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Challenges in sample processing within radiocarbon dating and their impact in ^{14}C-dates-as-data studies

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ABSTRACT

For decades, researchers have employed sets of radiocarbon dates to reconstruct trends in ancient human populations. The overarching assumption in this analysis is that the frequency of dates is proportional to the magnitude of past human activity. Thus, the distribution of summed or otherwise summarized dates is used to extrapolate population density and mobility patterns. There are, however, a number of underlying assumptions associated with this analysis that workers address to varying degrees and which, if false and not critically accounted for, will introduce bias, misrepresent the magnitude of activity, and ultimately prove misleading in archaeological interpretations. In this regard, research has so far mainly focused on correcting for the effects of time-dependent degradation of archaeological sites and constituent materials, calibration irregularities, and the efficacy of the statistical methods used. Assumptions directly related to sample processing in radiocarbon dating, however, are less discussed in '^{14}C-dates-as-data' analyses. It is, for example, assumed that all carbonaceous materials will yield sufficient, endogenous carbon for radiocarbon measurement. Yet sample failure in radiocarbon dating is common and contingent on, largely, deterministic factors such as post-depositional environment. Sets of radiocarbon dates analyzed, therefore, represent successful measurements independent of reliability. In this work, we discuss the biases introduced by challenges in radiocarbon processing and their impact on ^{14}C-dates-as-data studies.

1. Introduction

Within archaeological research, sets of radiocarbon dates are often used as a proxy to study the past (see Appendix). In this analysis, the overarching assumption is that the frequency of radiocarbon dates is proportional to the magnitude of past human activity and, in turn, the size of a population. As such, the distribution of summed or otherwise summarized radiocarbon dates is used to extrapolate population density, growth rate, mobility patterns, or occupational phases. There are, however, a number of underlying assumptions associated with this analysis:

1. The radiocarbon dataset used is a representative subsample of the archaeological record and this, in turn, adequately represents all past human activity.
2. Archaeological sites/features/items are uniformly preserved across time and space.
3. All archaeological sites, whether representative of ephemeral or long-lasting occupational events, carry the same importance in terms of population density.
4. All settlement patterns, modes of subsistence and cultural practices lead to the constant and equal deposition of material suitable for radiocarbon dating.
5. Population density events, e.g., booms and busts, as reflected by the archaeological record, are of sufficient duration and magnitude relative to the analytical uncertainties of the analysis for detection.
6. Research biases related to archaeological fieldwork and radiocarbon dating, e.g., interest, funding and access, have a negligible impact on the analysis.
7. All samples selected for radiocarbon dating directly relate to human activities and are a representative sample of the total occupational events at any given archaeological site.

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8. All carbonaceous materials yield sufficient, endogenous radiocarbon for measurement.
9. The radiocarbon dating process, as performed in all laboratories, produces robust chronometric results.
10. Chronometric data used, as obtained from original sources and/or pre-existing databases, are reliable. Where applicable, this includes adequate treatment of non-atmospheric reservoir effects.
11. The sample size of any given dataset analyzed is adequate for the statistical methods used and provides meaningful results.
12. Statistical methods used in summarizing radiocarbon dates generate an estimate that reasonably conforms to the original underlying distribution of the data.
13. The irregularities and fluctuations in the calibration curve have no significant effect.
14. Environmental proxies used in conjunction are appropriate and reliable.

If assumptions are false, the effect of the biases they introduce should be reasonably quantified and, if significant, critically accounted for. Otherwise, a distribution will likely misrepresent the magnitude of human activity and misguide interpretation. In the literature, biases are considered to varying degrees and research has mainly focused on identifying and correcting for the time-dependent survival of archaeological sites/features/items (assumption 2; Davies et al., 2016; Rhode et al., 2014; Surovell et al., 2009), effects caused by the calibration curve (assumption 13; Armit et al., 2013; Bamforth and Grund, 2012; Chiverrell et al., 2011; Contreras and Meadows, 2014; Kerr and McCormick, 2014; Williams, 2012), appropriate sample size of datasets (assumption 11; Contreras and Meadows, 2014; Michczyńska and Pazdur, 2004; Timpson et al., 2014; Williams, 2012), and the efficacy of statistical methods used to summarize datasets (assumption 12; Blackwell and Buck, 2003; Blockley et al., 2000; Bronk Ramsey, 2017). Biases stemming from challenges in sample processing for radiocarbon dating, particularly in relation to assumptions 1, 8 and 9, have gone largely undiscussed (but see Bradtmöller et al., 2012; Housley et al., 1997; Jöris et al., 2003). As such, this work aims to identify these biases and consider their potential effect in ^{14}C-dates-as-data studies, using the database of the Oxford Radiocarbon Accelerator Unit (ORAU) as reference. The remaining eleven assumptions are not further reviewed as they lie outside the scope of this work and are discussed at length already elsewhere in the literature (see Contreras and Meadows, 2014; van Andel et al., 2003; Williams, 2012).

2. Challenges in sample processing

The aim of radiocarbon dating is to obtain precise and accurate results for each sample measured. The main obstacles, prior to radiocarbon measurement and calibration, occur during laboratory processing[1] and include poor sample preservation and contamination. The former often results in insufficient yields of dateable material post-treatment and the latter introduces inaccuracy into the measurements obtained. Both are exacerbated when the true age of a sample nears the limit of radiocarbon dating, i.e., ~50 thousand radiocarbon years before present (ka BP), as diagenesis progresses and sensitivity to modern ^{14}C contamination increases exponentially.

[1] Sequentially, this involves sampling, chemical pretreatment, freeze drying, combustion, and graphitization (see Brock et al., 2010a, for more information and ORAU protocols). At the ORAU, bones may undergo an additional prescreening step, prior to chemical pretreatment, to assess collagen preservation using nitrogen content as a proxy—nitrogen derives exclusively from the protein component in bone (see Brock et al., 2010b for technique details).

Table 1
Main stable molecular forms targeted in radiocarbon dating (adapted from Bronk Ramsey, 2008).

Material	Molecular form
Bone and teeth	Collagen (protein)
Charred or carbonized plant remains	Amorphous carbon (elemental carbon)
Wood and plant remains	Cellulose (polysaccharide)
Arthropod exoskeletons	Chitin (polysaccharide)
Hair, horn, nails, claws and beaks	Keratin (protein)
Animal fats and vegetable oils	Lipids (glyceride)
Mollusk shells, corals, speleothems	Aragonite or calcite (calcium carbonate)

2.1. Poor preservation

In radiocarbon dating, stable fractions of any given material type are targeted during chemical pretreatment as these are likely to better retain the original radiocarbon ratio signal of the sample (Table 1). For example, radiocarbon specialists often focus on bone collagen rather than bioapatite, because the latter exchanges carbon with dissolved carbonate and generally yields minimum-age estimates (but see Zazzo and Saliège, 2011). If samples are poorly preserved, however, the yield of the targeted fraction is often insufficient for radiocarbon measurement. This is often the case for bones with low collagen content and plant remains that completely dissolve during humic-acid removal.[2] Apart from antiquity, sample preservation is largely dependent on the interaction between material type and post-depositional conditions. High temperatures and tropical conditions, for example, are known to accelerate the degradation of bone collagen (Ortner et al., 1972; Pestle and Colvard, 2012; Von Endt and Ortner, 1984; see Holmes et al., 2006, 2005 for predictive models involving collagen degradation and site distribution) and charcoal (Bird et al., 2002; T. F. G. Higham et al., 2009b). This is illustrated in (Wood et al., 2013), a research project designed to establish the chronologies of eleven Middle to Upper Palaeolithic (M-UP) sites in southern Iberia—a region of relatively high temperatures. Here, only ~12% of all prescreened bones analyzed were found suitable for collagen extraction and, consequently, only two sites yielded measurements (both sites found above 800 m.a.s.l.) In contrast, ~50% of prescreened bones from the cooler regions of northern Iberia—Asturias, Cantabria, the Basque Country and Catalonia—were found suitable for collagen extraction. Differences in soil pH can also drastically affect the preservation of archaeological materials. Generally, carbonates dissolve under low pH conditions (Berna et al., 2004), charred or carbonized organic remains readily degrade under high pH conditions (Braadbaart et al., 2009; Rebollo et al., 2008), and bone collagen deteriorates at pH extremes (Collins et al., 2002). In this sense, depending on the archaeological materials, regions with acidic soils often see a reduction in date frequency (see Bae, 2002; Berridge and Roberts, 1986). Unfortunately, sample failure due to poor preservation is quite common during laboratory processing, affecting samples from a wide range of locations (Fig. 1) and of different material types (Fig. 2). At the ORAU, 1041 sites have remained undated due principally to insufficient carbon yield.[3] This constitutes approximately 10% of all sites recorded in the laboratory database for which samples were processed. Moreover, materials like tooth, bone, antler, charcoal, charred seeds, and plant remains—all common archaeological samples—show failure rates at around 21%, 16%, 15%, 15% and 11%, respectively (Fig. 2).

[2] Evidence suggests that humic acids removed during pretreatment are not necessarily exogenous contaminants, but are sometimes derived from degradation of the actual sample (Ascough et al., 2011; Wild et al., 2013).

[3] Laboratory-processing error cannot be adequately disentangled from this estimate and is likely to be a contributing factor, albeit minor.

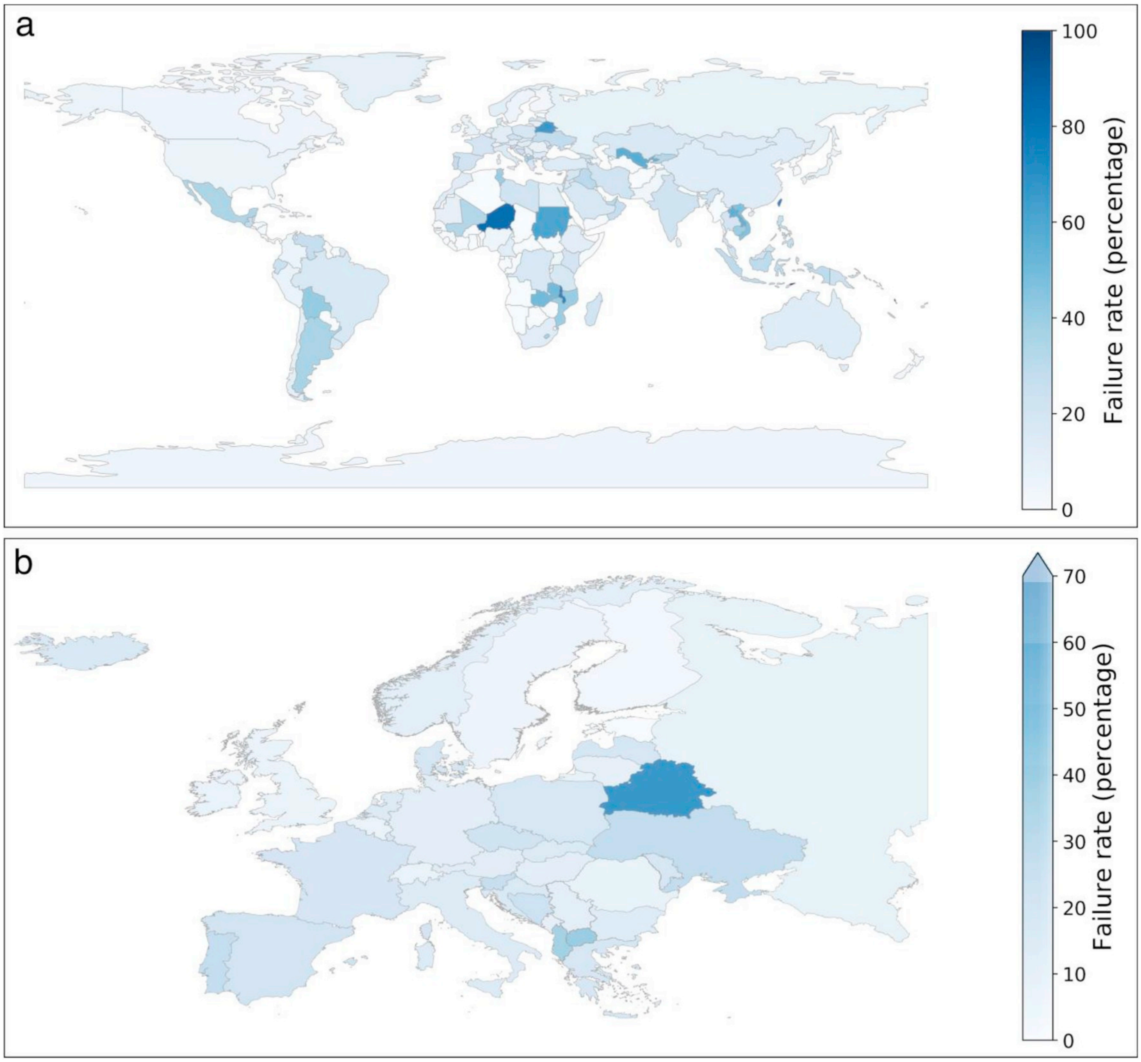

Fig. 1. (**a**) Map of countries from which samples have been submitted to the ORAU, between 1980 and 2019, and their individual failure rate. (**b**) subset of Europe. Factors like sample size and research focus vary for each country. As such, failure rates are not to be used comparatively but only serve to illustrate the ubiquity of sample failure due to, mainly, poor preservation. Failure rate = (total number of failed dates per country/total number of dates attempted per country)*100.

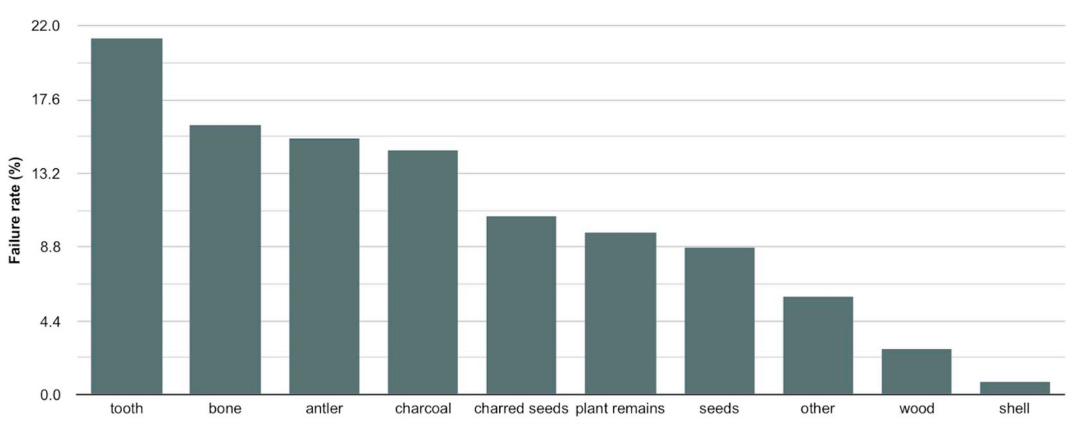

Fig. 2. Failure rate (%) of the top ten material types/categories (preset in the database) submitted to the ORAU between 1980 and 2019. "Other" is used as an editable database field by submitters and can represent a number of different material types not found within those preset. Factors like sample size and research focus vary for each material. As such, failure rates are not to be used comparatively but only serve to illustrate the ubiquity of sample failure due to, mainly, poor preservation. Failure rate = (total number of failed dates per material type/total number of dates attempted per material type)*100.

2.2. Contamination

For radiocarbon dating, sources of exogenous carbon include carbonates and humic acids from the sediment and consolidants applied during museum curation. The effects of contamination on a radiocarbon measurement largely depend on the age of the contaminant (from fully depleted to modern) and the "true" age of the sample. Modern contamination, for instance, has a more profound impact on older material (nearing 50 ka BP). For a sample dating to 40 ka BP, a 10% addition of modern carbon yields an age of 18 ka BP, and 40.8 ka BP if the sample contaminant is ^{14}C depleted. To remove contaminants, pretreatment protocols tailored to sample type, fragility, and age are employed (see Brock et al., 2010a for ORAU methods). In brief, carbonates are often leached in dilute acids to remove surface contamination, whilst non-carbonates, such as plant remains, often undergo simple "ABA" (acid-base-acid) pretreatment. This involves sequential immersions in acid, base and acid solutions, which remove carbonates, humic contaminants, and dissolved atmospheric carbon dioxide, respectively. If consolidants are suspected to be present, chemical pretreatment is preceded by a series of solvent washes. For bone collagen,[4] gelatinization (Longin, 1971) follows demineralization by ABA or acid-only treatment. Whereas these routine treatments normally remove sufficient contaminants from young samples to produce accurate dates, lengthier, more rigorous protocols are often required for Pleistocene-aged (>25–30 ka BP; Higham, 2011) or heavily contaminated samples (Deviese et al., 2017). These include acid-base-wet oxidation-stepped combustion for charcoal (ABOx-SC; Bird et al., 1999), and ultrafiltration (Brown et al., 1988) or hydroxyproline extraction (Deviese et al., 2017; Gillespie et al., 1984; Stafford et al., 1982) for bone. Discrepancies of several thousand radiocarbon years can exist between samples treated using routine and more stringent methods, with the latter yielding more reliable, often older results (Brock and Higham, 2009; Devièse et al., 2017; Douka et al., 2010b; Higham, 2011; Higham et al., 2009a, 2006b; Higham et al., 2009b, 2006a; Jacobi et al., 2006; Kosintsev et al., 2019; Marom et al., 2012; Wood et al., 2013, 2012).

Quality assurance parameters are often used to identify large amounts of contamination in many of the materials radiocarbon dated. For bone collagen, %C and C:N values, stable carbon isotopes and collagen yield might identify the presence of contaminants following pretreatment (van Klinken, 1999). For charcoal, %C values outside the expected yield of 50–70 (Braadbaart et al., 2009; Braadbaart and Poole, 2008) can denote contamination or diagenesis. With the exception of X-ray diffraction on aragonite shells, which can identify less than 1% of calcite contamination (Douka et al., 2010a; Sepulcre et al., 2009), these techniques are coarse, generally only identifying contaminants when they comprise a large percentage of the carbon in a sample. Although less common than failure due to poor preservation, some grossly contaminated samples will go undated if measurement accuracy is expected to be seriously compromised following quality control assessment.

3. Discussion

Not all carbonaceous materials yield sufficient, endogenous carbon for radiocarbon measurement (assumption 8) due to poor preservation—the main reason for sample failure. This is due to factors that, depending on the spatio-temporal unit studied, may be deterministic. Therefore, large radiocarbon datasets of the type often used in ^{14}C-dates-as-data analyses are not necessarily representative of the archaeological record (assumption 1). For this reason, archaeological and chronometric records should be considered as separate; an ancient butchering site affected by repeated groundwater flooding may persist in the former, but will be removed from the latter if collagen fails to survive in that post-depositional environment (Hedges and Millard, 1995) and is solely targeted for dating. It is important to assess how well both records represent each other, and to what degree the complex and highly localized factors driving sample preservation can bias radiocarbon datasets. This last point is shared by (Torfing, 2015), who argues that the acid soils of Jutland, Denmark (Montanarella, 2010), have reduced the number of radiocarbon dates available from bone, skewing distributions produced (Hinz et al., 2012; Shennan et al., 2013) toward shell middens and periods of shell midden construction, and ultimately misrepresenting past human activity in the region.

Quantifying the effect of the preservation bias might prove impossible or, at the very least, challenging and time-consuming. This because poor preservation is best assessed on a site-by-site basis and, often, site reports only publish successful radiocarbon measurements, making no reference to the total number of samples submitted for dating or preservation issues. In addition, due to slight differences in pretreatment protocols and quality assurance practices, sample failure rates almost certainly vary between laboratories. At the ORAU, for example, if the sample is near background (50 ka BP) and the amount of collagen extracted is <1% of the starting weight, it is usually failed and goes undated (Brock et al., 2010a,b). In some instances, a minimum age is a useful measure of time and, under these circumstances, an "OxA-X-"[5] prefix is added to the result. If, however, the collagen yield is 0.5% and the sample yields a "greater than" age, e.g., >48 ka BP, the measurement is passed and receives a routine "OxA-" notation. On the other hand, due to the pronounced differences in contamination effects for material of a different age, a younger sample with a low collagen yield will not often be failed, but given an OxA-X- prefix and a caution comment. In many instances, pretreatment chemistry will be repeated using a larger starting weight in an attempt to improve yield or reassess the original measurement and its reproducibility. The same criteria might not be employed by all laboratories.

As for assumption 9, published chronometric data is not always accurate as contaminants may not be fully removed during pretreatment. In general, we would expect older, Pleistocene measurements to be underestimated, shifting the dataset toward younger ages. To preclude skewing caused by unreliable measurements, some ^{14}C-dates-as-data studies apply quality criteria to vet datasets. These vary in detail and depend greatly on the judgement of the authors. Other approaches intentionally avoid this practice to keep from biasing a dataset. Bias is a possibility, particularly if filters are applied without careful consideration of how contamination-derived inaccuracies might impact the spatio-temporal units studied and the archaeological sites therein. For example, if more stringent protocols are rated higher than routine methods—ABOx-SC vs. ABA—younger (<20 ka BP) measurements obtained using the latter might be unnecessarily excluded. This because, as mentioned earlier, routine protocols like ABA are generally efficient in the decontamination of young material and the dates produced are no less reliable. Moreover, generalized screening treats dates as independent variables. In archaeology, however, dates are not separate from each other or their context and must be viewed and assessed according to the archaeological context from which they derive.

If unrecognized, unreliable dates have the potential to bias results in different ways. In Wood et al. (2013), for example, the application of improved ultrafiltration pretreatment methods identified a ~10,000-year age discrepancy in several archaeological samples in southern Iberia. This challenged the notion that the region served as a refugium for late-surviving Neanderthals (d'Errico et al., 1998; Guy Straus, 2005; Zilhao, 2006; Zilhão, 1993), which had been based on minimum-age

[4] We follow DeNiro and Weiner (1988) in their use of the term "collagen".

[5] Unlike the OxA prefix, OxA-X denotes samples that are research measurements using non-standard or experimental methods, or determinations that come with a caution based on the chemical parameters observed during pretreatment and measurement.

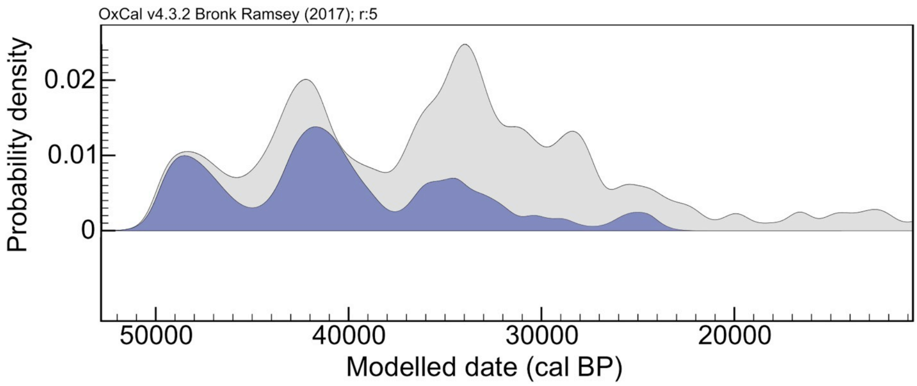

Fig. 3. Kernel density estimate (KDE) models for Middle-to-Upper Palaeolithic Iberia before (gray; n = 365) and after (blue; n = 141) the routine use of ultrafiltration and Bayesian age modelling. Data were compiled by Wood (2011). All >50,000 BP and infinite ages were excluded (it is thus likely that the probability density around the limit of radiocarbon dating is artificially low). The density peak for the gray distribution centered at ~34 ka cal BP possibly represents minimum-age estimates obtained from unsuitable, i.e., less stringent than required given the antiquity of the samples, protocols and materials. The models were made using the KDE_Model function in the OxCal software (Bronk Ramsey, 2017, 2009a). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

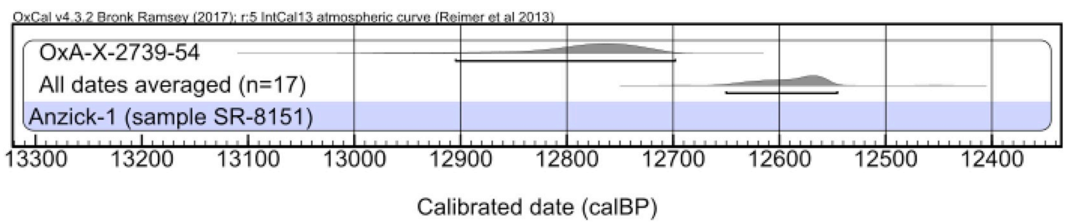

Fig. 4. Calibrated dates for sample SR-8151 (Anzick-1 individual from Anzick site, Montana; Becerra-Valdivia et al., 2018). At the top, the calibrated date for OxA-X-2739-54 (10.9 ka BP) at 12905-12695 cal BP (95.4% confidence). At the bottom, the average of 17 dates obtained for the same individual at 12650–12545 cal BP (95.4% confidence). At a 95.4% confidence level, both probability density functions fail to overlap. This illustrates the confounding effects that averaging, one approach used in ^{14}C-dates-as-data studies to offset data overrepresentation, can have at a small scale.

estimates obtained from unreliable sample types, e.g., bulk sediment (Pessenda et al., 2001; Wang et al., 1996) and gentler, less robust pretreatment protocols (Wood et al., 2013). When combined with Bayesian age modelling (see Bronk Ramsey, 2009a), ultrafiltered measurements have contributed to a revised chronology for the M-UP transition in Iberia (Wood, 2011; Wood et al., 2013). Notably, this yields a distribution that is dissimilar to that derived from earlier chronometric analysis, prior to the routine use of ultrafiltration in the region (Wood, 2011), with a reduced peak at ~34 ka cal BP (Fig. 3). Both distributions can be reasonably expected to yield different archaeological interpretations. Given that it is estimated that as many as 70% of published dates for this period may be too young (Higham, 2011), this is likely to be echoed in other M-UP studies.

Confounding effects can also be observed at a smaller scale, when combining dates for single archaeological events. The site of Anzick, Montana, for example, contains the likely curated remains of a male infant (Anzick-1; sample SR-8151), from which a total of 17 radiocarbon dates—averaged[6] to 12650-12545 cal BP (95.4% confidence)—have been produced in an effort to obtain a contaminant-free measurement (Becerra-Valdivia et al., 2018). So far, only one (OxA-X-2739-54) has been found to be reliable[7] and in agreement with the stratigraphic context (Becerra-Valdivia et al., 2018), dating Anzick-1 to 12905-12695 cal BP (95.4% confidence). This is relevant to ^{14}C-dates-as-data studies because some researchers combine multiple dates from a single cultural feature, layer or phase, to offset data overrepresentation (in relation to assumption 7). In the case of Anzick-1, however, distributions for OxA-X-2739-54 and the seventeen-date average do not overlap at a 95.4% confidence level (Fig. 4). Therefore, depending on how overrepresentation is dealt with, the difference between inclusion and exclusion of unreliable estimates into calculations should be tested. To do this, a critical assessment of site-specific chronometric data is necessary.

4. Conclusion

Challenges in radiocarbon sample processing have a direct impact on three of the fourteen underlying assumptions made in ^{14}C-dates-as-data studies, as identified earlier. For assumptions 1 and 8, we have shown that the radiocarbon dataset cannot always be considered an entirely representative subsample of the archaeological record, because not all carbonaceous materials yield sufficient endogenous carbon for radiocarbon measurement. This is generally due to preservation issues influenced by sample antiquity and unique post-depositional conditions. These factors may be deterministic, depending on the spatio-temporal unit studied. Moreover, failure rates differ between radiocarbon laboratories, introducing further uncertainty. As for assumption 9, the radiocarbon dating process has long been known to produce unreliable results, primarily due to partial sample decontamination. Large discrepancies have become increasingly apparent over the years with the development of more rigorous pretreatment methods (that only a subsection of radiocarbon laboratories employ) and the concomitant production of robust measurements in line with stratigraphic evidence—particularly for Pleistocene-aged and heavily contaminated samples. If unidentified, these inaccuracies have the potential to skew radiocarbon datasets and artificially alter a distribution. The critical assessment of site-specific chronometric data is, therefore, necessary and requires a basic understanding of the radiocarbon dating process and its complexities, as well as the spatio-temporal unit studied and each archaeological site.

We recommend that each study assesses the impact of sample preservation and contamination on a site-by-site basis. The success of

[6] Calibration was undertaken using OxCal 4.3 (Bronk Ramsey, 2009a) and the IntCal13 calibration curve (Reimer et al., 2013). Averaging was done in the same platform, following Ward and Wilson (1978), using OxCal's "R_Combine" command.

[7] This reliability is based on the fact that it was obtained using a single amino-acid method (Deviese et al., 2017), thus removing all potential contaminants.

identifying bias and offset results will inevitably vary according to each study, with those analyzing large spatial units spanning the spectrum of radiocarbon dating likely to encounter the greatest challenges. It might also prove informative to perform sensitivity testing by producing a number of distributions using different parameters and comparing the results. Assessing the impact of database vetting is one example. In case the effect of a single bias is unquantifiable or significant, the reliability of the method for the purpose of elucidating population dynamics should be questioned and not employed as the sole analytical tool; a number of ^{14}C-dates-as-data studies use the frequency of archaeological sites as a comparative proxy (e.g., French and Collins, 2015; Schmidt et al., 2012; Tallavaara et al., 2010). Moreover, when studying human dispersals, we suggest the use of Bayesian site-based age modelling instead. The same has been proposed by others in the past (see Bayliss et al., 2007; Blackwell and Buck, 2003; Culleton, 2008). In contrast to the ^{14}C-dates-as-data approach, Bayesian age modelling allows the incorporation of relative information, e.g., stratigraphy, with dates, and can be employed to objectively identify and down-weight outlier dates (Bronk Ramsey, 2009b), obtain quantitative age estimates for archaeological events or calculate the commencement of individual archaeological sequences (Bronk Ramsey, 2009a). Given that human presence likely predates the archaeological record, the latter is key in the study of population dynamics. Literature on Bayesian age modelling is vast (see Derek Hamilton and Krus, 2018, and references therein) and the analysis can be performed on the same platforms used for summarizing radiocarbon datasets, e.g., OxCal (Bronk Ramsey, 2009a, 2001).

Declaration of competing interest

None.

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Appendix

This is a compilation of published sources reviewed, which utilize or discuss the ^{14}C-dates-as-data approach on the archaeological record.

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