of ShanDong populations in the simulated populations are close to our qpAdm result (see the table below). In f4 (X, Nagabaka; BQGroup, Mbuti), the value of f4 reaches the 0 value much earlier (in the range of yellow lines of Fig S8 and #3.1, test by BQGroup) than when we tested with SDEN/Xiaojingshan. This is because comparing to SDEN and Xiaojingshan, the BQGroup possess additional YR related components (~13%, Table S5), which decreased the estimated ShanDong ancestries by ~ 13%.

Table. Comparison of simulation results and qpAdm results.

ShanDong population used for simulation	Proportion of ShanDong components in the simulated population when F4 ~ 0	Proportion of ShanDong components in the qpAdm modelling
YJCGroup	75%	72-78%
CZYGroup	75%	72-78%

We then used simulated data and f4 (X, Nagabaka_historic; Kofun, Mbuti) to verify that relative to the Kofun, Nagabaka_historic possesses excess ShanDong-related ancestry. In this simulation, X is a simulated population ((1-x)% Kofun+x% ShanDong populations, sample size = 30) (Fig S9 and #3.2), and we simulated X using 4 ShanDong populations (SDEN, Xiaojingshan, SDLS, and BQGroup) that are most likely representative of the source population for Nagabaka_historic. The f4 values decrease and get closer to 0 with an increase in ShanDong components in the simulated populations, suggesting the presence of ShanDong components in Nagabaka_historic. However, the f4 value does not reach 0 in this test, which is due to the additional genetic difference between Nagabaka_historic and Kofun, as we discussed earlier that Nagabaka_historic could not be modeled with the Kofun population.

We revised the manuscript to include the above analyses as: “To further analyze the northern coastal ancestry in the historical Nagabaka population and connect it to ShanDong populations, we designed a simulation test to use with the f4-analysis. We found two major patterns: (1) First, the simulation analysis further confirms that there is an additional genetic connection between the historical

Nagabaka and ShanDong populations. We tested f_4 (X, Nagabaka_historic; SDEN/Xiaojingshan/BQGroup, Mbuti), where X was a simulated population ((1-x)% Jomon+x% LongShan ShanDong populations (YJCGroup, CZYGroup), Fig S8). In the two sets of simulation tests (ShanDong populations = SDEN/Xiaojingshan), when x% is within the range of the proportion of the LongShan ShanDong population (~75%) calculated by the rotating qpAdm, the value of the f_4 -tests approximate zero (between the blue lines, Fig. S8). This supports the rotating qpAdm mixture model for the historical Nagabaka population containing a northern coastal population component related to ShanDong populations. In the set of simulation tests (ShanDong population = BQGroup), when x% is within the range of about 87% of the proportion of the LongShan ShanDong population calculated by qpAdm (~0.87 * 75%, between the yellow lines), the value of f_4 is equal to about 0. This is because the BQGroup population is a mixture of 87% ShanDong-related ancestry and 13% YR-related ancestry. These patterns support that there were additional genetic connections between ShanDong populations and the historical Nagabaka population beyond general northern East Asian connections. (2) In order to test for the additional contribution of a northern coastal component related to ShanDong populations to the historical Nagabaka population compared to the Kofun population, we tested f_4 (X, Nagabaka_historic; Kofun, Mbuti), where X was a simulated population ((1-x)% Kofun+x% ShanDong populations, sample size = 30, Fig S9). In all four sets of simulation tests, f_4 values gradually decreased in all four groups as the different ShanDong components in population X increased, suggesting that the historical Nagabaka population does share additional genetic connections with the ShanDong population compared to the Kofun population. In addition, because it was not possible to model the Nagabaka population using the Kofun population as the ancestral source in the rotating qpAdm analysis, the values of f_4 -tests approximately equal to 0 was not observed.” in Line 385-414.

Table #3.1

P1	P2	P3	P4	F4	Z
Kofun	Nagabaka_historic	GSGroup	Mbuti	0.0102	2.486
Kofun	Nagabaka_historic	FJGroup	Mbuti	0.0112	2.023
Kofun	Nagabaka_historic	BQGroup	Mbuti	0.0044	1.703
Kofun	Nagabaka_historic	YJCGroup	Mbuti	0.0061	2.144
Kofun	Nagabaka_historic	CZYGroup	Mbuti	0.008	2.948
Kofun	Nagabaka_historic	TLGroup	Mbuti	0.0113	4.237
Kofun	Nagabaka_historic	HLGroup	Mbuti	0.0058	1.355
Kofun	Nagabaka_historic	LJZGroup	Mbuti	0.0077	2.959
Kofun	Nagabaka_historic	YXGroup	Mbuti	0.0093	3.293
Kofun	Nagabaka_historic	XZGroup	Mbuti	0.0116	4.23
Kofun	Nagabaka_historic	XCGroup	Mbuti	0.0081	2.135

Fig #3.1

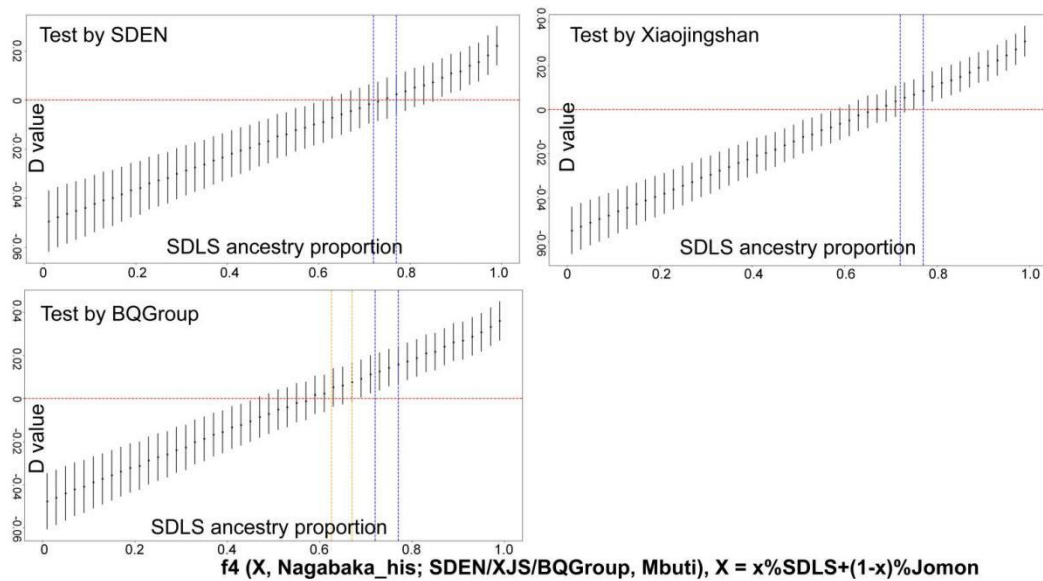
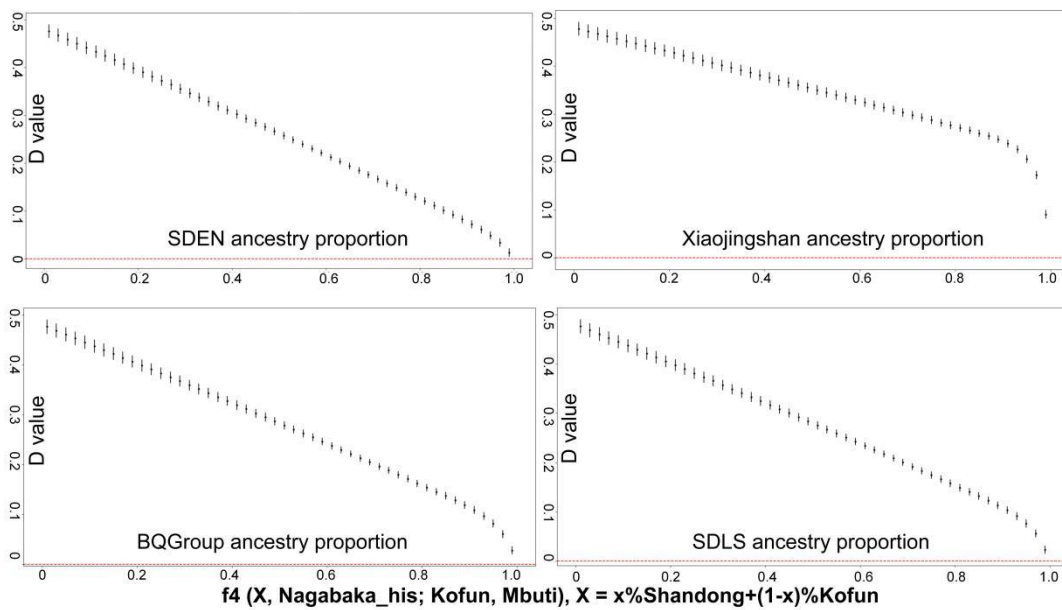


Fig #3.2



3.2 Secondly, I again appreciate the authors' discussion of the estimated admixture timing in Nagabaka_historic (between 1600–1400 years BP) and its contextualization with the dynastic period in the Japanese archipelago (Lines 446–452 in the revised main text). However, it is crucial to emphasize that this timing corresponds to the Aceramic period in the Ryukyu Islands, including the site where the bones associated with Nagabaka_historic were excavated, before the dynastic period began. The cultural transitions in the Ryukyu Islands are distinct from those on mainland Japan. As Yamagiwa (2022) discusses in "Early human cultural and communal diversity in the Ryukyu Islands," the dynastic period (or Ryukyu Kingdom as phrased in the text) in the Ryukyus only began around the 11th or 12th century AD, much later than the period referenced in the manuscript. Moreover, there is very limited—if not entirely absent—archaeological evidence supporting a direct connection between the Ryukyu Islands and China during the Aceramic period, which adds

uncertainty to the genetic results and interpretations. This lack of archaeological corroboration weakens confidence in the analysis and the conclusions drawn in this context.

Response: Thanks for pointing this out. As the reviewer suggested, this timing (1600-1400 BP) does not fit the dynastic Ryukyu Kingdom period (900-800 BP). Therefore, we rephrased the related section to interactions between the ShanDong population and pre-dynastic Ryukyu populations (i.e., before the establishment of the Ryukyu Kingdom), which can be potentially linked to conflicts between the Sui Dynasty (~1,400 BP) and Ryukyu populations (Sui Shu, Chuzan-sefu, and Chuzan-sekan) (Li, 2010). It is also worth mentioning that the DATES analysis calculates the timing of the admixture event under several assumptions (such as constant migration rates, constant effective population size, etc.), which may not be entirely consistent with the practical population history. Thus, it can only serve as an estimate of a range of average times when the event could have occurred.

Also, like the reviewer suggested, apart from the historical records, little archaeological evidence supporting the ShanDong and Ryukyu population interactions during this period is found. This again emphasizes the importance of both paleogenetic and archaeological studies to determine human prehistory. We hope with additional studies on East Asian history, further insights can be revealed in the near future.

We also revised the discussion section as “Compared to the Hondo Japanese, pre-dynastic populations in the Ryukyu Islands show ancestry related to northern coastal populations in the post-Yayoi period (~1,600 to 1,400 BP), likely derived from a population carrying ancestry related to ShanDong populations. The timing of admixture (~1,600-1,400 BP) can be potentially linked to conflicts that occurred between the Sui Dynasty and Ryukyu populations around 1,400 BP, as recorded in both ancient Chinese historical records (e.g. “Sui Shu”) and Japanese historical records (e.g. “Chuzan-sefu” and “Chuzan-sekan”)53. However, more fine-scale sampling in ShanDong and nearby regions is needed to pinpoint the exact historical event and timing of the population admixture.”, in Lines 489-497.

Given these concerns, I recommend that the authors reconsider the claims related to the gene flow from ShanDong to the Ryukyu Islands unless more compelling evidence can be provided. Alternatively, removing these claims would help maintain the manuscript’s focus the ShanDong region where the data and story are stronger and more conclusive.

Response: As described in the responses to Reviewer 3’s comments (#3.1 and #3.2, Lines 30 - 170), we have now added additional evidence and rephrased the related sections. We find that the qpAdm and simulation analyses additionally highlight that there is a connection between ancestry from ShanDong populations and ancestry in the Nagabaka_historic beyond the f4-analyses. Furthermore, historical texts highlight exchange between mainland East Asia and the Ryukyu Islands at the estimated time period. However, we acknowledge that additional evidence would help to clarify the nature of the connection. Thus, we have pointed out our observations, but added language that de-emphasizes gene flow from ShanDong to Ryukyu Islands as the only possibility. For instance, “more sampling is needed in nearby regions to help determine whether the ancestry really came from ShanDong”.

Minor Comments

3.3 The claim regarding the lesser impact of YR in coastal DWK and a higher impact in inland DWK, as presented in Figure 4, requires further clarification. It is not clear where these different sites are located geographically, and given that there are only three groups within this period, it may be premature to draw strong conclusions about geographic variation. Specifically, the data indicate one inland group and two coastal groups. Even if this pattern holds, the data presented appear too limited to confidently support such a conclusion. I recommend that the authors either provide additional evidence to strengthen this claim or revise the argument to reflect the current limitations of the data.

Response: Thanks for your suggestion. We rephrased the conclusion and described this result as confined to the limited data among 3 newly sampled ShanDong DWK populations. We now revised the main text to say “Based on our finding of greater YR-related ancestry in the inland GSGroup relative to the coastal BQGroup and FJGroup, we suspect that YR-related ancestry had decreasing impact with proximity to the coast. With only three DWK sites represented, however, finer sampling of DWK sites in ShanDong is needed to confirm this hypothesis.”, in Lines 239 - 242.

3.4 In Lines 480-486 of the rebuttal letter, the explanation raises a question: if a two-way admixture between GSGroup (one of the DWK populations) and YR is proposed, why do the qpAdm results not support this admixture for TLGroup and XZGroup? I suggest further clarification or additional evidence to explain this discrepancy, as relying only on DATES may not be sufficient without qpAdm support.

Response: Apologies for the confusion. In the previous response letter, we proposed that TLGroup and XZGroup are a mixture of ancestry related to the LongShan ShanDong (represented by CZYGroup and YJCGGroup) and YR populations, as supported by results from the DATES analysis (Table S7). This conclusion does not necessarily conflict with the qpAdm results, and we tested different scenarios.

Specifically, the dynastic TLGroup/XZGroup could be one-way modeled by Dawenkou GSGroup (4900-4800 BP), while the rest of the dynastic ShanDong populations can be one-way modeled by Longshan populations (4,500-4,000 BP), which is chronologically closer to the dynastic period and contain a reduced YR component compared to the GSGroup. It is important to note that, apart from the level of YR-related ancestry, the ancient ShanDong populations are overall highly similar to each other. Therefore, two possible scenarios can fit the above observed qpAdm result of the TLGroup/XZGroup: 1) genetic continuity between the GSGroup and the TLGroup/XZGroup or 2) additional YR introgression that led to genetic similarity between the GSGroup and the TLGroup/XZGroup. The major difference between the two scenarios is the timing of introduction of the YR components, and that's why we used DATES to test these two scenarios. We found that the YR component was introduced 4.6 to 18.9 generations prior to the dynastic period, or ~2,880 - 2,030 BP. Since the GSGroup possesses YR components that was likely introduced at least 4,900-4,800 BP ago, we proposed that the YR components in the TLGroup/XZGroup was introduced by more recent admixture.

We have now clarified this in the main text as “The TongLin (TLGroup) and XinZhi (XZGroup) populations can only be modeled by a single source ancestry related to the “GSGroup”, the DaWenKou population who showed an elevated YR-related ancestry (50-71%). This suggests that the two populations may share more YR-related ancestry, more similar to DaWenKou populations than LongShan populations. However, it is important to note that, apart from the level of YR-related ancestry, the ancient ShanDong populations are overall highly similar to each other. Two possible scenarios could have given rise to the observed qpAdm model for the TLGroup and XZGroup: (a) genetic continuity between the GSGroup and these two early dynastic groups or (b) additional YR-related introgression leading to genetic similarity between the GSGroup and these two groups. The major difference between these two scenarios is the timing of when the YR-related ancestry was introduced into the population.”, in Lines 288-299.

3.5 Why did the authors present selected combinations of the tests by qpAdm in Table S5?

Response: Thanks for your question. The combination of tests presented in Table S5 show successful qpAdm models in a rotating qpAdm analysis, to help identify the best mixture models. Compared to qpAdm without rotation, in a rotating qpAdm analysis, each potential source population is sequentially included as the left_population, whereas unused potential sources in that qpAdm analysis are included in the right_population. Finding a limited number of feasible mixture models using this rotation strategy allows determination of optimal source populations. The shown tests in Table S5 are thus all successful mixture models, where $\text{Tail_prob} > 0.05$, each ancestral mixture proportion $>$ standard error, $p_{\text{nest}} < 0.05$. Using this exhaustive strategy combined with a small number of source populations tends to reveal optimal combinations of sources, because the source populations can only be identified when they outperform the rest of the tested populations. Therefore, by this method, we can find the most suitable combination of source population among all combinations of source populations. Thus, Table S5 shows all feasible models (and thus the best results) without biasing towards any one particular mixture model.

We have now added more information regarding the rotating qpAdm analysis in the methods and materials to clarify this as “The identification of a set of best-fitting mixture models is a major advantage of a rotating qpAdm analysis. Compared to qpAdm without rotation, in a rotating qpAdm analysis, each potential source population is sequentially included as the left_population, where all unused potential sources in that qpAdm analysis are included in the right_population. Finding successful mixture models for one or a few potential sources highlights the optimal sources relative to the other potential sources (where $\text{Tail_prob} > 0.05$, each ancestral mixture proportion $>$ standard error, $p_{\text{nest}} < 0.05$). Using this exhaustive strategy, combined with a smaller number of source populations tends to reveal optimal combinations of sources, because the source populations can only be identified when they outperform the rest of the tested potential source populations. Therefore, by this method, we can find the most suitable combination of source populations among all combinations of source populations.”, in Supplementary Text: Materials and Methods (10) Admixture modeling with qpAdm.

3.6 In Lines 486-494 of the rebuttal letter, the authors successfully modeled additional gene flow

from YR in the YXGroup, and given the similar DATES results for TLGroup and XZGroup, they suggest that this scenario could apply to those groups as well, despite qpAdm modeling not being successful. However, this conclusion may be somewhat optimistic without further evidence. To strengthen this argument, I recommend providing additional evidence that YXGroup is genetically similar to TLGroup and XZGroup, and exploring in more detail why the admixture scenario worked for YXGroup but not for the other two groups. This would help clarify whether the proposed conclusion is fully supported by the data.

Response: Thanks for your suggestion. We have now clarified the consistency between the qpAdm of TLGroup/XZGroup and DATES in response #3.4 above. Also, we have emphasized in our qpAdm analysis that YXGroup and TLGroup/XZGroup are two different patterns. In fact, YXGroup is mixed with more YR-related ancestral components ($> 75\%$), compared to other ShanDong populations. The best model is the “2-way” model, which incorporates the Yellow River-related ancestry (Table S5). Therefore, it is reasonable that for the YXGroup population, we also successfully calculated the timing of the mixture of YR-related components through DATES.

3.7 In Lines 290-306 of the main text, the qpAdm results do not support additional YR contribution for TLGroup and XZGroup. Given this, I would recommend caution in concluding that these groups have recent YR ancestry based on gene flow alone, as this could potentially weaken the robustness of your results. To strengthen the argument, it may be beneficial to either provide additional evidence for this gene flow or reconsider how this aspect is presented to ensure it aligns with the qpAdm findings.

Response: Thanks for your comments. Please see detailed discussion on this issue in the above responses #3.4 and #3.6. We have now rephrased this section accordingly to improve clarity. In the main text, we describe a different pattern of population admixture in the TLGroup/XZGroup than in the ShanDong population during the Longshan period. This different pattern suggests that the YR component may be elevated in the TLGroup/XZGroup population compared to the Longshan populations. Then, the DATES analysis was used to further test the possibility that these two populations may have been additionally influenced by the YR population after 3,000 BP.

Reviewer #3 (Remarks to the Author):

I would like to thank the authors for their efforts in addressing the concerns I raised in my previous comments to the first round of revision. I appreciate the new simulation analysis, which enhances the validation of their results from the rotating qpAdm models. However, I was unable to fully evaluate the details of this simulation in the revised manuscript, as the methodology remains unexplained in the Materials & Methods section. I also sought this information in the papers cited in the rebuttal letter (Loosdrecht et al. 2018; Bai et al. 2024), but was again unable to find the necessary details. If the authors intend to retain this analysis in the manuscript, rather than just presenting it as their response, I recommend that the authors should include a thorough explanation of the methodology in the manuscript.

Response: We thank the reviewers for this suggestion. We have now added a detailed description about the simulation in the manuscript in the “Supplementary Text: Materials and Methods”:

“Here, we included a simulation to test the possible connections between the tested population (Population A) and the possible ancestral component related populations (Population C), which leverages the linear relation between proportion of arbitrary components in the simulated population (Population B) and the value of f_4 , the line where a series of f_4 values are located will pass through the zero point when the ratio of the two components is consistent with that in the population being tested (Loosdrecht et al. 2018; Bai et al. 2024).

Specifically, in $f_4(A, B; C, O)$, (1) Population A contains two population components, $i\% x$ and $j\% y$, where $i+j=1$ and is a fixed constant; (2) Population B is a series of populations generated by the simulation method and consists of $a\%x+b\%y$ with $a+b=1$; Population C is a 100% x component; and (4) O is an outgroup.

We know $f_4(A, B; C, O) = (p_A - p_B) \times (p_C - p_O)$, where p_X denotes the frequency of a given allele in population X. So, $p_A = i \times p_x + j \times p_y$; $p_B = a \times p_x + b \times p_y$; $p_C = p_x$; $p_O = 0$.

Then, $f_4(A, B; C, O) = (i-a) \times p_x^2 + (a-i) \times p_x p_y$ is a linear equation with respect to the variable a .

Finally, $f_4(A, B; C, O) = (p_x \times (i-a) + p_y \times (j-b)) \times p_x = 0$, only if all 3 of the following conditions are met at the same time:

(1) $i+j=1$; (2) $a+b=1$; (3) $a=i$ ($j-b=(1-i)-(1-a)=a-i$).

Also, the conditions that need to be satisfied simultaneously in more complex populations ($A=i\%x+j\%y+k\%z+\dots$) can be further derived from the equation:

(1) $i+j+k+\dots=1$; (2) $a+b+c+\dots=1$; (3) $a=i, b=j, c=k, \dots$

So the simulation can be used to demonstrate whether the population components in the tested population A contain only the population components calculated by qpAdm, and to verify if the corresponding component proportions are the same as the proportions of each component calculated by qpAdm when the value of the linear distribution f_4 crosses the zero point. ”

I remain unconvinced by the authors' response regarding the absence of archaeological evidence for

the timing of admixture. While paleogenomic data can indeed offer valuable insights where historical records are sparse, it's crucial to recognise that the Aceramic period is marked not by a total absence of archaeological artifacts but specifically by the sudden disappearance of pottery use. Given that this period spans nearly 2,000 years, it raises significant questions about the extent to which any inferred genetic connections between the Southern Ryukyu Islands and the Shandong region can be supported without corresponding archaeological evidence.

One specific point I noticed is that the authors presented, in Table S5(b), a three-way admixture model, involving YR_LN, YJGroup (a Shandong population), and Jomon, that significantly better explains the genetic makeup of Nagabaka_historic than a two-way admixture model of YJGroup and Jomon. This is supported by a nested p-value of 0.04. This finding is noteworthy when compared with the Kofun genetic ancestry, which is sufficiently explained by a three-way admixture model involving YR_LN, AR/WLR, and Jomon. Therefore, the key difference between genetic ancestry of Kofun and Nagabaka_historic populations appears to lie in the origin of the second ancestry component—Shandong populations in one case versus AR/WLR in the other.

The primary evidence supporting a link between the Southern Ryukyu Islands and Shandong during the Aceramic period is the admixture timing estimated under the two-way admixture model of YJGroup and Jomon. However, given the potential limitations of this model, I am cautious about interpreting this result as strong evidence of a connection specifically during the Aceramic period.

Additionally, I would like to point out that the literature cited in the revised manuscript to support this connection does not appear to be relevant to the Aceramic period in the Ryukyu archipelago. Instead, these sources document interactions during the Ryukyu Kingdom, which began in the 14th-15th centuries AD, significantly later than the period estimated by the authors. If the authors wish to make a strong claim about a connection between these regions during the Aceramic period, it is essential to identify and cite literature that specifically addresses this era. The current references do not adequately support such a claim.

[Response: Thanks for for your good suggestions and advices. As you suggestion, we responded and revised as follow.](#)

Overall, the claim of gene flow from the Shandong region to the Southern Ryukyu Islands around 1400-1600 BP would benefit from a more cautious framing. I recommend the following revisions to clarify the interpretations in the manuscript:

1. Emphasise the three-way admixture model: Highlight the three-way admixture model involving YR_LN, YJGroup, and Jomon, as this seems to most accurately represent the genetic profile of the Nagabaka_historic population based on the rotating qpAdm analysis.

[Response: Thanks for your comments. We have now highlighted both the three-way and two-way admixture fits the data, and the overall results are consistent with each other. Specifically, while the fitness of this model \(YR_LN + YJGroup + Jomon\) is slightly lower than the two-ancestry model \(\$p_{\text{nest}} = 0.04 < 0.05\$ \), there is no contradiction between the two models, since the ShanDong population is itself mixed with YR-related components. We have now further emphasized the three-way admixture model of the Nagabaka population in the main text: “We also found that the YR_LN, the LongShan ShanDong \(YJGroup/CZYGroup\), and the Jōmon populations were included in the](#)

best three-ancestry model (but when comparing with two way model, the $p_{\text{nest}} = 0.04$, which is smaller than 0.05, Table S5). The three-ancestry and two-ancestor models do not contradict with each other, because these ShanDong populations (CZYGroup and YJCGroup) already carry northern inland East Asian (YR related) and coastal East Asian components, where the coastal East Asian component is specific to ShanDong populations and was not identified in previously sampled ancient East Asian populations. Previous analysis of the historical Nagabaka population showed that they had a three-ancestry model composed of Jōmon ancestry, northern East Asian ancestry, and an ambiguous ancestry related to the present-day Han population. Here we found that a coastal East Asian ancestry related to the ShanDong population better represents the ancestry represented previously by the Han, where the historical Nagabaka population is best described by a three-ancestry model as a mixture of Jōmon ancestry, northern inland East Asian related to YR populations, and additional northern coastal East Asian ancestry related to ShanDong populations.” in Line 358 - 373.

2. Reconsider the use of DATES in the two-way admixture model: For Nagabaka_historic, please reconsider the inclusion of the DATES analysis under the two-way admixture model (YJCGroup and Jomon). Since DATES is generally less suited for admixture models with three distinct ancestral sources, it may lead to less reliable results in this case.

Response: Thanks for your suggestion. We now only include DATEs results as complementary information, and also highlighted that the link to the historical events is potential and needs to be verified by additional archeological data.

We now revised this part as “The most likely timing of the admixture is estimated to be 1,600 - 1,400 BP with a generation time of ~28 years (LJZGroup is the best fit for the ancestral source, with smallest $\text{nrmsd} = 0.180$, $\text{mean} = 43.3$, Table S7). While this can be potentially linked to the population interaction events of the time between the Sui Dynasty (around 1,400 BP) and the Ryukyu Islands populations in historical documentations (e.g. “Sui Shu”, “Chuzan-sefu” and “Chuzan-sekan”)⁵⁴, the specific historical events needs to be further supported by archaeological evidence.”, in Line 428 - 434.

3. Revise Figure 4B to depict potential connections more tentatively: Rather than specifying gene flow dating from Shandong to Nagabaka, consider using a dotted or dashed line to indicate a potential connection between the regions. This will allow you to illustrate the possible relationship without implying a precise date, as the DATES results may be affected by model misspecification.

Response: Thanks for your comments. We revised it now as your suggestion.