nature research

Peer Review Information

Journal: Nature Human Behaviour
Manuscript Title: Earliest Evidence for Human Use of Tobacco in the Pleistocene Americas
Corresponding author name(s): Daron Duke

Editorial Notes:

Reviewer Comments & Decisions:

Decision Letter, initial version:

2nd March 2021

Dear Dr Duke,

Thank you once again for your manuscript, entitled "First Evidence for Nicotiana (Tobacco) and its Human Use in Pleistocene North America," and for your patience during the peer review process.

Your manuscript has now been evaluated by 3 reviewers, whose comments are included at the end of this letter. Although the reviewers find your work to be of interest, they also raise some important concerns. We are interested in the possibility of publishing your study in Nature Human Behaviour, but would like to consider your response to these concerns in the form of a revised manuscript before we make a decision on publication.

To guide the scope of the revisions, the editors discuss the referee reports in detail within the team, including with the chief editor, with a view to (1) identifying key priorities that should be addressed in revision and (2) overruling referee requests that are deemed beyond the scope of the current study. We hope that you will find the prioritised set of referee points to be useful when revising your study. Please do not hesitate to get in touch if you would like to discuss these issues further.

1) Please incorporate in your manuscript the additional data requested by Reviewer 3, including details of the Anatidae genera in the faunal assemblage, and a map of the distribution of sampled areas outside the hearth. Please discuss these data in your main text, as requested by Reviewer 3.
2) Please also respond to all other reviewer suggestions, ensuring that your manuscript is well situated in existing data and theory, and is accessible to a broad audience.

Finally, your revised manuscript must comply fully with our editorial policies and formatting requirements. Failure to do so will result in your manuscript being returned to you, which will delay its consideration. To assist you in this process, I have attached a checklist that lists all of our requirements. If you have any questions about any of our policies or formatting, please don't hesitate

to contact me.

In sum, we invite you to revise your manuscript taking into account all reviewer and editor comments. We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

We hope to receive your revised manuscript within two to three months. We understand that the COVID-19 pandemic is causing significant disruption for many of our authors and reviewers. If you cannot send your revised manuscript within this time, please let us know - we will be happy to extend the submission date to enable you to complete your work on the revision.

With your revision, please:

• Include a “Response to the editors and reviewers” document detailing, point-by-point, how you addressed each editor and referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be used by the editors to evaluate your revision and sent back to the reviewers along with the revised manuscript.

• Highlight all changes made to your manuscript or provide us with a version that tracks changes.

Please use the link below to submit your revised manuscript and related files:

[REDACTED]

Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work. Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

Sincerely,

Charlotte Payne
Editor
Nature Human Behaviour

Reviewer expertise:

Reviewer #1: Archaeobotany; north American archaeology

Reviewer #2:Archeaology of intoxicant use; paleoethnobotany; American archaeology

Reviewer #3:Pleistocene north American archaeology

REVIEWER COMMENTS:

Reviewer #1:
Remarks to the Author:
The paper is generally well written, although I would suggest a little expansion/clarification of the ideas in sentences 88-90 to help the non-archaeologist readers. Also possibly inserting “Current” before “Possible” in line 144 (so as to not implying that there might not have been other areas 12,300 years ago.) The backup data on archaeological methods for recovery of the materials in and around the hearth are clearly spelled out, as are the dating activities, and the additional archaeo-botanical results divided.
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Catherine S. Fowler

Reviewer #2:
Remarks to the Author:
Reviewer Report
“First Evidence for Nicotiana (Tobacco) and its Human Use in Pleistocene North America”
Key results: The authors report on the recovery of the first archaeological Nicotiana seeds that can be dated by association to the late Pleistocene.
Validity: The support for cultural deposition is strong based on the number and volume of macrobotanical control samples. Similarly, the assigned dates appear sound given the stratigraphic context on and off site, as well as the precision in AMS radiocarbon of associated samples of charred wood.
Significance: Evidence for tobacco consumption by Pleistocene hunter-gatherer communities is of utmost importance for the understanding for the deep-time relationship between humans and psychoactive substances. The authors’ findings add several thousand years to the confirmed archaeological record for the use of mind-altering drugs on a global scale.
Data and methodology: The recovery of macrobotanical materials followed established protocols. Contextual and environmental control samples were applied and confirm the in situ evidence. Similarly, the discussion of the non-botanical evidence associated with the hearth feature strengthens the argument of cultural deposition. The selection of previously identified Salix charcoal for dating is well-reasoned given the impossibility of submitting the Nicotiana seeds themselves, as well as the post-Pleistocene changes in floral associations in the GSLD.
Analytical approach: Given the scarcity of Nicotiana seed specimens, the analysis of macrobotanical data is limited to presence/absence. The analysis of radiocarbon dates followed established standards.
Suggested improvements: None regarding additional experiments / data. Otherwise, please see point-by-point comments.
Clarity and context: As suggested below, the initial discussion regarding the natural distribution /

spread of members of the Nicotiana genus should integrate recent findings in genomics-based research. Additionally, the conclusions could benefit from an expansion of the discussion surrounding non-domestication plant management.
References: See list of suggested additional references below.

Point-by-point comments:
l. 43 As the authors lead the introductory discussion on the genus (l. 29) rather than a species-specific level, the statement about the South American origin is somewhat misleading. While initial speciation events appear to have developed in South America, the endemic character of some of the genus' members in North America, as well as Australia and Africa, indicates the (geological) time depth of such developments (Särkinen et al. 2013: Table 3). Recent genomics research has shown that even wild N. tabacum and N. rustica emerged around 200 kya (Chen et al. 2014; Sierro et al. 2018). Wild Nicotiana species therefore developed and spread prior to human existence/arrival in the Americas. Clarifying this situation is crucial for the overall argument of the article as the tentative identification for the macrobotanical findings is N. attenuata, a wild tobacco. This supports the authors' idea of early North Americans engaging with (different species of) wild tobacco (l. 61-63, 177) long before the eventual domestication of N. tabacum and N. rustica in the southern hemisphere.
l. 116 Please provide a brief verbal summary of overlapping characteristics such as seed shape, size, seed coat reticulation (as photographs in Fig. 1 only depict the archaeological yet no reference specimens). It might be worthwhile to add Planella et al. (2012) to the list of references to illustrate the potential of combining morphometric and multivariate ordinal data for taxonomic clustering.
l. 128 Are these the authors' own observations or is citable information available? Makings and Solves, for example, provide data for Arizona.
l. 159-170 Given the cultural formation processes described, as well as the pubescent nature of Nicotiana leaves, phytolith analysis could be discussed as an additional data type for hypothesis testing.
l. 167 Typo
l. 178-180 I suggest rephrasing for two reasons: Farming communities established by the latest around 4,000 BP in Western Mesoamerica, a region equidistant to the study area when compared to the Eastern Woodlands. The current phrasing also implies N. tabacum and N. rustica were domesticated as part of the earliest American cultivars. To my knowledge, research on tobacco domestication has not yet produced a timeline which allows for this type of claim.
l. 182-183 The authors could expand on this argument by briefly discussing the perspective of ethnobotanists focusing on plant management by communities closer to the food forager than the food producer end of the spectrum (i.e., Nancy Turner; Kristen Gremillion).
l. 214; 221 References are missing authorship
Methods section: Citations co-occur in two formats.
l. 367 "recovery and collection" or "recovery collection of charred plant remains … collected using …"
l. 379 typo
ED Table 1: Given the acknowledged human interest, do the authors believe it is significant that Chenopodium seeds occur only at the study site and TO-35, the only other locale reporting Potamogeton remains. Could Chenopodium be another taxon whose presence is caused by human introduction / a hearth at TO-35?
Suggested references:

Chen, Ke, François Dorlhac de Borne, Ernö Szegedi, and Léon Otten 2014 Deep sequencing of the ancestral tobacco species Nicotiana tomentosiformis reveals multiple T-DNA inserts and a complex evolutionary history of natural transformation in the genus Nicotiana. The Plant Journal 80(4):669–

682. DOI:10.1111/tpj.12661.

Planella, M . Teresa, Kathy Collao-Alvarado, Hermann M. Niemeyer, and Carolina Belmar 2012 Morfometría comparada de semillas de Nicotiana (Solanaceae) e identificación de semillas carbonizadas provenientes de un sitio arqueológico en Chile Central. Darwiniana 50(1/2):207–217.

Sierro, N., J. N.D. Battey, L. Bovet, V. Liedschulte, S. Ouadi, J. Thomas, H. Broye, H. Laparra, A. Vuarnoz, G. Lang, S. Goepfert, M. C. Peitsch, and N. V. Ivanov 2018 The impact of genome evolution on the allotetraploid Nicotiana rustica - An intriguing story of enhanced alkaloid production 06 Biological Sciences 0604 Genetics. BMC Genomics 19(1):1–18. DOI:10.1186/s12864-018-5241-5.

Mario Zimmermann
Postdoctoral Research Associate
Institute of Biological Chemistry
Washington State University

Reviewer #3:
Remarks to the Author:
In the manuscript “First Evidence for Nicotiana (Tobacco) and its Human Use in Pleistocene North America” the authors present the case for the identification of charred Nicotiana seeds in sediments obtained from a hearth dated to approximately 12,300 cal years ago. As stated, the age of this hearth suggests tobacco was in the Great Basin of North America by the end of the Pleistocene. The researchers discuss how tobacco entered the region and when, given this new information. In addition, the authors discuss the possible ways tobacco seeds entered the hearth and became charred. Likely avenues include use of plant stalks as fuel, inclusion of seeds within the digestive tract (gizzard) of waterfowl that were cooked or discarded in the hearth, and intentional discard of unusable tobacco plant portions. Evidence brought to bear on the various hypotheses was obtained from distribution of plants within the basin and distribution of identified seeds from sediment flotation samples from outside the hearth vicinity.

The authors argue that it is unlikely that tobacco was used as fuel because the location of the site, on a spit between water basins, was an unlikely place for tobacco plants to naturally grow. This conclusion is supported in part by the lack of tobacco seeds in the flotation samples obtained from outside the hearth area. More discussion of this possibility could have included a discussion of soil seed banks and that most tobacco species are annuals that are found in disturbed areas. One could argue that the area of the hearth between water basins is likely a place where early successional seed plants, including tobacco, may have grown naturally.

Another possibility that the seeds were contained in the gizzards of waterfowl that had been discarded into the fire is prompted by the recovery of waterfowl bones, including 586 charred bone fragments and three avian gastroliths within the hearth and an additional 20,000 plus fragments of bone in the area surrounding the hearth. Other charred seeds within the hearth included species known to be waterfowl foods. The seeds from samples distant from the hearth were not dominated by known waterfowl foods. This discussion could have been strengthened by presentation of data about the varieties of waterfowl that were represented in the faunal remains. The family Anatidae includes ducks, geese, and swans. Even at the family level broad differences in seed use or disuse can be

made. The size of faunal elements should provide an easy means to segregate the sample into these categories. Furthermore, ducks can also be divided into divers and dappling (puddle) ducks based on skeletal element morphologies. The implication here is that diving ducks do not eat seeds and thus if the faunal remains represented mainly diving species, duck entrails would immediately be eliminated as a source for the tobacco seeds. If dappling duck genera are indicated, then a more thorough discussion of changing food preferences by season would be helpful as terrestrial seeds are preferred over other sources during some seasons. Because of the controversy of the toxicity of lead shot in ducks, there is a plethora of studies on gizzard and other digestive tract contents of waterfowl in various regions of North America that could be drawn upon for this discussion.

In addition, though the authors state that three gastroliths (gizzard stones) were found, if the gizzards of multiple ducks were thrown into the fire then there should be considerably more gizzard stones in the deposits. Duck gizzard grit size is generally in the very coarse sand particle size 1 to 2 mm in greatest dimension. The sediment within the hearth is stated by the authors to be fine silty clay. Fine silty clay has particles in the 3.9 – 62.5 μm size; considerably finer than the 1 – 2 mm size. The lack of coarser grit size in the hearth sediments would counter-indicate the presence of gizzard contents in the deposits. This, however, does not rule out the presence of esophagus and colon contents. Again, knowing the number of waterfowl in the assemblage would be helpful in this discussion.

The last hypothesis offered by the authors as the most parsimonious is that the tobacco seeds were introduced into the fire directly by site occupants as refuse from intentional use of tobacco leaves and/or flowers. In this scenario the seeds were unintentional components of plant parts consumed, smoked, chewed, or sucked. This scenario is supported primarily on the disqualification of the other two possibilities. There is no archaeological evidence that people used (chewed, smoked, spat, sucked) tobacco at the site. Here the authors could have expanded on the topic that the collection and use of tobacco by Paleoamericans is the most intriguing conclusion for archaeologists, particularly as it relates to the development of tobacco as a cultivated crop some 9000 years later. It has long been asserted that early cultivation of crops centered on early successional weed seed plants, including goosefoot, marshelder, and pigweed, to name a few. Adding tobacco to the list of early important weed seeds that would later become cultivated could draw on the understanding of the relationship between people and wild seed resources and the processes developed by hunter-gatherer groups to bolster that relationship, culminating in domestication of the seed crop. For tobacco, it is not the seed that is the main consumable, but the leaves, stems, and flowers. The seeds, however, are the target of the evolutionary process that eventually led to the domestication of tobacco as we know it today.

I encourage the authors to expand on this study by including a discussion and map of the distribution of sampled areas outside the hearth; include identifications of the Anatidae genera in the faunal assemblage, and expand the discussion of how wild seed domestication processes furthers archaeological understanding of human/plant interactions and the development of cultigens, whether the plants are raised for ritual or subsistence purposes.

The authors tend to use vague terms such as “several” and “few” in instances where exact numbers should be known such as the number of bones exhibiting tool cutmarks and the number of medium and large-mammal bone fragments. The use of exact numbers reinforces the scientific level of the analysis, and thus, perhaps, the results.

Overall the manuscript is well-written and follows a logical presentation of topics.

Leland C. Bement

Author Rebuttal to Initial comments

MEMO

June 3, 2021

To: Editors and Reviewers

From: Daron Duke (corresponding author)

Subject: Review responses

We have addressed all the comments provided by the reviewers and editors, as presented in the table below. To a few of the points more broadly, there were two comments by Dr. Zimmermann (Reviewer #2) of particular relevance to our revision, all for the better. This first is the fact that phylogenetic research has resolved the issue of the distribution of wild species of *Nicotiana* in North America; i.e., that it was widely distributed well before people came to the Americas. This is a relatively recent scholarly development compared to the amount of archaeological literature written prior to the possibility that humans were critical to dispersing tobacco across the continent. We prioritized the latter in our first draft to frame our finding at the Wishbone site, but upon review agree with Dr. Zimmermann and completely overhauled this background.

Secondly, was the request that we add seed description and clarification about overlapping traits among species. We did this, but also took the request to imply that a species call (we were cautious about this and left our determination at the genus level) would be preferable. One of our authors, Adams, has done extensive research on the issue of identifying *Nicotiana* species and has a published record of finding it challenging at best. We took a closer look at the geography of the relevant species and are confident with a classification of *N. attenuata*. We expanded the paragraph referred to by Dr. Zimmerman to justify this.

Reviewer #3 (Dr. Bement) did not make line-specific comments but provided narrative input and concerns. We have noted these by paragraph of his comments in the attached matrix. More broadly, his comments imply, but do not directly state, that he is concerned we have underestimated the potential for tobacco to grow in the wetland and overestimated (or otherwise misinterpreted) the potential for duck entrail contents to have contributed the seeds. We have done our best to address these concerns at scale with further details on the bone assemblage, the subject of hearth erosion, soil conditions

required for tobacco (which are not consistent with the ORBD marsh), clarifications of the gastroliths, and more detailed accounting of the assemblage components altogether.

We made word changes where we thought an improvement or clarification was needed but otherwise refrained from making changes outside of those needed to address the reviewer comments. We also made small changes to our manuscript title based on our revisions related to Dr. Zimmermann's comments; i.e., that finding tobacco naturally in North America is not in itself meaningful given the phylogenetic data. We emphasized human use in our title change.

Thank you very much for your consideration.

Sincerely,
Daron Duke

Reviewer	Line No.	Reviewer Comment	Response and Action Taken
#1	88-90	Expand/clarify ideas to help non-archaeologist readers	We expanded on the subject of Haskett to better frame it in early America but sought to stay brief. We can do more if desired.
#1	144	Insert "current" before "possible"	We chose neither term and instead qualified our description of habitat and the area better in the sentence ahead and behind this line. This more directly addresses reviewer's concern about what we might be implying for the past.
#1	general	Bibliographic style needs attention	References are now clean and consistent with Nature HB style
#1	362, 365-366, 383	Footnote and author/date citations both included	Removed the author/date citations

#2	43	Integrate integrate genomics research demonstrating that wild *Nicotiana* species developed and spread (naturally) across North America well ahead of human entry.	We overhauled the second paragraph of the main text (containing line 43), made some changes to the third paragraph, and added discussion of the implications for the distribution of *N. attenuata* at the end of the Pleistocene at the end of the first paragraph of the final section.
#2	116	Provide a summary of seed characteristics and consder adding Planella et al. (2012) as a citation regarding overlapping traits	We did both of these things and made substantive changes to the paragraph containing line 116. Upon these clarifications we made a more definitive call on the species present at the Wishbone site to be *N. attenuata*.
#2	128	Clarify wether statement about tobacco habitat is an observation or citable	This information is well documented, and we have added key citations, included the one offered by the reviewer.

#2	159-170	Suggests discussing phytolith analysis potential	We had some difficulty with this comment but ultimately added a sentence to the end of the paragraph in this section (line 170). The reviewer is correct that phytolith analysis would be a way of identifying tobacco use (residual), such as on a tobacco-filled quid or other used object, but these microscopic and sparse remains would be highly unlikely to be found in hearth sediment by any practical method. They would also be lost through the manual flotation technique used in our study, which is the preferred and recommended method for finding small macrobotanical remains. In short, the reviewer is not wrong, but to discuss it in full would require more space to express it than seems economical here, and we think the value of residue studies in general is implied. Alternately, if we misunderstood the reviewer's comment, we are happy to make a more directed change.
#2	167	Typo	Fixed "on" to "one"
#2	178-180	Suggests we are underestimating the beginning of agriculture in Mesoamerica as part of our timeline and that we have tied tobacco's domestication with the beginning of agriculture	Our intent in this closing paragraph was to be generalizing with regard to the broader trajectory of agriculture and domestication, so we retained the sentence at large but made some wording clarifications that we hope clarifies. We definitely did under-appreciate the Mesoamerican aspect, but we just changed the date instead of detailing regions of the continent.
#2	182-183	Suggests we discuss further the implications of plant management by foragers	We addressed this subject in the discussion where indicated and made added some preface to the subject at the end of the introduction

#2	214, 221	References missing authorship	Fixed
#2	367	Should read "recovery and collection"	Fixed
#2	379	Typo	Fixed "on" to "one"
#2	ED Table 1	Do we think the Chenopodium seeds in one of the environmental control samples (TO-35) could be cultural?	We do not. This sample is very clearly environmental and not cultural, but we see the value of the question. Related to this reviewers above question about plant management by foragers (lines 182-183), we have addressed the other seed components in the hearth to better contextualize all these issues. We DO interpret the C. berlandieri in the Wishbone hearth to be cultural, but we cannot be certain about the C. spp. given its presence in the TO-35 sample. Chenopodium species are diverse and some may populate the damp areas around of the wetlands, unlike Nicotiana or C. berlandieri, which are well away from their normal habitat at Wishbone.
#3	n/a	In his paragraph 2, suggests tobacco may well have grown on the dry landforms of the marsh	In our second paragraph under "Cultural versus Natural Deposition" we clarified the difference between the "dry" regions of the marsh and tobacco's natural habitat. The main difference is the drainage of the sediments, which are limiting to tobacco and other similarly adapted early successional weedy species (such as others in the hearth, as we also discuss further).

#3	n/a	In his paragraph 3, suggests we could strengthen our discussion about the possibility of the tobacco seeds having been from duck entrail contents by considering the different taxa at the site, diving vs. dabbling ducks, etc.	We provided a more thorough discussion of the bone assemblage to address this issue (paragraphs 3 and 4 under Results), and others presented by reviewer #3 (see below), but we are unable to do so fully. Because of the fragmentary nature of the bone, we could not make confident taxonomic determinations. We instead emphasized body size, consistent with our primary scope to understand human subsistence priorities. We can exclude geese and swans as substantial contributors and add to the assertion that ducks are the primary prey target. As it is, the notion that ducks of any sort would have fed on tobacco plants is remote for all the reasons already presented in the manuscript (and bolstered by some of our revisions), but we addressed it because of the other evidence presented for waterfowl entrails in the hearth. Thus, we have done our best to expand on the waterfowl assemblage and address #3's comments, but we do not feel that what we cannot provide detracts from our central argument for human use of tobacco at the site.

#3	n/a	In his paragraph 4, suggests we should have more gizzard stones in the assemblage	This is addressed to some extent above, but we do not know what the appropriate amount would be because we cannot control for which genera of ducks were present in what proportions or whether they were processed in a consistent manner (i.e., entrails into the hearth or deposited elsewhere for various case-specific reasons) or the eroded extent of the hearth (which we address now in paragraph 5 of the Results). We did not examine grain size differences between the hearth fill and surrounding areas and have no indication from retained sediments that the the differences are substantial. With regard to the counts of gizzard stones, we reduced the number from three to one to emphasize the only definitive item among nine potentials in the site (three in the hearth fill). We added a section of 270 words to the end of the Methods section describing these distinctions. This may be more than is needed, but the implication that gastroliths further attest to the presence of duck entrail contents in the hearth.
#3	n/a	In his paragraph 5, suggests we could expand on discussion of tobacco as part of a complex of early succession weedy plants with big implications for archaeologists' understanding of the ultimate use of tobacco as a cultivated crop	We take this comment to be much as that suggested by reviewer #2, to which we have made several changes to accommodate, as described above. We have added text to the Discussion in this regard, as well as expanded our Results to include description of the other likely culturally used seeds in the hearth and more context for the fostering of these weedy species through burning, etc.

#3	n/a	In his paragraph 6, requests a plan view map of the sampled areas outside the hearth, identifications of Anatidae genera, and more discussion of the human/plant interactions involved in tobacco and any other eventual cultigens	We have added a figure to the manuscript with a plan view of the site area. Further description supporting it is provided in the Methods. The latter two comments have been addressed above, with revisions made for both reviewers #2 and #3.
#3	n/a	In his paragraph 7, suggests we provide more specific accounting of assemblage components	We did this. Our primary changes came with the bone results, but we also added exact counts and clarified our writing for other materials. We sought not to get too far with this, prioritizing aspects that relate directly to the core purpose of the manuscript to explain the finding of tobacco. For example, we gave numbers for flaked stone tools and debris but no further details while we expanded more on the fauna and gastroliths for their pertinence to the hearth contents. In short, we resisted making this an extended site report and have plans for publishing other aspects separately.

Decision Letter, first revision:

8th June 2021

Dear Dr Duke,

RE: "First Evidence for Human Use of Nicotiana (Tobacco) in the Pleistocene Americas"

Thank you for submitting your revised manuscript and for all your work on the revision.

Although your manuscript has been revised in response to reviewer comments, the response to editors and reviewers document does not fully comply with our editorial requirements.

We ask that you please include all reviewer comments verbatim and in full in this document, with your corresponding responses. That is, please include the text of each review in full, and not as paraphrased points. This is to make the review process as straightforward as possible for our reviewers.

Before we can send your manuscript back to reviewers, we ask that you please update your response to editors and reviewers document to ensure that it includes all text (verbatim) from all three reviews.

Please use the link below to submit your revised manuscript and related files:

[REDACTED]

Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

Thank you in advance for attending to these requests and I look forward to receiving your revised submission.

Sincerely,

Charlotte Payne
Editor
Nature Human Behaviour

Author Rebuttal, first revision:

MEMO

June 8, 2021

To: Editors and Reviewers

From: Daron Duke (corresponding author)

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scholarly development compared to the amount of archaeological literature written prior to the possibility that humans were critical to dispersing tobacco across the continent. We prioritized the latter in our first draft to frame our finding at the Wishbone site, but upon review agree with Dr. Zimmermann and completely overhauled this background.

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Daron Duke

Reviewer Comments and Response Matrix:

Reviewer #1:

Remarks to the Author:

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Other editorial comments have to do with the bibliographic style: I’m not sure what your style is, but item 9 lacks an author, as does item 12 (editor?). Is 24 a dissertation or an independent publication? Item 28 lacks the author’s first name. In addition, under “Methods” lines 362, 365-366, and 383 contain both footnote references and the author/date citations. I assume that both are not needed.

Catherine S. Fowler

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Reviewer	Line No.	Reviewer Comment	Response and Action Taken
#1	362, 365-366, 383	In addition, under "Methods" lines 362, 365-366, and 383 contain both footnote references and the author/date citations. I assume that both are not needed.	Removed the author/date citations consistent with NHB style

Reviewer #2:

Remarks to the Author:

Reviewer Report

"First Evidence for Nicotiana (Tobacco) and its Human Use in Pleistocene North America"

Key results: The authors report on the recovery of the first archaeological Nicotiana seeds that can be dated by association to the late Pleistocene.

Validity: The support for cultural deposition is strong based on the number and volume of macrobotanical control samples. Similarly, the assigned dates appear sound given the stratigraphic context on and off site, as well as the precision in AMS radiocarbon of associated samples of charred wood.

Significance: Evidence for tobacco consumption by Pleistocene hunter-gatherer communities is of utmost importance for the understanding for the deep-time relationship between humans and psychoactive substances. The authors' findings add several thousand years to the confirmed archaeological record for the use of mind-altering drugs on a global scale.

Data and methodology: The recovery of macrobotanical materials followed established protocols. Contextual and environmental control samples were applied and confirm the in situ evidence. Similarly, the discussion of the non-botanical evidence associated with the hearth feature strengthens the argument of cultural deposition. The selection of previously identified Salix charcoal for dating is well-reasoned given the impossibility of submitting the Nicotiana seeds themselves, as well as the post-Pleistocene changes in floral associations in the GSLD.

Analytical approach: Given the scarcity of Nicotiana seed specimens, the analysis of macrobotanical data is limited to presence/absence. The analysis of radiocarbon dates followed established standards.

Suggested improvements: None regarding additional experiments / data. Otherwise, please see point-by-point comments.

Clarity and context: As suggested below, the initial discussion regarding the natural distribution / spread of members of the Nicotiana genus should integrate recent findings in genomics-based research. Additionally, the conclusions could benefit from an expansion of the discussion surrounding non-domestication plant management.

References: See list of suggested additional references below.

Point-by-point comments:

l. 43 As the authors lead the introductory discussion on the genus (l. 29) rather than a species-specific level, the statement about the South American origin is somewhat misleading. While initial speciation events appear to have developed in South America, the endemic character of some of the genus' members in North America, as well as Australia and Africa, indicates the (geological) time depth of such developments (Särkinen et al. 2013: Table 3). Recent genomics research has shown that even wild N. tabacum and N. rustica emerged around 200 kya (Chen et al. 2014; Sierro et al. 2018). Wild Nicotiana species therefore developed and spread prior to human existence/arrival in the Americas. Clarifying this situation is crucial for the overall argument of the article as the tentative identification for the macrobotanical findings is N. attenuata, a wild tobacco. This supports the authors' idea of early North Americans engaging with (different species of) wild tobacco (l. 61-63,

177) long before the eventual domestication of N. tabacum and N. rustica in the southern hemisphere.

l. 116 Please provide a brief verbal summary of overlapping characteristics such as seed shape, size, seed coat reticulation (as photographs in Fig. 1 only depict the archaeological yet no reference specimens). It might be worthwhile to add Planella et al. (2012) to the list of references to illustrate the potential of combining morphometric and multivariate ordinal data for taxonomic clustering.

l. 128 Are these the authors' own observations or is citable information available? Makings and Solves, for example, provide data for Arizona.

l. 159-170 Given the cultural formation processes described, as well as the pubescent nature of Nicotiana leaves, phytolith analysis could be discussed as an additional data type for hypothesis testing.

l. 167 Typo

l. 178-180 I suggest rephrasing for two reasons: Farming communities established by the latest around 4,000 BP in Western Mesoamerica, a region equidistant to the study area when compared to the Eastern Woodlands. The current phrasing also implies N. tabacum and N. rustica were domesticated as part of the earliest American cultivars. To my knowledge, research on tobacco domestication has not yet produced a timeline which allows for this type of claim.

l. 182-183 The authors could expand on this argument by briefly discussing the perspective of ethnobotanists focusing on plant management by communities closer to the food forager than the food producer end of the spectrum (i.e., Nancy Turner; Kristen Gremillion).

l. 214; 221 References are missing authorship

Methods section: Citations co-occur in two formats.

l. 367 “recovery and collection” or “recovery collection of charred plant remains … collected using …”

l. 379 typo

ED Table 1: Given the acknowledged human interest, do the authors believe it is significant that Chenopodium seeds occur only at the study site and TO-35, the only other locale reporting Potamogeton remains. Could Chenopodium be another taxon whose presence is caused by human introduction / a hearth at TO-35?

Suggested references:

Chen, Ke, François Dorlhac de Borne, Ernö Szegedi, and Léon Otten 2014 Deep sequencing of the ancestral tobacco species Nicotiana tomentosiformis reveals multiple T-DNA inserts and a complex evolutionary history of natural transformation in the genus Nicotiana. The Plant Journal 80(4):669–682. DOI:10.1111/tpj.12661.

Planella, M . Teresa, Kathy Collao-Alvarado, Hermann M. Niemeyer, and Carolina Belmar 2012 Morfometría comparada de semillas de Nicotiana (Solanaceae) e identificación de semillas carbonizadas provenientes de un sitio arqueológico en Chile Central. Darwiniana 50(1/2):207–217.

Sierro, N., J. N.D. Battey, L. Bovet, V. Liedschulte, S. Ouadi, J. Thomas, H. Broye, H. Laparra, A. Vuarnoz, G. Lang, S. Goepfert, M. C. Peitsch, and N. V. Ivanov 2018 The impact of genome evolution on the allotetraploid Nicotiana rustica - An intriguing story of enhanced alkaloid production 06 Biological Sciences 0604 Genetics. BMC Genomics 19(1):1–18. DOI:10.1186/s12864-018-5241-5.

Mario Zimmermann
Postdoctoral Research Associate
Institute of Biological Chemistry
Washington State University

Reviewer	Line No.	Reviewer Comment	Response and Action Taken
#2	43, 61-63, 177	l. 43 As the authors lead the introductory discussion on the genus (l. 29) rather than a species-specific level, the statement about the South American origin is somewhat misleading. While initial speciation events appear to have developed in South America, the endemic character of some of the genus' members in North America, as well as Australia and Africa, indicates the (geological) time depth of such developments (Särkinen et al. 2013: Table 3). Recent genomics research has shown that even wild N. tabacum and N. rustica emerged around 200 kya (Chen et al. 2014; Sierro et al. 2018). Wild Nicotiana species therefore developed and spread prior to human existence/arrival in the Americas. Clarifying this situation is crucial for the overall argument of the article as the tentative identification for the macrobotanical findings is N. attenuata, a wild tobacco. This supports the authors' idea of early North Americans engaging with (different species of) wild tobacco (l. 61-63, 177) long before the eventual domestication of N. tabacum and N. rustica in the southern hemisphere.	We overhauled the second paragraph of the main text (containing line 43), made some changes to the third paragraph, and added discussion of the implications for the distribution of *N. attenuata* at the end of the Pleistocene at the end of the first paragraph of the final section.

Reviewer	Line No.	Reviewer Comment	Response and Action Taken
#2	116	l. 116 Please provide a brief verbal summary of overlapping characteristics such as seed shape, size, seed coat reticulation (as photographs in Fig. 1 only depict the archaeological yet no reference specimens). It might be worthwhile to add Planella et al. (2012) to the list of references to illustrate the potential of combining morphometric and multivariate ordinal data for taxonomic clustering.	We did both of these things and made substantive changes to the paragraph containing line 116. Upon these clarifications we made a more definitive call on the species present at the Wishbone site to be *N. attenuata*.
#2	128	l. 128 Are these the authors' own observations or is citable information available? Makings and Solves, for example, provide data for Arizona.	This information is well documented, and we have added key citations, included the one offered by the reviewer.
#2	159-170	l. 159-170 Given the cultural formation processes described, as well as the pubescent nature of Nicotiana leaves, phytolith analysis could be discussed as an additional data type for hypothesis testing.	We had some difficulty with this comment but ultimately added a sentence to the end of the paragraph in this section (line 170). The reviewer is correct that phytolith analysis would be a way of identifying tobacco use (residual), such as on a tobacco-filled quid or other used object, but these microscopic and sparse remains would be highly unlikely to be found in hearth sediment by any practical method. They would also be lost through the manual flotation technique used in our study, which is the preferred and recommended method for finding small macrobotanical remains. In short, the reviewer is not wrong, but to discuss it in full would require more space to express it than seems economical here, and we think the value of residue studies in general is implied. Alternately, if we misunderstood the reviewer's comment, we are happy to make a more directed change.
#2	167	l. 167 Typo	Fixed "on" to "one"

Reviewer	Line No.	Reviewer Comment	Response and Action Taken
#2	178-180	l. 178-180 I suggest rephrasing for two reasons: Farming communities established by the latest around 4,000 BP in Western Mesoamerica, a region equidistant to the study area when compared to the Eastern Woodlands. The current phrasing also implies N. tabacum and N. rustica were domesticated as part of the earliest American cultivars. To my knowledge, research on tobacco domestication has not yet produced a timeline which allows for this type of claim.	Our intent in this closing paragraph was to be generalizing with regard to the broader trajectory of agriculture and domestication, so we retained the sentence at large but made some wording clarifications that we hope clarifies. We definitely did under-appreciate the Mesoamerican aspect, but we just changed the date instead of detailing regions of the continent.
#2	182-183	l. 182-183 The authors could expand on this argument by briefly discussing the perspective of ethnobotanists focusing on plant management by communities closer to the food forager than the food producer end of the spectrum (i.e., Nancy Turner; Kristen Gremillion).	We addressed this subject in the discussion where indicated and made added some preface to the subject at the end of the introduction. Turner and Gremillion are now cited multiple times as appropriate for the issue as integrated more broadly.
#2	214, 221	l. 214; 221 References are missing authorship	All references are now clean and consistent with Nature HB style
#2	Methods	Methods section: Citations co-occur in two formats.	Removed the author/date citations consistent with NHB style
#2	367	l. 367 “recovery and collection” or “recovery collection of charred plant remains … collected using …”	Fixed
#2	379	l. 379 typo	Fixed "on" to "one"
#2	ED Table 1	Given the acknowledged human interest, do the authors believe it is significant that Chenopodium seeds occur only at the study site and TO-35, the only other locale reporting Potamogeton remains. Could Chenopodium be another taxon whose presence is caused by human introduction / a hearth at TO-35?	We do not. This sample is very clearly environmental and not cultural, but we see the value of the question. Related to this reviewers above question about plant management by foragers (lines 182-183), we have addressed the other seed components in the hearth to better contextualize all these issues. We DO interpret the C. berlandieri in the Wishbone hearth to be cultural, but we cannot be certain about the C. spp. given its presence in the TO-35 sample. Chenopodium species are diverse and some may populate the damp areas around of the wetlands, unlike Nicotiana and C. berlandieri.

Remarks to the Author:

In the manuscript “First Evidence for Nicotiana (Tobacco) and its Human Use in Pleistocene North America” the authors present the case for the identification of charred Nicotiana seeds in sediments obtained from a hearth dated to approximately 12,300 cal years ago. As stated, the age of this hearth suggests tobacco was in the Great Basin of North America by the end of the Pleistocene. The researchers discuss how tobacco entered the region and when, given this new information. In addition, the authors discuss the possible ways tobacco seeds entered the hearth and became charred. Likely avenues include use of plant stalks as fuel, inclusion of seeds within the digestive tract (gizzard) of waterfowl that were cooked or discarded in the hearth, and intentional discard of unusable tobacco plant portions. Evidence brought to bear on the various hypotheses was obtained from distribution of plants within the basin and distribution of identified seeds from sediment flotation samples from outside the hearth vicinity.

The authors argue that it is unlikely that tobacco was used as fuel because the location of the site, on a spit between water basins, was an unlikely place for tobacco plants to naturally grow. This conclusion is supported in part by the lack of tobacco seeds in the flotation samples obtained from outside the hearth area. More discussion of this possibility could have included a discussion of soil seed banks and that most tobacco species are annuals that are found in disturbed areas. One could argue that the area of the hearth between water basins is likely a place where early successional seed plants, including tobacco, may have grown naturally.

Another possibility that the seeds were contained in the gizzards of waterfowl that had been discarded into the fire is prompted by the recovery of waterfowl bones, including 586 charred bone fragments and three avian gastroliths within the hearth and an additional 20,000 plus fragments of bone in the area surrounding the hearth. Other charred seeds within the hearth included species known to be waterfowl foods. The seeds from samples distant from the hearth were not dominated by known waterfowl foods. This discussion could have been strengthened by presentation of data about the varieties of waterfowl that were represented in the faunal remains. The family Anatidae includes ducks, geese, and swans. Even at the family level broad differences in seed use or disuse can be made. The size of faunal elements should provide an easy means to segregate the sample into these categories. Furthermore, ducks can also be divided into divers and dappling (puddle) ducks based on skeletal element

morphologies. The implication here is that diving ducks do not eat seeds and thus if the faunal remains represented mainly diving species, duck entrails would immediately be eliminated as a source for the tobacco seeds. If dappling duck genera are indicated, then a more thorough discussion of changing food preferences by season would be helpful as terrestrial seeds are preferred over other sources during

some seasons. Because of the controversy of the toxicity of lead shot in ducks, there is a plethora of studies on gizzard and other digestive tract contents of waterfowl in various regions of North America that could be drawn upon for this discussion.

In addition, though the authors state that three gastroliths (gizzard stones) were found, if the gizzards of multiple ducks were thrown into the fire then there should be considerably more gizzard stones in the deposits. Duck gizzard grit size is generally in the very coarse sand particle size 1 to 2 mm in greatest dimension. The sediment within the hearth is stated by the authors to be fine silty clay. Fine silty clay has particles in the 3.9 – 62.5 ❍m size; considerably finer than the 1 – 2 mm size. The lack of coarser grit size in the hearth sediments would counter-indicate the presence of gizzard contents in the deposits. This, however, does not rule out the presence of esophagus and colon contents. Again, knowing the number of waterfowl in the assemblage would be helpful in this discussion.

The last hypothesis offered by the authors as the most parsimonious is that the tobacco seeds were introduced into the fire directly by site occupants as refuse from intentional use of tobacco leaves and/or flowers. In this scenario the seeds were unintentional components of plant parts consumed, smoked, chewed, or sucked. This scenario is supported primarily on the disqualification of the other two possibilities. There is no archaeological evidence that people used (chewed, smoked, spat, sucked) tobacco at the site. Here the authors could have expanded on the topic that the collection and use of tobacco by Paleoamericans is the most intriguing conclusion for archaeologists, particularly as it relates to the development of tobacco as a cultivated crop some 9000 years later. It has long been asserted that early cultivation of crops centered on early successional weed seed plants, including goosefoot, marshelder, and pigweed, to name a few. Adding tobacco to the list of early

important weed seeds that would later become cultivated could draw on the understanding of the relationship between people and wild seed resources and the processes developed by hunter-gatherer groups to bolster that relationship, culminating in domestication of the seed crop. For tobacco, it is not the seed that is the main consumable, but the leaves, stems, and flowers. The seeds, however, are the target of the evolutionary process that eventually led to the domestication of tobacco as we know it today.

I encourage the authors to expand on this study by including a discussion and map of the distribution of sampled areas outside the hearth; include identifications of the Anatidae genera in the faunal assemblage, and expand the discussion of how wild seed domestication processes furthers archaeological understanding of human/plant interactions and the development of cultigens, whether the plants are raised for ritual or subsistence purposes.

The authors tend to use vague terms such as “several” and “few” in instances where exact numbers should be known such as the number of bones exhibiting tool cutmarks and the number of medium and large-mammal bone fragments. The use of exact numbers reinforces the scientific level of the analysis, and thus, perhaps, the results.

Overall the manuscript is well-written and follows a logical presentation of topics.

Leland C. Bement

Reviewer	Line No.	Reviewer Comment	Response and Action Taken
#3	Suggested References	Chen, Ke, François Dorlhac de Borne, Ernö Szegedi, and Léon Otten 2014 Deep sequencing of the ancestral tobacco species Nicotiana tomentosiformis reveals multiple T-DNA inserts and a complex evolutionary history of natural transformation in the genus Nicotiana. The Plant Journal 80(4):669–682. DOI:10.1111/tpj.12661. Planella, M . Teresa, Kathy Collao-Alvarado, Hermann M. Niemeyer, and Carolina Belmar 2012 Morfometría comparada de semillas de Nicotiana (Solanaceae) e identificación de semillas carbonizadas provenientes de un sitio arqueológico en Chile Central. Darwiniana 50(1/2):207–217. Sierro, N., J. N.D. Battey, L. Bovet, V. Liedschulte, S. Ouadi, J. Thomas, H. Broye, H. Laparra, A. Vuarnoz, G. Lang, S. Goepfert, M. C. Peitsch, and N. V. Ivanov 2018 The impact of genome evolution on the allotetraploid Nicotiana rustica - An intriguing story of enhanced alkaloid production 06 Biological Sciences 0604 Genetics. BMC Genomics 19(1):1–18. DOI:10.1186/s12864-018-5241-5.	We read and included each of these references where appropriate in the second paragraph of the main text regarding phylogenetic studies.

Reviewer	Line No.	Reviewer Comment	Response and Action Taken
#3	n/a	The authors argue that it is unlikely that tobacco was used as fuel because the location of the site, on a spit between water basins, was an unlikely place for tobacco plants to naturally grow. This conclusion is supported in part by the lack of tobacco seeds in the flotation samples obtained from outside the hearth area. More discussion of this possibility could have included a discussion of soil seed banks and that most tobacco species are annuals that are found in disturbed areas. One could argue that the area of the hearth between water basins is likely a place where early successional seed plants, including tobacco, may have grown naturally.	In our second paragraph under "Cultural versus Natural Deposition" we clarified the difference between the "dry" regions of the marsh and tobacco's natural habitat. The main difference is the drainage of the sediments, which are limiting to tobacco and other similarly adapted early successional weedy species (such as others in the hearth, as we also discuss further).

Reviewer	Line No.	Reviewer Comment	Response and Action Taken
#3	n/a	Another possibility that the seeds were contained in the gizzards of waterfowl that had been discarded into the fire is prompted by the recovery of waterfowl bones, including 586 charred bone fragments and three avian gastroliths within the hearth and an additional 20,000 plus fragments of bone in the area surrounding the hearth. Other charred seeds within the hearth included species known to be waterfowl foods. The seeds from samples distant from the hearth were not dominated by known waterfowl foods. This discussion could have been strengthened by presentation of data about the varieties of waterfowl that were represented in the faunal remains. The family Anatidae includes ducks, geese, and swans. Even at the family level broad differences in seed use or disuse can be made. The size of faunal elements should provide an easy means to segregate the sample into these categories. Furthermore, ducks can also be divided into divers and dappling (puddle) ducks based on skeletal element morphologies. The implication here is that diving ducks do not eat seeds and thus if the faunal remains represented mainly diving species, duck entrails would immediately be eliminated as a source for the tobacco seeds. If dappling duck genera are indicated, then a more thorough discussion of changing food preferences by season would be helpful as terrestrial seeds are preferred over other sources during some seasons. Because of the controversy of the toxicity of lead shot in ducks, there is a plethora of studies on gizzard and other digestive	We provided a more thorough discussion of the bone assemblage to address this issue (paragraphs 3 and 4 under Results), and others presented by reviewer #3 (see below), but we are unable to do so fully. Because of the fragmentary nature of the bone, we could not make confident taxonomic determinations. We instead emphasized body size, consistent with our primary scope to understand human subsistence priorities. We can exclude geese and swans as substantial contributors and add to the assertion that ducks are the primary prey target. As it is, the notion that ducks of any sort would have fed on tobacco plants is remote for all the reasons already presented in the manuscript (and bolstered by some of our revisions), but we addressed it because of the other evidence presented for waterfowl entrails in the hearth. Thus, we have done our best to expand on the waterfowl assemblage and address #3's comments, but we do not feel that what we cannot provide detracts from our central argument for human use of tobacco at the site.

Reviewer	Line No.	Reviewer Comment	Response and Action Taken
		tract contents of waterfowl in various regions of North America that could be drawn upon for this discussion.	

Reviewer	Line No.	Reviewer Comment	Response and Action Taken
#3	n/a	In addition, though the authors state that three gastroliths (gizzard stones) were found, if the gizzards of multiple ducks were thrown into the fire then there should be considerably more gizzard stones in the deposits. Duck gizzard grit size is generally in the very coarse sand particle size 1 to 2 mm in greatest dimension. The sediment within the hearth is stated by the authors to be fine silty clay. Fine silty clay has particles in the 3.9 – 62.5 mm size; considerably finer than the 1 – 2 mm size. The lack of coarser grit size in the hearth sediments would counter-indicate the presence of gizzard contents in the deposits. This, however, does not rule out the presence of esophagus and colon contents. Again, knowing the number of waterfowl in the assemblage would be helpful in this discussion.	This is addressed to some extent above, but we do not know what the appropriate amount would be because we cannot control for which genera of ducks were present in what proportions or whether they were processed in a consistent manner (i.e., entrails into the hearth or deposited elsewhere for various case-specific reasons) or the eroded extent of the hearth (which we address now in paragraph 5 of the Results). We did not examine grain size differences between the hearth fill and surrounding areas and have no indication from retained sediments that the the differences are substantial. With regard to the counts of gizzard stones, we reduced the number from three to one to emphasize the only definitive item among nine potentials in the site (three in the hearth fill). We added a section of 270 words to the end of the Methods section describing these distinctions. This may be more than is needed, but the implication that gastroliths further attest to the presence of duck entrail contents in the hearth.

Reviewer	Line No.	Reviewer Comment	Response and Action Taken
#3	n/a	The last hypothesis offered by the authors as the most parsimonious is that the tobacco seeds were introduced into the fire directly by site occupants as refuse from intentional use of tobacco leaves and/or flowers. In this scenario the seeds were unintentional components of plant parts consumed, smoked, chewed, or sucked. This scenario is supported primarily on the disqualification of the other two possibilities. There is no archaeological evidence that people used (chewed, smoked, spat, sucked) tobacco at the site. Here the authors could have expanded on the topic that the collection and use of tobacco by Paleoamericans is the most intriguing conclusion for archaeologists, particularly as it relates to the development of tobacco as a cultivated crop some 9000 years later. It has long been asserted that early cultivation of crops centered on early successional weed seed plants, including goosefoot, marshelder, and pigweed, to name a few. Adding tobacco to the list of early important weed seeds that would later become cultivated could draw on the understanding of the relationship between people and wild seed resources and the processes developed by hunter-gatherer groups to bolster that relationship, culminating in domestication of the seed crop. For tobacco, it is not the seed that is the main consumable, but the leaves, stems, and flowers. The seeds, however, are the target of the evolutionary process that eventually led to the domestication of tobacco as we know it today.	We take this comment to be much as that suggested by reviewer #2, to which we have made several changes to accommodate, as described above. We have added text to the Discussion in this regard, as well as expanded our Results to include description of the other likely culturally used seeds in the hearth and more context for the fostering of these weedy species through burning, etc.

Reviewer	Line No.	Reviewer Comment	Response and Action Taken
#3	n/a	I encourage the authors to expand on this study by including a discussion and map of the distribution of sampled areas outside the hearth; include identifications of the Anatidae genera in the faunal assemblage, and expand the discussion of how wild seed domestication processes furthers archaeological understanding of human/plant interactions and the development of cultigens, whether the plants are raised for ritual or subsistence purposes.	We have added a figure to the manuscript with a plan view of the site area. Further description supporting it is provided in the Methods. The latter two comments have been addressed above, with revisions made for both reviewers #2 and #3.
#3	n/a	The authors tend to use vague terms such as “several” and “few” in instances where exact numbers should be known such as the number of bones exhibiting tool cutmarks and the number of medium and large-mammal bone fragments. The use of exact numbers reinforces the scientific level of the analysis, and thus, perhaps, the results.	We did this. Our primary changes came with the bone results, but we also added exact counts and clarified our writing for other materials. We sought not to get too far with this, prioritizing aspects that relate directly to the core purpose of the manuscript to explain the finding of tobacco. For example, we gave numbers for flaked stone tools and debris but no further details while we expanded more on the fauna and gastroliths for their pertinence to the hearth contents. In short, we resisted making this an extended site report and have plans for publishing other aspects separately.

Decision Letter, second revision:

Our ref: NATHUMBEHAV-210113947B

12th July 2021

Dear Dr. Duke,

Thank you for submitting your revised manuscript "First Evidence for Human Use of Nicotiana (Tobacco) in the Pleistocene Americas" (NATHUMBEHAV-210113947B). It has now been seen by the original referees and their comments are below. As you can see, the reviewers find that the paper has improved in revision. We will therefore be happy in principle to publish it in Nature Human Behaviour, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Please do not hesitate to contact me if you have any questions.

Sincerely,

Charlotte Payne

Charlotte Payne, PhD
Senior Editor
Nature Human Behaviour

Reviewer #1 (Remarks to the Author):

You have done a good job in answering the questions/criticisms of the reviewers and the article is improved and much clearer.

Lines 165-82: discussion of the presence of tobacco in quids in the region and its role in potential movements of people into the region:

1) Consideration of quids as a means of tobacco use: Given that Danger Cave and Hogup Cave, both fairly close to this site, contained many quids, it might be worthwhile to point out when and if any of those analyzed to date contained tobacco seeds and if so, what was their dates?

2) I personally find the sentence "should figure prominently in the initial move..." a little strong. People might have been pleased to find tobacco (if already familiar with it) in any new lands they explored, but this almost sounds like they were specifically seeking it as a reason for migration. I don't require the change, but perhaps consider it.

Reviewer #2 (Remarks to the Author):

I very much appreciate and am satisfied with the authors' attention to the comments I provided during review. From my point of view, the revised manuscript is ready for publication and would be a great asset to Nature Human Behavior.

Reviewer #3 (Remarks to the Author):

In the revised manuscript, the authors have adequately addressed my reviewer comments, including those directed at further consideration of the role of waterfowl as a potential source of the seeds. The authors did not add any new concerns from my viewpoint. As it now stands, the article is direct as well as comprehensive in its discussion.

Decision letter, final requests:

** Please ensure you delete the link to your author homepage in this e-mail if you wish to forward it to your co-authors. **

nature research

Our ref: NATHUMBEHAV-210113947B

23rd July 2021

Dear Dr. Duke,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Human Behaviour manuscript, "First Evidence for Human Use of Nicotiana (Tobacco) in the Pleistocene Americas" (NATHUMBEHAV-210113947B). Please carefully follow the step-by-step instructions provided in the attached file, and add a response in each row of the table to indicate the changes that you have made. Please also check and comment on any additional marked-up edits we have proposed within the text. Ensuring that each point is addressed will help to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (preferably within two weeks). Please get in contact with us if you anticipate delays.

When you upload your final materials, please include a point-by-point response to any remaining reviewer comments.

If you have not done so already, please alert us to any related manuscripts from your group that are under consideration or in press at other journals, or are being written up for submission to other journals (see: https://www.nature.com/nature-research/editorial-policies/plagiarism#policy-on-duplicate-publication for details).

Nature Human Behaviour offers a Transparent Peer Review option for new original research manuscripts submitted after December 1st, 2019. As part of this initiative, we encourage our authors to support increased transparency into the peer review process by agreeing to have the reviewer comments, author rebuttal letters, and editorial decision letters published as a Supplementary item. When you submit your final files please clearly state in your cover letter whether or not you would like to participate in this initiative. Please note that failure to state your preference will result in delays in accepting your manuscript for publication.

In recognition of the time and expertise our reviewers provide to Nature Human Behaviour's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "First Evidence for Human Use of Nicotiana (Tobacco) in the Pleistocene Americas". For those reviewers who give their assent, we will be publishing their names alongside the published article.

Cover suggestions

As you prepare your final files we encourage you to consider whether you have any images or illustrations that may be appropriate for use on the cover of Nature Human Behaviour.

Covers should be both aesthetically appealing and scientifically relevant, and should be supplied at the best quality available. Due to the prominence of these images, we do not generally select images featuring faces, children, text, graphs, schematic drawings, or collages on our covers.

We accept TIFF, JPEG, PNG or PSD file formats (a layered PSD file would be ideal), and the image should be at least 300ppi resolution (preferably 600-1200 ppi), in CMYK colour mode.

If your image is selected, we may also use it on the journal website as a banner image, and may need to make artistic alterations to fit our journal style.

Please submit your suggestions, clearly labeled, along with your final files. We'll be in touch if more information is needed.

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Best regards,
Chloe Knight
Editorial Assistant
Nature Human Behaviour

On behalf of

Charlotte Payne

Charlotte Payne, PhD
Senior Editor
Nature Human Behaviour

Reviewer #1:
Remarks to the Author:
You have done a good job in answering the questions/criticisms of the reviewers and the article is improved and much clearer.

Lines 165-82: discussion of the presence of tobacco in quids in the region and its role in potential movements of people into the region:

1) Consideration of quids as a means of tobacco use: Given that Danger Cave and Hogup Cave, both fairly close to this site, contained many quids, it might be worthwhile to point out when and if any of those analyzed to date contained tobacco seeds and if so, what was their dates?

2) I personally find the sentence "should figure prominently in the initial move..." a little strong. People might have been pleased to find tobacco (if already familiar with it) in any new lands they explored, but this almost sounds like they were specifically seeking it as a reason for migration. I don't require the change, but perhaps consider it.

Reviewer #2:
Remarks to the Author:
I very much appreciate and am satisfied with the authors' attention to the comments I provided during review. From my point of view, the revised manuscript is ready for publication and would be a great asset to Nature Human Behavior.

Reviewer #3:
Remarks to the Author:
In the revised manuscript, the authors have adequately addressed my reviewer comments, including those directed at further consideration of the role of waterfowl as a potential source of the seeds. The authors did not add any new concerns from my viewpoint. As it now stands, the article is direct as well as comprehensive in its discussion.

Author Rebuttal, second revision:

Response to Referees

August 12, 2021

Reviewer	Line No.	Reviewer Comment	Response and Action Taken
#1	-	You have done a good job in answering the questions/criticisms of the reviewers and the article is improved and much clearer.	-
#1	165-82	Discussion of the presence of tobacco in quids in the region and its role in potential movements of people into the region:	There seems to be a misalignment between the line numbers given (165-182) and the actual comments relative to our working version, but we believe we have addressed the comments nonetheless. We did the following, per the reviewer's numbering:
#1	-	1) Consideration of quids as a means of tobacco use: Given that Danger Cave and Hogup Cave, both fairly close to this site, contained many quids, it might be worthwhile to point out when and if any of those analyzed to	1) In the second paragraph of the section titled "Manner of Human Use" we added discussion and citation of the quids reported in Danger Cave. There are no quids reported from Hogup Cave, although we double-checked with the Natural History Museum of Utah, where that site's collections are stored, and asked Reviewer #1 (Kay Fowler) if she knew of something we weren't aware of. We confirmed it's just Danger Cave with quids and none from Hogup.

		date contained tobacco seeds and if so, what was their dates?	
#1	-	2) I personally find the sentence "should figure prominently in the initial move..." a little strong. People might have been pleased to find tobacco (if already familiar with it) in any new lands they explored, but this almost sounds like they were specifically seeking it as a reason for migration. I don't require the change, but perhaps consider it.	2) At the end of the first paragraph of the "Discussion," we modified the sentence to read "…should have figured into the traditional practices of human populations moving into the western North American interior shortly thereafter." This removes the unintended suggestion that tobacco played a role in the motives for migration.
#1	-	-	Addressing Reviewer #1's comments entailed a few other mentions and citations to tie off the issue of smoking versus quids. We added a statement in the "Manner of Human Use" section mentioning that other intoxicants could have been used in quids, especially *Datura*, which was recently published and would be conspicuously absent if not cited. We also added to paragraph four of the Introduction a statement and overlooked citation to evidence for ceramic smoking pipes in the Great Basin at ~4,800 cal BP, another meaningful mention in framing the issue of smoking and quids in the region to the reader.
#2	-	I very much appreciate and am satisfied with the authors' attention to the comments I provided during review. From my point of view, the revised manuscript is ready for publication and would be a great	No action required

		asset to Nature Human Behavior.	
#3	-	In the revised manuscript, the authors have adequately addressed my reviewer comments, including those directed at further consideration of the role of waterfowl as a potential source of the seeds. The authors did not add any new concerns from my viewpoint. As it now stands, the article is direct as well as comprehensive in its discussion.	No action required

Final Decision Letter:

Dear Dr Duke,

We are pleased to inform you that your Article "Earliest Evidence for Human Use of Tobacco in the Pleistocene Americas", has now been accepted for publication in Nature Human Behaviour.

Before your manuscript is typeset, we will edit the text to ensure it is intelligible to our wide readership and conforms to house style. We look particularly carefully at the titles of all papers to ensure that they are relatively brief and understandable.

Once your manuscript is typeset and you have completed the appropriate grant of rights, you will receive a link to your electronic proof via email with a request to make any corrections within 48 hours. If, when you receive your proof, you cannot meet this deadline, please inform us at rjsproduction@springernature.com immediately. Once your paper has been scheduled for online

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We look forward to publishing your paper.

With best regards,

Charlotte Payne

Charlotte Payne, PhD
Senior Editor
Nature Human Behaviour

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