

Ancient DNA and spatial modeling reveal a pre-Inca trans-Andean parrot trade

Corresponding Author: Dr George Olah

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)

Authors provide a robust and thoroughly argued study. They integrated an innovative set of methods that highlights novel contributions to understanding pre-Inca trade networks and the symbolic importance of Amazonian species.

With these productive contributions in mind, the authors would benefit from further reflection on their rejection of locally raised and bred macaws and parrots. First, they mention that the nucleotide diversity measure convinces them of procurement from the Amazon, but they do not define a threshold that would be clear evidence for this interpretation. Furthermore, a clear idea of origin would benefit from strontium analyses (in particular after reading the supplemental information that the authors analyzed strontium isotopes in human burials from this site). Finally, the dietary interpretation that these animals were fed C4 plants, like maize, to account for enriched carbon and nitrogen isotope results is complicated by the fact that these are bulk analyses from feathers that were likely growing in the months and years leading up to their deaths. Can these authors account for the temporal growth of feathers? How does this complicate their interpretations? Any idea of the age of animals from which these feathers were plucked? Does that add another layer to their argument for non-local procurement and not local breeding?

Line comments:

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Line 136: How do the authors quantify or qualify "highly variable" mitochondrial genomes to interpret non-local procurement rather than local breeding?

Line 137-140: The authors use nucleotide diversity measures, but how many generations of local breeding would lower this diversity measure? Is it within one generation?

Line 145-147: Which features from which species were isotope analyses applied to?

Line 148-149: Wouldn't strontium analysis be another measure to elucidate origin?

Line 179-182: Earlier Moche period iconography also often depicts Amazonian species, including monkeys and plants from the selva, thus indicating long-held ties to the Amazonian Basin in northern Peru.

Line 220-222: It would be useful to consider strontium isotope analyses to clarify the origin of these feathers.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)

The authors present a highly interesting and well-written manuscript that investigates ancient trade patterns and natural resource use in the Andean region. They demonstrate an excellent example on how to use and integrate a range of different methodologies to reconstruct behaviours in the past by linking archaeological and modern data, and this study will serve as a good example for future research.

I am no expert on all of the methods used in this paper, but the analyses seem sound and conclusions are well founded on the relatively clear results. The paper is also well written and structured. I thus have no major concerns about this work. I understand that this is a highly explorative study, but I would, however, have liked to see a clearer definition of the research questions (hypotheses) that they are investigating, and some more background on various past theories/evidence on the subject.

Minor points:

- The identified trade patterns are partly based on the fact that these parrot species are rainforest species that are not found in the area today. That is a sound argument, but for those who are not familiar with the history of the region it would be good with some text about the environmental conditions being similar also in the study period (i.e. no rainforest in the area or surrounding area), or if there are any faunal historical studies of the species.
- It is a bit hard to identify which bundles/bundle contexts that were actually sampled. Is it only A, C and W? Or also B, or more?
- Main text, lines 46-73: This reads much like the abstract. It would be great to have some more background info, context and research questions here (see e.g. various points above)

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)

Olah et al. use an interdisciplinary approach to identify bird species from feathers found in a pre-Incan archaeological site and model trade routes, revealing new insights into the culture, subsistence practices, and behaviours of the pre-Hispanic peoples of Peru. They use a novel modelling approach to predict trade routes, which may be applied to other contexts. I thought this was a very interesting way of integrating different data streams together and found the amount of information that was able to be gleaned impressive. The results were also unexpected and change the way pre-Incan society is viewed.

I reviewed this paper for another journal, and I found it noteworthy and publishable then. Since that time, the minor revisions I suggested have been addressed, and additional methods have been integrated, namely, dating, stable isotope analysis, and extra genetic analysis, which has further strengthened the paper. The paper is well-written and clear, and methods are sound (from what I can judge, which is mostly the genetic aspect, not the modelling), and are detailed extensively in text and the Supplementary Information. Conclusions are supported by the results and their arguments are convincing. I don't think I can offer any suggestions that would make substantial improvements to the paper and would be happy to see it published as-is.

(Remarks on code availability)

The link above to the code didn't work for me, perhaps it's not been made publicly available yet?

Reviewer #4

(Remarks to the Author)

Very interesting and well-written article. I am a spatial ecologist, not an archaeologist, so I reviewed only the species

distribution model (SDM) and connectivity model sections. In general, it seems best practices were followed, and no obvious flaws in the analysis, but please see my comments below, which include suggestions to explain important methodological details.

Regarding code availability, I could not confirm anything because the Figshare link did not work. It seems the authors will eventually archive the R code to reproduce the SDMs, but it would be good if they could also archive the Julia code to reproduce the Circuitscape models.

Note: Throughout, instead of referring to the SDMs as "paleobiogeographic models", I think it is more accurate to use the term "hindcasted species distribution models".

L164: I wouldn't call species distribution models "landscape models" because they are modeling species, not the landscape.

L165: Please call these "potential distributions" instead of "likely habitats".

L519: Please use "were implemented with the application Wallace EcoMod, available from the wallace R package". Also, please include the exact version number.

L523: What does "made by humans" refer to? In comparison to what kind of occurrence data? I believe nearly all the data on eBird and GBIF is made by humans.

L524: Please define "geospatial issues" here. And how did you define each species' "distributional ranges"? What reference was used?

L526: The reference to R and citation only needs to be included once.

L527: I would call these bioclimatic variables.

L529: How was spatial thinning done? If you used a package, please cite it.

L531: Please fix the grammar in this sentence. Maybe an "and" before 532?

L533: If you used Wallace, Maxent did not sample the background points, Wallace did. Also, please capitalize Maxent.

L538: What threshold was used to calculate omission rates? This is important for reproducibility. Also, in what order were these conditions applied? In other words, how were they considered together?

L539: You don't need to include the fact that you looked at the models. I would just specify how you made your projections (cloglog transformation).

L542: Please change to "Continuous predictions of habitat suitability were converted into binary predictions that indicate presence/absence predictions for each species using 'minimum training presence or 10 percentile' as threshold..." -- but here, why did you use two thresholds? Minimum training presence is the minimum predicted value, so it's different from 10 percentile. Were two thresholds used for all species? The explanation after this refers only to the 10 percentile threshold (L545-546).

L563: Did you use river line data for this analysis? If so, please cite the dataset. If not, how did you identify these rivers?

L565: Aren't these typically called "cost values" or "resistance values"?

L571: "based on circuit theory"

L573: "A pairwise modeling approach"

L573-575: It would aid the reader to break down what "resistance" and "current" mean in this context.

L576: Do you mean here the potential distribution predictions? If so, please use this terminology because they are predictions.

L577: Why didn't you use Circuitscape to calculate the paths? Can you explain?

(Remarks on code availability)

The code was not available because the Figshare link did not work.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have greatly improved their manuscript. Previous comments were thoroughly addressed, and the manuscript is now ready for publication. I leave one comment below for consideration.

Line comments:

Line 106: The phrase "a region where environmental conditions have remained stable for millennia" does not explain fluctuations in the region from ENSO cycles. Please consider adding a short mention of these climatic phenomena.

(Remarks on code availability)

Reviewer #4

(Remarks to the Author)

Thanks for addressing the comments I made on the last draft, and I was able to access and verify the code this time. I am satisfied with the changes you have made, but I have a few last edits.

1) For the title, I think "spatial modeling" would more effectively describe both the species distribution models and circuitscape models than "computational modeling", which is not informative because nearly all model are "computational".

2) L635: Remove the v2 after Wallace because now you list the version of the package.

3) L640: Change to "the basis of record field was 'made by humans',"

4) L651: Change to "with the block setting". However, if you only used AICc (which does not use cross-validation) to select models and did not report the cross-validation statistics, there is no point in reporting that you did spatial cross-validation (because you did not use the results to select models). In that case, I would rephrase this paragraph as:

"For modeling, we first defined a training background of the country borders of Peru with a 0.5° buffer, then used 'wallace' to randomly sample 532 background points. We then built species distribution models using the algorithm Maxent, which estimates the species' responses to environment variables. We tuned the complexity of models by testing a set of feature classes (L = linear, LQ = linear-quadratic, H = hinge, LQH = linear-quadratic-hinge, and LQHP = linear-quadratic-hinge-product) with a regularization multiplier of value 1 (the default penalty against complexity in Maxent), without clamping (i.e., unconstrained extrapolation). We selected optimal model complexity settings by choosing the model with a delta AICc of 0."

I do not understand "in 10 parallel runs". Do you mean you ran this in parallel? Or do you mean you ran Maxent 10 times? If so, why? It doesn't seem like you explored uncertainty among runs, or something similar.

And here is a rewrite of the next paragraph:

"We transferred the optimal models to the environmental variables for the year 1000 CE in our study area (defined above). We made model predictions with the 'cloglog' transformation, which is bounded between 0 and 1. Continuous predictions of habitat suitability were converted into binary predictions that indicate presence/absence predictions for each species using the '10 percentile' threshold, omitting all regions with habitat suitability lower than the suitability values for the lowest 10% of occurrence records within Peru."

If these changes are made, I would be happy to recommend this for acceptance should the other reviewers agree.

(Remarks on code availability)

I reviewed the Wallace code briefly to confirm that it represents the output of the software, but I did not run the code. I did not review the Julia code because I am not proficient in this language.

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Detailed response to Reviewers
Manuscript ID: NCOMMS-25-65964-T
Title: Ancient DNA and computational modeling reveal a pre-Inca trans-Andean parrot trade

Reviewer #1

Authors provide a robust and thoroughly argued study. They integrated an innovative set of methods that highlights novel contributions to understanding pre-Inca trade networks and the symbolic importance of Amazonian species.

With these productive contributions in mind, the authors would benefit from further reflection on their rejection of locally raised and bred macaws and parrots.

We thank the reviewer for their constructive assessment and for the insightful comments that will undoubtedly strengthen our manuscript. We address each of their points below. Regarding the “rejection of locally raised and bred macaws and parrots” commented by the reviewer, please note that we have made pertinent comments in our manuscript including the absence of any documentation of the local and *in situ* (i.e. the site of Pachacamac) breeding/maintenance of these birds. The climatic condition at Pachacamac and the surrounding area of the Central Coast is inhospitable for these birds for much of the year, as noted in the text. In addition, we have already considered the possibility of these birds imported from the North Coast (being raised and maintained at Chan Chan, the capital of contemporary Chimú Empire that maintained outposts in the Upper Amazonia).

First, they mention that the nucleotide diversity measure convinces them of procurement from the Amazon, but they do not define a threshold that would be clear evidence for this interpretation.

The reviewer poses a reasonable question. A single pre-defined threshold for nucleotide diversity, however, is not feasible, and it depends on the species and on the study performed. Our conclusion is instead based on a comparative analysis of our Pachacamac samples against well-defined reference groups. Unfortunately, the number of available *Ara* mitochondrial genomes to be used as comparison is very low, but estimates of nucleotide diversity (π , π_i) are robust even with small sample sizes. This is why we can compare the values obtained for our three individuals (Haplogroup 4) with other individuals considered to be from wild and non-wild populations (see Table S13).

However, to strengthen this comparison, as per the reviewer’s suggestion, we have now incorporated data from a broad Scarlet Macaws study, although the mitochondrial sequence size is limited to ~2,200 bp (Schmidt et al. 2020. *Ibis*, 162:735–748), which might have a potential impact on the π calculation. This study reports an average nucleotide diversity for the wild Haplogroup 4, the same haplogroup we identified, of $\pi = 0.00231$ (SD = 0.00032). Our calculated diversity for the Pachacamac macaws ($\pi = 0.00223$) falls squarely within this range of the wild average. Schmidt et al. (2020: Table 1) also measured genetic diversity (π) in other *A. macao* haplogroups being higher (about 0.003–0.002) in those with a greater sample size ($n = 29–37$), whereas, smaller π values (0.00065 or 0.00095) are displayed for haplogroups with a smaller sample size ($n = 6$, but ~ 0.001 for haplogroups with also smaller sample size). So even the smaller π values, considered as low diversity haplogroups by these authors, are ~10x higher than the π values we calculated for Haplogroup 6 individuals

(0.00005), whereas higher π values in Schmidt et al. (2020), and thus considered as haplogroups with high diversity, are similar to our values calculated for the Pachacamac samples.

Furthermore, this value is over 40 times higher than the diversity found in ancient macaws from known captive breeding programs ($\pi = 0.00005$). This stark difference, combined with the direct match to wild population data, provides compelling evidence that the Pachacamac individuals were sourced from the wild. We have revised the main text to include this new comparative data and to explicitly state that our inference is based on this dramatic relative difference from captive populations and the match with wild populations.

Furthermore, a clear idea of origin would benefit from strontium analyses (in particular after reading the supplemental information that the authors analyzed strontium isotopes in human burials from this site).

Line 148-149: Wouldn't strontium analysis be another measure to elucidate origin?

Line 220-222: It would be useful to consider strontium isotope analyses to clarify the origin of these feathers.

The reviewer's suggestion to use strontium isotope analysis is highly insightful, and we thank them for pushing us to clarify our methodological choices. While strontium is a powerful provenancing tool, its application to our feather samples—although tested in modern individuals (e.g. Font et al. 2007. *J. Anal. At. Spectrom.*, 22:513–522; Evans & Bullman 2009. *Applied Geochem.*, 24:1927–1933; Crowley et al. 2021. *Ecosphere*, 12:e03765)—is complicated by significant geochemical challenges specific to our study site.

The primary issue is the well-documented daily 'sea spray effect' at the beach-front location of Pachacamac. This effect introduces marine-derived strontium, which has a globally homogenizing effect on $^{87}\text{Sr}/^{86}\text{Sr}$ ratio in coastal areas (e.g. Whipkey et al. 2000. *Chem. Geol.*, 168:37–48; Snoeck 2014. *Quat. News.*, 134:37–39; Alonzi et al. 2020. *J. Archaeol. Sci. Rep.*, 33:102462). This marine input can significantly modify the local overall terrestrial strontium ratio of our samples, potentially masking the true geological signature of the coast and making it indistinguishable from certain inland Amazonian or other regions.

Furthermore, a precise location of origin would require a much more accurate and detailed isotopic baseline than currently available for potential food sources. The diet of captive birds may have included items like maize grown in and transported from various unknown locations, which would further complicate the interpretation of a 'local' Sr signal in the feathers.

Given that any potential signal would be confounded by these factors, we concluded that the results would be possibly uninterpretable or would not provide information that would clarify our case. Therefore, we decided that the destructive sampling of these invaluable artifacts for strontium analysis was not justified.

Finally, the dietary interpretation that these animals were fed C4 plants, like maize, to account for enriched carbon and nitrogen isotope results is complicated by the fact that these are bulk analyses from feathers that were likely growing in the months and years leading up to their deaths. Can these authors account for the temporal growth of feathers? How does this complicate their interpretations? Any idea of the age of animals from which these feathers

were plucked? Does that add another layer to their argument for non-local procurement and not local breeding?

The reviewer raises several crucial points about the interpretation of stable isotope data from feathers. We appreciate the opportunity to elaborate on our reasoning. The reviewer is correct that we cannot determine the age of the birds from isolated feathers, unfortunately.

We agree that a feather provides a dietary ‘snapshot’ corresponding to its specific growth period (weeks to months for a large macaw), not a lifetime average. Our bulk analysis reflects the average diet during this window. But please note that macaws moult most of their feathers in average around once a year (a standard process in all avian species, Jenni & Winkler, 2020). The biology of moult in birds, so these feathers were not necessarily sourced after they died.

We argue that these acknowledged complexities strengthen our conclusion when combined with the genetic data. Our model is that of wild capture followed by transport and captive rearing on the coast. In this scenario the genetic signature reflects the birds’ wild origin, and a feather grown after arrival on the coast would incorporate the local diet (e.g. C4 maize). The isotopic signature we found is exactly this, a coastal dietary snapshot.

If the birds were from a multi-generational, locally bred population, we would expect a consistent coastal isotopic signature across all feathers grown at any point in their lives. However, finding a coastal diet signature in a feather from a bird with a “wild” genetic signature is the precise combination of evidence predicted by our capture-and-transport model. We now explicitly state the ‘snapshot’ nature of feather data in the main text.

Line comments:

Pg 66: Feathers are identified from four parrot species, and the native ranges are hundreds of kilometers away, but the authors don’t clearly identify if the parrots whose feathers were used in these ornaments could have been raised on the coast nearby Pachacamac.

While our manuscript addresses the possibility of local breeding through multiple lines of evidence, we acknowledge that this argument could be stated more directly. Our rejection of the local breeding hypothesis is based on the convergence of three key findings, presented in the last paragraph of the section “Genetic and isotopic data reveal wild origins and a coastal diet”.

First, our genomic data show a high nucleotide diversity, which is very similar in magnitude to wild Amazonian populations and orders of magnitude higher than known pre-Hispanic captive breeding populations (as we elaborated in our previous response). This refutes the idea of a small, inbred local breeding stock.

Second, despite extensive excavations at Pachacamac and other central coast sites, there is a complete absence of the physical evidence expected from a breeding program (e.g. adobe cages, eggshells, or a large number of skeletal remains).

Third, the temperate and humid climate of the central coast is not well-suited for breeding these tropical species. The site of Pachacamac that overlooks the Pacific is frequently subjected to dense and salty fogs and even salty splashes from the Pacific. The local climate is inhospitable to these Neotropical birds.

To make this argument clearer for the reader, we have added a summary statement at the beginning of the paragraph that explicitly states our conclusion.

Line 105: Were the 25 feathers from the same object?

No, they were sourced from eight different locations within the deposit. This is now included in the text.

Line 105: What class of clean lab was the “dedicated aDNA facility”?

Generally, the ancient DNA laboratories do not need to be accredited following any ISO category of clean laboratories, as the main purpose of the protective measures is to avoid contamination of the sample with exogenous and/or contaminant DNA. Ancient DNA laboratories (such as the one at ACAD used to process the samples) should at least be physically isolated from regular molecular biology laboratories where DNA is routinely amplified, have different rooms for different purposes (dressing up with PPE, sample processing, molecular work, etc.), automated UV lights, positive air pressure, HEPA filters, wearing PPE (e.g. single-use hooded overall, gloves, facemask, visor), etc. Other protective measures can be implemented, but it will depend on the laboratory. More details are available in Llamas et al. 2017. *STAR Science & Technology of Archaeological Research*, 3(1):1–14. We added the name of the laboratory in the main text to avoid any confusion.

Line 109: What number is the “majority” of colorful feathers?

We rephrased the sentence as all feathers were identified as parrots, except one that was a local gull.

Line 113: So the small seabird is from a local species?

The species winters along the Peruvian coast. We modified the sentence accordingly.

Line 116: So, the macaw and amazon parrot species are found all along the eastern region of Peru from Loreto, to Madre de Dios to Cusco?

Yes, these species are distributed across the eastern region of Peru, as shown in Figure S9.

Line 118: Interesting that the Sabine’s Gull is migratory. Maybe this factored into the significance of using this species in the feathered ornament?

Sabine Gull, being a pelagic (open sea) bird is commonly found on offshore islands during its breeding season. It may have been captured on or near an offshore island (there are a string of offshore islands near the site of Pachacamac). We believe white feathers from this gull were used because its tail provides good size white feathers. In addition, adult birds partially moult in their wintering area (i.e. offshore islands along the Peruvian coast) and their white feathers can be collected on the offshore islands close to Pachacamac.

Line 136: How do the authors quantify or qualify “highly variable” mitochondrial genomes to interpret non-local procurement rather than local breeding?

Our qualitative description “highly variable” is based on direct quantitative comparisons. We have quantified this in two ways: (1) we calculated the nucleotide diversity for our Pachacamac Scarlet Macaw samples as $\pi = 0.00223$; and (2) we qualified this value by comparing it to external datasets (as described above and Table S13). We have revised the text to ensure this explicit quantitative comparison is presented immediately alongside any qualitative description, making the basis for our interpretation clearer.

Line 137-140: The authors use nucleotide diversity measures, but how many generations of local breeding would lower this diversity measure? Is it within one generation?

The precise rate at which genetic diversity would be lost in a captive population depends on several unknown variables, including the effective population size of the founding flock and the specific mating system employed. Therefore, we cannot calculate an exact number of generations. However, based on population genetics principles, a significant drop in diversity would occur immediately in the first generation due to the founder effect, assuming the number of founding individuals was small, as would be expected for a pre-Hispanic trans-Andean transport scenario. Subsequent inbreeding would then accelerate this loss of heterozygosity in the following generations. Crucially, our data show nucleotide diversity that is not lost at all, and it is consistent with a large, outbred wild population. Our evidence indicates that no such bottleneck and subsequent inbreeding process occurred.

Line 145-147: Which features from which species were isotope analyses applied to?

The stable isotope analysis was performed on a subset of five ancient and five modern feathers due to the destructive nature of the analysis. The analysed ancient samples include one Scarlet Macaw feather, one Blue-and-yellow Macaw feather, two Red-and-green Macaw feathers, and one Southern Mealy Amazon feather. Modern samples include two Scarlet Macaw feathers, two Red-and-green Macaw feathers and one Blue-and-yellow Macaw feather. We have amended the text and added a reference to Table S1, where we clearly list the specific sample numbers and corresponding species that were subjected to stable isotope analysis.

Line 179-182: Earlier Moche period iconography also often depicts Amazonian species, including monkeys and plants from the selva, thus indicating long-held ties to the Amazonian Basin in northern Peru.

It is widely known that Cupisnique, Chavín, Moche, and other pre-Hispanic art styles depict animals that are presumably of the Amazonian origin. True taxonomic identity and geographical origins of the plants and animals depicted in them, however, are often difficult to define as many species that are often assumed to be Amazonian existed or still exist on the North Coast of Peru. Examples include the boa (*Boa constrictor*) and jaguar (*Felis onca*). There is also a debate to the true identity of the so-called Amazonian black cayman (*Melanosuchus niger*) and harpy eagle (*Harpia harpyja*) in the Chavín art; if they might be the Pacific crocodile (*Crocodylus acutus*) and osprey (*Pandion haliaetus*) instead. Amazonian plants are similarly difficult to identify conclusively from ceramic representations. We integrated this information into the paragraph also making a reference to the fact that spider monkey (*Simia paniscus*) and Amazonian parrots were depicted in the Moche, Nasca, and other pre-Hispanic ceramics styles.

Reviewer #2

The authors present a highly interesting and well-written manuscript that investigates ancient trade patterns and natural resource use in the Andean region. They demonstrate an excellent example on how to use and integrate a range of different methodologies to reconstruct behaviours in the past by linking archaeological and modern data, and this study will serve as a good example for future research.

I am no expert on all of the methods used in this paper, but the analyses seem sound and conclusions are well founded on the relatively clear results. The paper is also well written and structured. I thus have no major concerns about this work. I understand that this is a highly explorative study, but I would, however, have liked to see a clearer definition of the research questions (hypotheses) that they are investigating, and some more background on various past theories/evidence on the subject.

We thank the reviewer for their constructive and encouraging feedback. We appreciate their assessment of our study as a valuable example for future research. We have revised the manuscript to incorporate all suggestions, primarily by clarifying our research hypotheses, providing more explicit environmental context, and ensuring sample provenance is clear. We also extended the introduction with the information requested for a fuller background to the topics.

Minor points:

- The identified trade patterns are partly based on the fact that these parrot species are rainforest species that are not found in the area today. That is a sound argument, but for those who are not familiar with the history of the region it would be good with some text about the environmental conditions being similar also in the study period (i.e. no rainforest in the area or surrounding area), or if there are any faunal historical studies of the species.

We had implicitly assumed the reader would know that the Peruvian coast is generally hyper-arid except for roughly triangular valleys formed by over 50 perennial and seasonal rivers that drain the Andes, but this should be stated clearly. We have added a clause in the first paragraph of the section “An Elite Ychsma Tomb...” to explicitly state that Pachacamac is located in a hyper-arid desert.

- It is a bit hard to identify which bundles/bundle contexts that were actually sampled. Is it only A, C and W? Or also B, or more?

While we mention a few specific bundles for narrative context, the full set of samples came directly or indirectly (explained in Methods, section 1, and Table S2) from multiple bundles or from the floor of excavation levels but definitely related to funerary bundles. We have clarified this in the section where we first introduce the feathers analysed, we have added a direct reference to the Supplementary Information (Table S2) where a comprehensive list of each sample and its specific funerary bundle provenance is provided.

- Main text, lines 46-73: This reads much like the abstract. It would be great to have some more background info, context and research questions here (see e.g. various points above)

We have rewritten the final paragraph of the introduction. Instead of summarizing our findings, it now explicitly states the central hypotheses we are testing regarding the origin

and procurement of the feathers. It frames the paper as a direct investigation into these unanswered questions, setting a much clearer stage for the results that follow.

Reviewer #3

Olah et al. use an interdisciplinary approach to identify bird species from feathers found in a pre-Incan archaeological site and model trade routes, revealing new insights into the culture, subsistence practices, and behaviours of the pre-Hispanic peoples of Peru. They use a novel modelling approach to predict trade routes, which may be applied to other contexts. I thought this was a very interesting way of integrating different data streams together and found the amount of information that was able to be gleaned impressive. The results were also unexpected and change the way pre-Incan society is viewed.

I reviewed this paper for another journal, and I found it noteworthy and publishable then. Since that time, the minor revisions I suggested have been addressed, and additional methods have been integrated, namely, dating, stable isotope analysis, and extra genetic analysis, which has further strengthened the paper. The paper is well-written and clear, and methods are sound (from what I can judge, which is mostly the genetic aspect, not the modelling), and are detailed extensively in text and the Supplementary Information. Conclusions are supported by the results and their arguments are convincing. I don't think I can offer any suggestions that would make substantial improvements to the paper and would be happy to see it published as-is.

We are particularly grateful for the reviewer's continued engagement with our research, and we are very pleased that they recognize how the manuscript has been strengthened since their initial review.

Remarks on code availability:

The link above to the code didn't work for me, perhaps it's not been made publicly available yet?

Indeed, the DOI link of the code only works once the manuscript has been published. We added the new section "Code Availability" after the "Data Availability" following the journal's format. Please use the following direct access link during review:
<https://figshare.com/s/10790803bfd1f6ac3f4a>

Reviewer #4

Very interesting and well-written article. I am a spatial ecologist, not an archaeologist, so I reviewed only the species distribution model (SDM) and connectivity model sections. In general, it seems best practices were followed, and no obvious flaws in the analysis, but please see my comments below, which include suggestions to explain important methodological details.

We thank the reviewer for their time and their valuable feedback from the perspective of a spatial ecologist. We agree that adding methodological details will significantly improve the manuscript's transparency and value to other modelling experts. We have addressed each of the reviewer's specific comments below.

Regarding code availability, I could not confirm anything because the Figshare link did not work. It seems the authors will eventually archive the R code to reproduce the SDMs, but it would be good if they could also archive the Julia code to reproduce the Circuitscape models.

Indeed, the DOI link of the code only works once the manuscript has been published. We have added the codes to reproduce the Circuitscape models in Julia as well. Please use the following direct access link during review: <https://figshare.com/s/10790803bfd1f6ac3f4a> We added the new section “Code Availability” after the “Data Availability” following the journal’s format.

Note: Throughout, instead of referring to the SDMs as "paleobiogeographic models", I think it is more accurate to use the term "hindcasted species distribution models".

Thank you, we updated this terminology across the manuscript.

L164: I wouldn't call species distribution models "landscape models" because they are modeling species, not the landscape.

We agree and now refer to them collectively as “computational modeling approaches”. We also changed the title of the study accordingly.

L165: Please call these "potential distributions" instead of "likely habitats".

Corrected.

L519: Please use "were implemented with the application Wallace EcoMod, available from the wallace R package". Also, please include the exact version number.

Corrected and version number added to the package.

L523: What does "made by humans" refer to? In comparison to what kind of occurrence data? I believe nearly all the data on eBird and GBIF is made by humans.

This is a “basis of record” filter in GBIF, ignoring machine observations, preserved specimens at museums, etc. But you are right that most of the data is made by human observations.

L524: Please define "geospatial issues" here. And how did you define each species' "distributional ranges"? What reference was used?

The “geospatial issues” filter in GBIF is a standard data-cleaning flag that excludes records with common and significant coordinate errors (e.g. invalid or zero coordinates, country-coordinate mismatch, coordinate out of range, etc.).

We used the widely accepted expert range maps provided by BirdLife International to define the known geographic extent for each species within Peru. We have added the appropriate citation for these range maps to the Methods section and included the full reference in our bibliography.

L526: The reference to R and citation only needs to be included once.

Corrected.

L527: I would call these bioclimatic variables.

Corrected.

L529: How was spatial thinning done? If you used a package, please cite it.

We used the package 'spThin' v0.2.0 and now included it with citation.

L531: Please fix the grammar in this sentence. Maybe an "and" before 532?

L533: If you used Wallace, Maxent did not sample the background points, Wallace did. Also, please capitalize Maxent.

We corrected this sentence, which now reads as: "For modeling, occurrence data were first spatially partitioned with block ($k = 4$), then a training background of the area of Peru was used with a 0.5° buffer around the borders. Finally, 532 background points were sampled with 'wallace' by using the Maxent algorithm that estimates the species' response to the environment."

L538: What threshold was used to calculate omission rates? This is important for reproducibility. Also, in what order were these conditions applied? In other words, how were they considered together?

After careful revision, we noted that in the final version, we used the lowest AICc value (i.e. zero) to select the best model. We changed the sentence in the methods accordingly.

L539: You don't need to include the fact that you looked at the models. I would just specify how you made your projections (cloglog transformation).

We shortened the sentence.

L542: Please change to "Continuous predictions of habitat suitability were converted into binary predictions that indicate presence/absence predictions for each species using 'minimum training presence or 10 percentile' as threshold..." -- but here, why did you use two thresholds? Minimum training presence is the minimum predicted value, so it's different from 10 percentile. Were two thresholds used for all species? The explanation after this refers only to the 10 percentile threshold (L545-546).

Corrected and the sentence reads better this way. Indeed, we only used the '10 percentile' threshold in the final analysis. We must have left the 'minimum training presence' text from our preliminary testing.

L563: Did you use river line data for this analysis? If so, please cite the dataset. If not, how did you identify these rivers?

Yes, we used potentially navigable rivers for small boats (around 5–15 meters minimum extent) in South America, and we already cited this dataset (Schielein, 2017).

L565: Aren't these typically called "cost values" or "resistance values"?

Yes, we changed this to “resistance values” throughout this paragraph, thank you for pointing this out.

L571: "based on circuit theory"

Corrected.

L573: "A pairwise modeling approach"

Corrected.

L573-575: It would aid the reader to break down what "resistance" and "current" mean in this context.

We added an explanatory sentence that reads as: “In this framework, ‘resistance’ represents the difficulty of movement across a given landscape feature (e.g., high elevation poses high resistance), and ‘current’ represents the modeled flow of movement, with high-current areas indicating the most probable corridors.”

L576: Do you mean here the potential distribution predictions? If so, please use this terminology because they are predictions.

Yes, we changed the terminology and used “potential present distribution predictions” as the word ‘current’ is ambiguous in the present context.

L577: Why didn't you use Circuitscape to calculate the paths? Can you explain?

Our use of two different tools was a deliberate choice to visualize two distinct but complementary aspects of connectivity. Circuitscape is powerful because it uses circuit theory to model connectivity across all possible pathways simultaneously, highlighting broad corridors of movement (i.e. ‘current flow’) rather than a single optimal route. However, it does not output a single, discrete least-cost path. To complement this view of the broader connectivity landscape, we used a Least-Cost Path algorithm in QGIS. This allowed us to identify the single most efficient route of minimum cumulative resistance, which can be overlaid on the Circuitscape current maps for interpretation. This dual approach allowed us to visualize both the most probable regions for movement and the most efficient path within those regions. We added these reasonings to the corresponding section of the Methods.

Remarks on code availability:

The code was not available because the Figshare link did not work.

Please use the following link during review: <https://figshare.com/s/10790803bfd1f6ac3f4a>

Detailed response to Reviewers
Manuscript ID: NCOMMS-25-65964A

Title: Ancient DNA and spatial modeling reveal a pre-Inca trans-Andean parrot trade

Reviewer #1

The authors have greatly improved their manuscript. Previous comments were thoroughly addressed, and the manuscript is now ready for publication. I leave one comment below for consideration.

We thank the reviewer for their assessment of our revised manuscript and for supporting its publication.

Line 106: The phrase “a region where environmental conditions have remained stable for millennia” does not explain fluctuations in the region from ENSO cycles. Please consider adding a short mention of these climatic phenomena.

We agree that the original phrasing implied a level of climatic stability that overlooks the significant impact of El Niño events on the Peruvian coast. We have amended the text to explicitly acknowledge ENSO cycles while maintaining the point that the prevailing aridity has allowed for exceptional artifact preservation.

The sentence has been revised to read: “The famed religious-funerary center of Pachacamac, situated in the hyper-arid coastal desert of Peru—a region characterized by enduring aridity, punctuated by rains accompanying periodic El Niño Southern Oscillation (ENSO) events—presents a rare window into the sacred ritual practices of Andean societies.”

Reviewer #4

Thanks for addressing the comments I made on the last draft, and I was able to access and verify the code this time. I am satisfied with the changes you have made, but I have a few last edits.

We thank the reviewer for their time and effort in verifying our code and for their constructive guidance throughout the review process. We are glad to hear the previous revisions were satisfactory. We have addressed the final minor edits as detailed below.

1) For the title, I think "spatial modeling" would more effectively describe both the species distribution models and circuitscape models than "computational modeling", which is not informative because nearly all model are "computational".

Thank you, we changed the title accordingly.

2) L635: Remove the v2 after Wallace because now you list the version of the package.

We removed “v2” after Wallace throughout the manuscript, leaving only the version number at the package name: ‘wallace’ v2.1.0.

3) L640: Change to "the basis of record field was 'made by humans',"

We corrected the wording here.

4) L651: Change to "with the block setting". However, if you only used AICc (which does not use cross-validation) to select models and did not report the cross-validation statistics, there is no point in reporting that you did spatial cross-validation (because you did not use the results to select models). In that case, I would rephrase this paragraph as:

"For modeling, we first defined a training background of the country borders of Peru with a 0.5° buffer, then used 'wallace' to randomly sample 532 background points. We then built species distribution models using the algorithm Maxent, which estimates the species' responses to environment variables. We tuned the complexity of models by testing a set of feature classes (L = linear, LQ = linear-quadratic, H = hinge, LQH = linear-quadratic-hinge, and LQHP = linear-quadratic-hinge-product) with a regularization multiplier of value 1 (the default penalty against complexity in Maxent), without clamping (i.e., unconstrained extrapolation). We selected optimal model complexity settings by choosing the model with a delta AICc of 0."

Thank you for pointing out this, we changed the paragraph following your recommendations.

I do not understand "in 10 parallel runs". Do you mean you ran this in parallel? Or do you mean you ran Maxent 10 times? If so, why? It doesn't seem like you explored uncertainty among runs, or something similar.

Indeed, we ran these in 10 cores to speed up the computational process, but this detail is not so important to mention here, so we only kept it in the Wallace_session.Rmd file in FigShare.

And here is a rewrite of the next paragraph:

"We transferred the optimal models to the environmental variables for the year 1000 CE in our study area (defined above). We made model predictions with the 'cloglog' transformation, which is bounded between 0 and 1. Continuous predictions of habitat suitability were converted into binary predictions that indicate presence/absence predictions for each species using the '10 percentile' threshold, omitting all regions with habitat suitability lower than the suitability values for the lowest 10% of occurrence records within Peru."

Thank you, we changed the wording of this paragraph as well, following your recommendations.

If these changes are made, I would be happy to recommend this for acceptance should the other reviewers agree.

We made all these changes in our revised manuscript.

Remarks on code availability:

I reviewed the Wallace code briefly to confirm that it represents the output of the software, but I did not run the code. I did not review the Julia code because I am not proficient in this language.