

## Punic people were genetically diverse with almost no Levantine ancestors

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

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)

This is an exciting article that will attract a great deal of attention among Mediterranean historians and archaeologists, amid increasing interest in maritime connectivity and mobility in the Iron Age. The key results from that perspective are the suggestion that port city populations of the Phoenician-speaking west were unusually cosmopolitan but lacked significant Levantine presence, and the tantalising suggestion that population change in Sardinia, at least, tracks Carthaginian expansion (a notoriously difficult phenomenon to trace historically or archaeologically).

The study offers support and significant further detail for a model that was beginning to emerge from smaller datasets (Moots et al. 2023). It also offers a useful challenge to the widely-publicised study of modern Y haplogroups as a Phoenician ‘signature’ (Zalloua et al. 2008). It is a particular treat to have significant data from Carthage at last. And (with minor caveats noted below) the article is notably clear and careful in its use of historical population labels as well as its contextualisation of the data in the archaeological record.

I am not equipped to comment on the data, methodology, and use of statistics, but the conclusions seem to me robust and plausible in the historical context.

Leaving aside larger questions of historical interpretation, a debate that I am sure this article will provoke, and the clarity of the initial framing (on which more below), I have only a few minor suggestions for improvement.

Main article, lines 129-131: I know of no evidence that ‘Punic people’ in general “saw themselves as the descendants of earlier Phoenicians who arrived from the East”. There are specific foundation legends linked to Carthage and Cádiz and reported in antiquity to originate in those cities that do associate these two settlements (as well as Utica) with Levantine migrants. Beyond that though we are simply in the dark as to what ‘Punic people’ thought on this matter. It is very likely in my opinion that many of them did in more or less specific terms trace their culture or even their own ancestry to the Levant, but that is not a fact. If there is in fact evidence for this claim, it should be cited; otherwise I’d suggest deleting this point, or limiting its scope to the people of Carthage and Cádiz. (The only citation given before the end of this paragraph is to my own book, which certainly doesn’t say more than that – and should be Quinn 2018, not 2019, as throughout!)

Main article, line 135: I’d delete the phrase “leaving ample room for biases” here: the point is at least as well made without it. All historical sources contain ‘bias’ of one kind or another, and Phoenician sources would surely be just as ‘biased’ if not more so than outsiders in relation to their own societies.

Main article, lines 398-401: I don’t think the authors need to mention the Numidians here: they are a sub-set of a larger population of indigenous ‘Libyans’ (to Greek authors) or ‘Afri’ (to Romans) that only emerges clearly as a confederation (and then kingdom) in the late third century BCE, rather too late for the point being made here. More generally (and as this article notes in the introduction), Carthage was already a ‘major population center’ in the 7th century BCE (Telmini et al. 2014 (in Quinn & Vella 2014), 118-122), and there’s no reason to think that there wasn’t already a significant local component to the population then. Furthermore, the connection between that point and the changing population of Sardinia is currently unclear. What this expansion of North African ancestry does coincide with is evidence for the expansion and intensification of Carthaginian imperial control overseas, beyond western Sicily (eg Quinn 2018, 87-88).

However, if the authors do wish to mention the Numidians here, Horsted 2021 is inappropriate as a limited and non-academic summary of the evidence insofar as it relates to the Numidian 'army'. Better would be Brett & Fentress 1996 ('The Berbers') with updates in Mattingly 2023 ('Between Sahara and Sea'). For the more general indigenous population of North Africa, which I suggest as a more prudent reference point, Mattingly 2023 alone would now be sufficient.

Supplementary Fig. 1: I couldn't see the data for Kerkouane plotted on the 'North Africa' map, only that for Carthage.

Supplementary Section 1, p1 (and recurring): there are a number of mentions by of 'Sicanian' people/ancestry in Sicily, which are I think unnecessary: 'Sicanian' is not a clear historical/factual category, but a Greek label for some of the people living in western Sicily, usually understood to be the original population of the island. These people themselves exhibit no very clear ethnic (in the sense of self-identification as a community), political, or even cultural differentiation from other indigenous Sicilians (unlike, for instance, 'Elymians' and 'Sikels' who have left some evidence for distinctive languages). 'Indigenous Sicilian' would, I suggest, suffice here. (Further thinking on this topic, in case of interest: <https://crossreads.web.ox.ac.uk/article/about-arrow-shaped-alpha-and-sikel-identity>; Jonathan Prag's article on 'The Indigenous Languages of Ancient Sicily' in *Palaeohispanica* 2020.)

Supplementary Section 1, p2: the 'Punic' dating of the samples from Kerkouane and Carthage can be more confident. Kerkouane was established in the sixth century and abandoned in the mid-third, so any remains found in good archaeological context must date to the 'Punic' period defined here. Similarly, Carthage was destroyed in 146, and its cemeteries were not reused by the later Roman settlers, so since the remains come from an identifiable Punic-era cemetery (or at least I assume they do: the phrasing 'should have come from' (p35) is a little mysterious!) rather than the later Roman city there should be no question about relevance.

#### Site Descriptions:

Solar los Chinchurros: 'Opus signum' should be opus signinum

Birgi: what is 'a marine road'?

Lilybaeum: it is only in the third entry, for Corso Gramsci, that the relationship between Lilybaeum and other 'Punic' sites is explained; I suggest moving that to the first description of the site.

Selinunte: the historical summary here fails to clarify the imposition of Carthaginian rule on this (originally) Greek-speaking city (in 409 BCE), and it also fails to clarify which of the cemeteries described provided the relevant data: the basis of the dating of the sample to the relatively brief and intermittent period of Carthaginian control of the city (409-c. 250 BCE) is therefore unclear.

Carthage: 'Latin' sources should be 'Greco-Latin', so as to include the important evidence of Timaios. NB 'The' Tophet. And 'proof' seems a strong word to use here.

Finally, I would note one issue with the clarity of the framing of the project at the beginning: the explanation of the term 'Punic' in the Introduction. As the authors note, this is a Roman word (in fact a simple transliteration of the Greek 'Phoenician', as Latin long lacked aspiration: Prag, *Papers of the British School at Rome*, 2006). It is indeed often used by modern historians and archaeologists (though not consistently by Romans) to refer to Phoenician-speaking communities in the western Mediterranean, at least from the sixth century on, on the (only slightly anachronistic) grounds that that this is a decisive moment in the process of Carthaginian expansion over its neighbours. And I have no objection to the adoption of this term here to point to the same chronological and cultural boundaries: it is a neat modern solution to the problem of a fuzzy ancient set.

I do however think that the meaning of 'Punic' remains unclear here in significant ways, especially as the sites chosen range from certain or very probable Levantine foundations (eg Motya, Carthage, Cádiz), through certain or probable Carthaginian colonies (eg Ibiza, Kerkouane), to settlements whose origins remain obscure (eg Malaga, Villaricos, Villamar). Have the sites chosen for the study been "classified" as 'Punic' because the Phoenician language was spoken there? Or does 'Punic' in this article point to a shared material culture with Carthage and other sites with (more) definite origins in Levantine settlement? Or does it represent a judgement that these particular sites had fallen under Carthaginian imperial control (or, more vaguely, 'influence')? I think any of these definitions could work with the sites chosen (though there might be questionable cases in each case), but it would be useful to know which - or how many - were actually used to construct this corpus.

Josephine Quinn, Professor of Ancient History, Oxford University

#### Referee #2

(Remarks to the Author)

General Comment

This is an interesting and well-structured study. The authors have applied relatively standard analyses to a set of 210 ancient individuals, mostly from Phoenician and Punic sites. The main findings are

1. Punic peoples derive their ancestry mostly from an unknown Sicilian/Aegean source with little detectable input from the Levant. I believe a previous paper already showed as much for a Punic population from a Carthaginian town (Moots et al. 2023). This study comes no closer to identifying the origins of this Sicilian/Aegean ancestry or explaining how Phoenician culture spread without any detectable Levantine gene flow. Genomes from the eastern Mediterranean and pre-600 BC are

required to resolve this.

2. North African ancestry is widespread in Punic populations and appears to have been incorporated in sporadically, illustrating the important influence of Carthage. I believe this finding is more novel than the first, although it is not clear from lines 142-143 whether North African ancestry has previously been identified in Punic populations outside North Africa.

3. Genetic diversity is high in Punic populations, mainly due to varying amounts of North African ancestry. From this, claims are made about the cosmopolitan nature of the society, its openness to foreigners and the amount of continuous gene flow that occurred across the Mediterranean during the period.

4. Some Punic communities may also have tended towards endogamy and closed mating networks.

I have no real criticisms of the technical approaches used, which are robust and appropriate. My main concerns are

a) The novelty and significance of the findings (particularly number 1), given that multiple papers have previously described Phoenician and Punic ancestry in the Levant, North Africa and the western Mediterranean. A number of the conclusions also seem to rely heavily on archaeological and historical data without explicitly saying as much. For example, it is already well-established that the Phoenicians had a cosmopolitan culture and unprecedented levels of maritime mobility over centuries – whether there is strong genetic evidence for this is another matter.

b) Following from the previous point, some of the authors' claims seem a little dubious on the basis of the genetic data alone. I think more nuanced discussion is required in parts.

I outline my specific issues below

#### 1. Reference to prior work.

The introduction needs to provide more detail on what is already known from ancient DNA studies of the Phoenician and Punic peoples. For example, Phoenician populations of the Levant have been well-characterised (e.g. Haber et al. 2020), but these studies are only cited in the results section as sources of comparative data. Four other studies of Punic populations are mentioned in the introduction, but their findings are discussed in a very vague manner ("contributions from various ancestry sources") and then immediately written off due to sample size. It is hard to assess the novelty of the authors' work without proper context. These studies should also be appropriately cited in the discussion (e.g. lines 361-364)

#### 2. Genetic evidence for the exceptionally diverse and cosmopolitan nature of Punic societies

The authors claim in their abstract that the Punic people "were genetically exceptionally diverse relative to earlier Mediterranean people" and "the first genetically heterogeneous metapopulation of the ancient world". They also argue for high degrees of mobility and cosmopolitanism, with new people being continuously incorporated into Punic communities over centuries. These are strong claims and ones that I am not convinced by for a number of reasons detailed below.

A) Lack of appropriate comparative populations. The authors appear to have very few earlier Mediterranean populations to compare to, especially from Italy and the Aegean. In Fig 3b, it seems there are only two populations dating to the millennium before the Phoenician sites, one from the Levant and one from Central Europe. The Y chromosome plot (3a) also has very few data points during this period. Without having a genetic picture of the Mediterranean prior to Phoenician expansion it is hard to assess their impact on diversity.

B) What is the genetic signature of cosmopolitanism? The authors seem to conflate genetic diversity with cosmopolitanism. The logic appears to be the more genetically diverse a population is, the more culturally diverse/open it is, which allows the authors to claim the Punic population had an "exceptionally cosmopolitan culture" relative to comparative populations (line 278).

I can understand the authors' line of reasoning, but it doesn't sit quite well with me. For example, I don't think you could argue that a Viking port was less cosmopolitan or "open to foreigners" than a Phoenician port or an Avar settlement (steppe nomads), even though I'd wager genetic diversity would be much lower. The geographical context also matters. If a highly mobile culture open to trade and outside influences emerges at a continental periphery, the impact on genetic diversity is going to be much less than if it emerges at a continental crossroads. In the case of the author's dataset, it is admixture with North Africa that really inflates the 2D PCA diversity. But cosmopolitanism is a cultural phenomenon and I'm not sure the genetic distance between the admixing populations is that relevant. For this reason, to use mostly Central European sites as comparative populations (Fig. 3) does not seem completely fair.

For a word that is used in the title of the paper, I would like to see a more nuanced discussion of cosmopolitanism and how it can be inferred from the archaeological and genetic record.

C) Evidence from ROH. ROH provides a means to assess the openness of a community's mating networks. However, the ROH profiles do not seem particularly informative in this case as they are not discussed in the context of other populations. Is 44/73 individuals with no ROH >4cM to be expected for an "exceptionally diverse" and "genetically open" population. Looking at Figure S6b, it seems many Bronze and Iron Mediterranean individuals have no detectable ROH. Are the Punic and Phoenician profiles significantly different? If anything, it seems the Punic communities have a higher frequency of

endogamy. The authors do discuss endogamous sub-communities but fail to reconcile this behaviour with their vision of a culture “open to incorporating foreigners into their communities”. Again, I think more nuanced discussion is required here.

I'm also unsure how to interpret the effective population size, which is a standalone value with no comparative data. And is it meaningful to provide a single  $N_e$  estimate for a “metapopulation” that encompasses a wide range of geographical regions and ancestries. Would it make more sense to estimate  $N_e$  for specific Punic communities?

D) Evidence for continuous gene flow. The authors claim to show that admixture from North African and Sicilian/Aegean sources was constant, with people of different ancestries continually being incorporated into Punic sites (lines 245-248; lines 285 to 295). While believable, given what we know about the Phoenicians from the archaeological and historical record, I am not convinced the genetic data can be used to support such a claim. The authors' make the assumption that Punic populations experienced random mating and thus inward migration must have been continuous to keep ancestry heterogenous. But there is no reason to assume this. Cultural barriers to random mating can persist for centuries among different communities within a society. The authors themselves later discuss the discovery of an endogamous sub-community, whose ancestry from Figure 2 appears to be relatively homogenous. To argue for continuous mobility and incorporation of foreigners into a population, I think more data is needed. Isotopic data would be very useful, as would denser sampling of single burial communities (e.g. identification of large extended families of diverse ancestries).

E) Ancestry Distributions. The authors state that “ancestry distributions” in Punic people were similar despite being variable within sites (Lines 296-298). I do not quite understand this line and no figure/table reference was supplied. I take it to mean the north African-Sicilian/Aegean gradient seen in PCA. I am unclear how this demonstrates interconnectedness. Populations can fall in the same place on PCA due to independent demographic processes. And even if a singular demographic process created this gradient, what evidence is there that this process is ongoing? (see comment on random mating above)

Following from this, if most Punic populations are very similar in terms of their “ancestry distribution”, despite being geographically widespread, how does this fit with the hypothesised “genetically open” nature of the society? For example, it seems quite possible that many Punic populations across the Mediterranean were not very open to local communities. The authors suggest this is the case for Iberia and Sardinia, but it could also be the case for Sicily. The authors do not know the source of this “Sicilian/Aegean” ancestry in Punic groups – it does not necessarily derive from local Sicilian groups. And if Punic populations derive their ancestry mostly from just two sources, can you really make the argument that this is an exceptionally cosmopolitan/culturally diverse population from the genetic data?

F) IBD. The discovery of a pair of relatives between Sicily and Kerkouane is a really nice find, but as it is only a single observation it is hard to draw any firm conclusions about the extent of mobility across the Mediterranean, especially as these sites are actually quite close to one another. What do the broader patterns of IBD sharing look like? Is most sharing within or between sites? Do individuals who share IBD tend to have similar ancestry profiles?

Minor

-Please provide proper table contents for Supplementary.

Line 124-127: Hard to follow the logic of this sentence. Are you choosing to distinguish between “Punic” and “Phoenician” peoples because there is a genetic difference? If there was no genetic difference would you recommend that historians cease making a distinction? I think history and archaeology should guide the usage of the term “Punic”, not an ancestry profile (especially given the demonstrated diversity of these populations).

Line 128: Do you mean “gene flow” from Levantine populations? Genetic continuity is typically used in the context of continuity within a geographical region, not migration between regions.

Line 144: Do you mean all studies combined only looked at 20 individuals?

Lines 281 and 349: What is meant by “extreme” or “ultra” Y haplogroup diversity? These seem like very hyperbolic terms. The Punic values are only extreme in relation to a very small dataset of mostly central European sites. One Italian site has a similarly high value.

Where is the archaeological supplementary (site descriptions/broader context)? For this number of ancient genomes, I think it is important to provide sufficient archaeological detail.

Referee #3

(Remarks to the Author)

I am not a DNA expert, so I will confine my remarks to the radiocarbon data, which is my expertise.

The radiocarbon data is basically summarized in the supplemental Excel file S1, pages 1 and 2. The details are in page 2. Everything looks in good order to me, except.

1. Why is (Delta 14C) presented in columns K and L, when this is just  $(F-1)*1000$  and not the usual usage of Delta 14C

which includes an age correction (where the age is known). I suspect this is solely because it's generated by the BATS program for the MICADAS machine, so I recommend deleting these two columns as they convey no additional information. 2. The last sample S-8135 is stated to be run at "CIRAM". CIRAM is a sample-preparation only laboratory and does not do <sup>14</sup>C analyses, they are all run elsewhere. The information about WHERE the AMS measured was made should be included.

Timothy Jull

(Remarks on code availability)

I believe the radiocarbon calculations are correctly done in OxCal using the INTCAL-20 calibration curve.

Version 2:

Reviewer comments:

Referee #2

(Remarks to the Author)

I thank the authors for their thorough response to my previous review. I raised two main issues – reference to prior work and the genetic evidence for cosmopolitanism.

The first issue has been rectified, with ample context from prior aDNA studies now provided. However, there is one thing I think should be corrected. In lines 151-153, it is said that whole genome data from 12 Punic individuals at Kerkouane “suggested that” Punic people in North Africa “might have” substantial European and North African ancestry (Moots et al. 2023). Why multiple qualifiers? The Moots study definitively demonstrated both North African and European ancestry in this population and this should be made clear.

In response to the second issue I raised, the authors have provided new analyses and softened their interpretations. I think this has greatly improved the study. However, a few concerns still remain.

A. Definition of cosmopolitanism: The authors agree that it is misleading to conflate genetic diversity and admixture with cosmopolitanism. However, they then go on to provide their own non-standard definition of cosmopolitanism that does just that, allowing them to then apply this term to the interpretation of their results.

Line 302: “a population that is the product of the ongoing mixing of people of diverse cultural, geographic, and genetic backgrounds”

But this is a poor definition of cosmopolitanism and is provided without citation. Indeed, many sampled ancient populations are the product of ongoing genetic/geographic/cultural mixtures, resulting in heterogeneous ancestry profiles. However, no one is calling the Neolithic or Bronze Age populations of western Europe cosmopolitan, despite there being high variability in diverse ancestries in some regions, sometimes within single sites (Olalde et al. 2018; Seguin-Orlando et al. 2021). This is because the term cosmopolitan refers primarily to a set of cultural values that are based on openness, tolerance and inclusivity. A cosmopolitan society is one that is open to people who possess many different ethnic, cultural and religious identities, and emphasises shared universal rights and values among its diverse members. It follows that cosmopolitan populations are likely to harbour diverse and mixing ancestries. However, it does not follow that all populations that harbour diverse and mixing ancestries are cosmopolitan.

If the authors are committed to having the term “cosmopolitan” in the text, I'd ask them to not treat it as a buzzword, but instead engage with its full meaning and prior literature on the inference of cosmopolitanism in the classical world and archaeological record more broadly (e.g. urbanisation, cultural exchange, religious syncretism, rules of citizenship). This includes providing a well-accepted and cited definition of the term. Also, Lines 304-6 do not make sense - “Punic individuals were cosmopolitan in this sense ... but also genetically” - There is no such thing as a population being “genetically cosmopolitan”. Cosmopolitanism is a cultural phenomenon. Admixture between divergent populations does not equal “genetic cosmopolitanism”. Please rephrase this section.

Finally, the authors compare the Punic people to the “later cosmopolitan cultures of Rome and Byzantium” in their abstract (Line 103), but do not expand on this anywhere in the main text or archaeological supplementary, which could be useful. It is an interesting comparison, as I believe the cosmopolitan nature of Carthage (primarily economic and functional) is thought to have been more limited than that of Rome (e.g. integration of conquered peoples through citizenship), with the elites of Punic society less open to outside influence. It could be useful to consider these different manifestations of cosmopolitanism when interpreting the genetic data. An ethos of openness and “free mixing” (Line 488) need not have penetrated all strata of society, which could be a potential explanation for some seemingly contradictory genetic patterns (see next section).

B. Genetic support for claims of cosmopolitanism (and general exceptionalism):

The authors still make several very strong claims that I remain uneasy about.

Firstly, to say that the Punic people are the “first genetically heterogeneous metapopulation of the ancient Mediterranean” is quite sweeping statement (Line 102), as well as vague. Firstly, does the ancient Mediterranean refer to the entirety of Mediterranean prehistory or just classical antiquity? Secondly, what is meant by “heterogeneous metapopulation”? This is not

a term that is used often in ancient genetics and is not defined in the text. I take it to mean a set of subpopulations with some level gene flow between them and some level of heterogeneity within them (what those levels are I do not know). How can the authors claim that “heterogeneous metapopulations” did not exist in the Mediterranean before the Punic people? Indeed, I would hesitate to call any sampled ancient population the first anything, given the patchy nature and Eurocentric bias of reference databases. I recommend the authors qualify this statement or remove it.

Secondly, I am still not convinced the genetic findings alone are strong enough to “document a highly cosmopolitan culture” (line 426-427) and would remove this phrase. In their revision, the authors have now laid out five lines of genetic evidence for their own (flawed) definition of a cosmopolitan society under the header “One of the first cosmopolitan cultures of the ancient world”. Two of these lines of evidence relate to genetic diversity (Y chromosome and autosomal), two to mobility (similar ancestry distributions and IBD sharing) and one to ancestral diversity within a family. However, the strength of this evidence is medium at best. There are two main issues:

1. As I put forward in my original review, neither high genetic diversity, nor high mobility, are necessarily indicative of a “highly cosmopolitan culture”. For example, I think it would be an overstatement to describe the Avar as highly cosmopolitan or culturally open, despite their mobility and likely having higher 2D PCA diversity than the Punic people (Gnecchi-Ruscone et al. 2024). The authors seemed to agree that this conflation is problematic in their response letter, but it would be useful for them to acknowledge this in the main text.

Importantly, the authors note themselves that the genetic diversity observed in Punic populations is not due to them deriving from a large diversity of sources (which you might expect for a cosmopolitan society), but a limited set of similar or identical sources (Lines 321-322), including a divergent North African population. New analyses (Fig S6b) confirm this North African ancestry is the primary driver of the exceptional diversity levels seen at Punic sites (although this is downplayed somewhat in lines 317-318 – see comment below).

I do think the wide variance in North African ancestry in Punic populations and extended families is of great interest. However, I’m unclear if the authors are concluding that the integration of individuals from this ancestral background was a sporadic occurrence (lines 287-289), or if it occurred quite freely (lines 422-424). Could they clarify this? In any case, it is clear the social dynamics underlying this admixture (including cosmopolitan cultural values) are still not well-defined and any interpretations drawn should be careful and considered.

2. Some observations seem to actively contradict the interpretation of cosmopolitanism (and these are interesting in their own right – societies can be closed in some ways and open in others). The Punic populations are described as highly mobile and genetically diverse, but also with persistent endogamy (line 481) and very similar ancestry distributions between sites, with little local admixture in a number of regions. These results do not paint a neat picture of a “highly cosmopolitan” (Line 427) and “free mixing” (Line 488) culture. Indeed, the results present the possibility that Punic society was relatively closed in some regions (e.g. ~50% of Iberians have ROH >50cM and little local admixture) or possibly in some social strata. As the authors provide no data on the inferred social status of the individuals sampled here (is there a reason for this stated anywhere in the text?) it is hard to make firm conclusions.

To be clear, I do not have a problem with the authors discussing how their results interact with archaeological and historical evidence for cosmopolitanism in the Punic world. What I take issue with is that lines 301-306 and lines 424-427 read as if the genetic data provides a strong independent line of evidence that Punic society was highly cosmopolitan, when the data presents a more complex picture. I worry that if geneticists are not explicit that their interpretations of the data are being shaped by prior knowledge from other fields, it can lead to circular referencing between disciplines. I would change the header of line 301 to reflect the actual results within (diversity, mobility). I would also provide a brief overview of the current thought on the nature of Punic cosmopolitanism in the discussion and whether the genetic results provide some support for these ideas or challenge them. When “free mixing” is discussed, it should be carefully caveated.

I make some other specific suggestions below:

1. Lines 487-488 seems to suggest that endogamy cannot co-exist with high individual mobility, which is obviously not the case. Indeed, in elite societal strata both often co-occur (look at Europe’s aristocratic networks).

2. I don’t think the sample size is large enough to conclude that endogamy only occurred sporadically across Punic populations (Line 353). It could have been quite frequent in some regions like Iberia. Also, I think it is speculative to conclude that “endogamy was likely practiced only by sub-communities” (Lines 487-488) and would soften this. While this could well have been the case, endogamous marriages can also occur alongside exogamous ones in the same sub-community due to different material circumstances of the individuals involved (inheritance is the big one – heirs may be more restricted in their choice of marriage partner relative to other children; couples without sons may choose a son-in-law from among their relatives)

3. The authors rely heavily on the words “exceptionally”, “unprecedentedly” and “remarkably”, when describing the genetic diversity of Punic populations. This seems a little sensational to me, as they are only exceptional with respect to a limited comparative dataset, although I appreciate the authors have done their best to include as many references as possible. But there are still many geographic gaps (e.g. almost all earlier pops are European with none from North Africa itself). I think the descriptive language could be softened a little without reducing the novelty of these results.

4. The new analysis (Fig S6) seems to confirm that the high diversity of Punic populations is primarily driven by North African ancestry, although I think the description of these results in lines 317-318 are a bit misleading. Only 2/6 Punic

populations have 2D PCA diversity levels higher than earlier sites (Fig S6b). Is there a statistically significant difference here that allows the authors to state that “in Punic populations, diversity levels remain higher than in earlier populations even when this specific contribution is factored out”? Also, there are near-contemporaneous Italian and Iberian populations with higher diversity in Fig S6b (and similar diversity in Figure 3), which could indicate that these changes in population structure and mobility are not just a Punic phenomenon. Also, the two Punic contexts with highest 2D PCA diversity in Fig S6b, Tharros and Lilybaeum/Birgi/Motya, have individuals with dates that may overlap with Roman expansion. Can I check that these were excluded from this analysis? Also were a minimum number of individuals per context used for Fig 6b, as was done for Fig. 3?

5. Line 290 – please be clear here that it is also quite possible that Punic populations in Sicily did not receive substantial local input. Given the widespread geographical distribution of this Aegean-Sicilian ancestry (and lack of local contribution in other regions) both scenarios have similar probabilities.

6. Figure 3a Y axis should be Y haplogroups, not haplotypes. Also, it seems very arbitrary to use the first four characters of the ISOGG classification system as a cut-off (corresponds roughly to last glacial divergences). Why not use the full haplogroup label for each individual? By choosing such a threshold you will deflate diversity values in certain geographical regions where Y chromosome diversity underwent more recent homogenising events (e.g. R1b1 expansion during steppe migrations). As you are trying to demonstrate the exceptional nature of Punic Y haplotype diversity, it would be a more conservative approach to use full haplogroup labels for comparative sites. Please justify why this was not done in the text.

7. Pedigree in S7a is hard to understand. Are I24215 and I24194 inferred to be descendants of parents of I24494 and I24193?

8. The provided numbers of new samples generated and analysed are inconsistent. In the abstract it says 196 from Punic/Phoenician sites were analysed. (Line 86). But from the intro (Line 166) it seems only 131 individuals produced analysable data? The number 196 does not appear anywhere else in main text. I think the number used in the abstract should reflect that in the main text to avoid confusion.

9. Line 329 – it is more appropriate to say “relationships up to the 5th-7th degree” as kinship is typically viewed as a social phenomenon.

10. Line 336. I think it would be better to be more explicit on the estimated number of generations that could separate two ancient individuals sharing >20cM. “Several” is quite vague.

11. Line 455 – This is odd phrasing. While I know it is not the authors’ intention, it could be misinterpreted as saying that the Greek and Phoenician civilisations were fuelled due to them carrying a particular biological ancestry. I would rephrase to avoid confusion.

## References

Gnecchi-Ruscone, G.A., Rácz, Z., Samu, L. et al. Network of large pedigrees reveals social practices of Avar communities. *Nature* 629, 376–383 (2024). <https://doi.org/10.1038/s41586-024-07312-4>

Olalde, I., Brace, S., Allentoft, M. et al. The Beaker phenomenon and the genomic transformation of northwest Europe. *Nature* 555, 190–196 (2018). <https://doi.org/10.1038/nature25738>

Seguin-Orlando, A. et al. Heterogeneous Hunter-Gatherer and Steppe-Related Ancestries in Late Neolithic and Bell Beaker Genomes from Present-Day France. *Curr. Biol.* 31, 1072–1083.e10680 (2021).

## Referee #3

(Remarks to the Author)

They said in reply to my comment:

2. The last sample S-8135 is stated to be run at “CIRAM”. CIRAM is a sample-preparation only laboratory and does not do 14C analyses, they are all run elsewhere. The information about WHERE the AMS measured was made should be included. We have extracted the location of the accelerator mass spectrometer from the C14 report that we received and added the information on where the CIRAM AMS date was measured to our methods section: “For the CIRAM date, the different carbon isotopes were separated using a 250 kV accelerator mass spectrometer in a joint venture with JSC Barnas.”

Well BARNAS (barnas.lt) doesn't do AMS measurements either. They do sample preparation. So, it was run somewhere else. My guess is Vilnius or Poznan.

## Referee #4

(Remarks to the Author)

I am leaving the assessment of the scientific methods and results to those experts.

Like the first reviewer, I am certain that this article will be of interest to people working on Punic and Phoenician topics. It is significant for its sample size and draws some exciting conclusions about the movement of and intermarriage among

populations in specific Mediterranean regions during the first millennium BCE. I enjoyed reading about the genetic work very much.

I am less sanguine than the first reviewer concerning the historical and ethno-cultural framing and its reliance on archaeological and linguistic evidence. The updated text in L134-137, for example, does not strike me as clarifying the working definition of "Punic", as no evidence is offered of the claimed "shared language and culture" for the selected sites in the article. The site summaries, while appreciated, add little more evidence of the purported connections. Later in the same sentence, we read: "inhabitants of Carthage saw themselves as the descendants of earlier Phoenicians who arrived from the East no later than the 9th century BCE", which is not a central argument of the only supplied reference, Quinn 2018.

While the authors can certainly revise this sentence, it is a symptom of larger (but correctable) difficulties with their methodology, which begins with a definition of "Punic" that is not very rigorous. Punic archaeological and linguistic data are imperfect, and I do not wish to suggest that an article dedicated to new genetic research should attempt to work out serious problems of other fields. Yet the limitations of the evidence need to be accounted for, and, more critically, caution must be taken when moving from genetic data to the discussion of identity and related social concepts. I agree with the authors that genetic data will be helpful in ways that the historical record and archaeological material are not, but that is because they are not the same kinds of evidence. Conflating them causes problems, as when the authors suggest that "Punic people" have "no genetic link to Levantine Phoenicians" (L226-227) after defining them as Phoenicians who travelled north and west in the first millennium BCE (L80-83). By comparison, the Moots et al. study of 2023 is admirably cautious about the relationships between the geographic distribution of people, their genetic makeup, and ethnic/cultural identities.

I encourage the authors of the present study to be similarly careful when revising their work. I recommend two specific steps for the framework: 1) offer a clear and consistent working definition of "Punic" for the purposes of this study, making it clear that "Punic" is a historically bounded geographical and cultural label; and 2) anticipate criticism of that definition by addressing how the difficulty of defining a "Punic person" might impact the conclusions drawn. Most of all, I encourage the authors to reconsider how accurate and productive it is to assign a "Punic" label to the burials or genetic material of individuals. I find it more persuasive to restrict that language to discussion of the selected sites, namely, sites "traditionally identified as Punic" in certain historical periods. Last, I do not think the discussion of cosmopolitanism works, as it has nothing to do with genetics so far as I know and there are many examples in the first millennium BCE Mediterranean of shared social behaviors independent of biological mixture.

Specific suggestions:

L2: "few genetic links" vs L226-227 "no genetic links". Which is it?

L91: "archaeological, historical, linguistic, and religious links" is vague in a few ways. I think instead of "archaeological" the authors mean "material culture". But what material culture in particular? Can the authors offer some examples? L127 is also unspecific. L135 is commented on above.

L319: a place where "individuals" are called "Punic". I recommend avoiding such assertions.

L389-390 and Figure 5a: mention and illustration of this unique plaque is perplexing, especially as the only example of material culture illustrated in the article. I'd take it out.

L401 and L410: I do follow "Canaanite Phoenicians from the Levant" or "Canaanite-Phoenician culture". I don't think "Canaanites" should be mentioned at all in this period. "Levantine Phoenician" is better and maybe "Phoenician" is enough, depending on how the authors clean up the framework and general use of these cultural terms.

L454-456: reference to a "traditional view" about Greek-Phoenician competition is not very developed or supported with references and is not, to my mind, disproven by the new genetic evidence. Maybe it can be dropped?

Version 3:

Reviewer comments:

Referee #2

(Remarks to the Author)

Thanks to the authors for their considered response to my last review. I have only a few small comments remaining:

Y chromosome diversity

L296-298: "While varying North African ancestry is a primary driver of the high diversity we observe in Punic sites, Y haplogroup diversity levels in several sites remain higher than in earlier populations, even when this specific contribution is factored out (Extended Data Figure 6 and Supplementary Information S4)"

I note that the two sites that retain highest Y chromosome diversity after North African ancestry is controlled for are both in North Africa (Kerkouane, Carthage). I understand individuals were filtered by autosomal ancestry, but could this high Y

chromosome diversity be due to the presence of North African haplogroups? If so, the above lines are a bit misleading.

L670-677: In my last review, I queried why only the first four characters of the ISOGG classification system were used for Y chromosome diversity estimates. I was not quite satisfied with the answer. I take the authors point that not all samples can be placed further downstream of a four letter clade due to low coverage or absence from the 1240k panel. But why remove information for those that are able to be confidently placed in a haplogroup further downstream? What harm is there in retaining information where it is available? Could the authors please justify this decision and flag to the reader the potential issues in interpretation that might arise from truncating haplogroup information to pre-Holocene divergences.

#### Generational Distances

L316-317: The authors state that individuals who share >20cM in IBD share a common ancestor within the last “several” generations. In my previous review, I asked them to be more specific, but in their response they tell me they used this term to be purposefully vague. I do not think this is appropriate for a scientific journal. If they are not able to provide an estimated range of generations that might separate these individuals then they should not use “several” as a placeholder, as that term is often taken to mean ~3-7. It is possible other readers might interpret “several” in a different manner, which makes it even more problematic.

As the authors say themselves, estimating the minimum or maximum genealogical distance between individuals based on IBD is tricky, due to stochasticity in recombination and variability in background demography. Indeed, it seems to me that the average length of shared segments (or max segment length) would be more informative for estimating the maximum genealogical distance, rather than total IBD shared (which might be in quite short segments).

Please be clearer about the uncertainty here and remove the word “several”.

#### General Comment

This is an important and timely paper that I believe it will attract a great deal of general interest. In my opinion, one of most interesting aspects is the heterogeneity of North African ancestry across regions, through time and within pedigrees, as well as the inter-site connectivity. The high genetic diversity of Punic populations relative to earlier (mostly European) populations is only of secondary interest, as it is simply a corollary of this North African admixture.

Given how the text has evolved throughout the review process, I wonder if the authors should update the structure of the section on “Genetic heterogeneity and interconnectedness” to better highlight the more exciting findings (heterogeneity within sites and pedigrees, IBD connectivity, similar patterns of variation in distinct regions), rather than getting bogged down in proving how diverse Punic populations were relative to a limited set of earlier groups.

#### Referee #4

##### (Remarks to the Author)

I think the authors have addressed my comments and suggestions well. The modified framing makes their argument much more persuasive. They have refrained from referring to individuals or burials as “Punic” as much as possible and made the good decision to omit the discussion of genetic cosmopolitanism. They acknowledge how the term “Punic” is simultaneously difficult and useful so long as it is clear how it is being used, which the authors have shown in their revisions.

I encourage the authors to add references to Helene Sader's work on Levantine Phoenicia throughout the text as appropriate. The authors have yet to cite Lebanese (or Syrian) archaeologists to my knowledge (bibliographical information and explanation below, re: L418-420) and have seemingly missed a key point of connectivity in burial practices.

I have a few additional comments on specific parts of the revised document:

L82-83: I caution the authors to avoid implying that “culture” “spreads” without the very human agents at the center of their study. They might instead say: “The spread of Phoenicians and their culture from the Levant transformed the Mediterranean during the first millennium BCE.”

L91-92: I'll just note that the archaeological record linking Levantine and north African/western Mediterranean sites is sometimes quite weak. The simultaneous heterogeneity and connectivity observed in Punic & Phoenician sites is part of what makes your results (L393-394 and L409-428) interesting to archaeologists. (It will of course also make them crave more data from Lebanese sites, Tyre most of all.)

L113-129: The revision here is excellent. I only call attention again to the use of Quinn 2018 without further specification. It is acceptable to use this book, as the author herself suggested, but I think the authors of the article must include page numbers, so the reader understands what part of this long and layered work they are drawing from.

L367-369 and Figure 5a: I take the authors' point but still find the plaque distracting as none of the Punic material culture is given this much attention.

L413: A small but important point is that the use of “local” is vague, as it obscures the phenomenon and blurs the timing of Greek (Aegean) colonization of Sicily. I think it can be deleted. I see that “local” is used often in the article and suggest looking over each case to see if a more precise word or phrase might suit.

L418-420: The authors refer to a "shift in burial practices". Upon re-reading, I think they mean the shift from cremation to inhumation in the 6th c. observed in central and western Mediterranean Punic sites. This is a valid point but one that suffers from too narrow of a focus. While so far as I know, a shift from cremation to inhumation is not observed in the Aegean in the 6th c., move from cremation to inhumation is attested in Levantine Phoenicia in the late 6th c., notably in elite burials of Sidon. The apparently simultaneous change might be related or unrelated. The authors can use Helene Sader's handbook to address the Levantine phenomenon quickly:

Sader, H. S. (2019). *The history and archaeology of Phoenicia*. SBL Press.

Time permitting, the authors would benefit from familiarity with Helen Dixon's work, because she specializes in the topic of Phoenician burial practices of the 1st millennium BCE:

Dixon, H. M. (2013) *Phoenician Mortuary Practice in the Iron Age I-III (ca. 1200-ca. 300 BCE) Levantine "Homeland"*. Diss. Doctor of Philosophy (Near Eastern Studies) in the University of Michigan.

<https://www.proquest.com/openview/a7f62d11f7bb37be9d3e9570a6a2234f/1?pq-origsite=gscholar&cbl=18750>

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## Reviewer #1

This is an exciting article that will attract a great deal of attention among Mediterranean historians and archaeologists, amid increasing interest in maritime connectivity and mobility in the Iron Age. The key results from that perspective are the suggestion that port city populations of the Phoenician-speaking west were unusually cosmopolitan but lacked significant Levantine presence, and the tantalising suggestion that population change in Sardinia, at least, tracks Carthaginian expansion (a notoriously difficult phenomenon to trace historically or archaeologically).

The study offers support and significant further detail for a model that was beginning to emerge from smaller datasets (Moots et al. 2023). It also offers a useful challenge to the widely-publicised study of modern Y haplogroups as a Phoenician ‘signature’ (Zalloua et al. 2008). It is a particular treat to have significant data from Carthage at last. And (with minor caveats noted below) the article is notably clear and careful in its use of historical population labels as well as its contextualisation of the data in the archaeological record.

I am not equipped to comment on the data, methodology, and use of statistics, but the conclusions seem to me robust and plausible in the historical context.

Leaving aside larger questions of historical interpretation, a debate that I am sure this article will provoke, and the clarity of the initial framing (on which more below), I have only a few minor suggestions for improvement.

Main article, lines 129-131: I know of no evidence that ‘Punic people’ in general “saw themselves as the descendants of earlier Phoenicians who arrived from the East”. There are specific foundation legends linked to Carthage and Cádiz and reported in antiquity to originate in those cities that do associate these two settlements (as well as Utica) with Levantine migrants. Beyond that though we are simply in the dark as to what ‘Punic people’ thought on this matter. It is very likely in my opinion that many of them did in more or less specific terms trace their culture or even their own ancestry to the Levant, but that is not a fact. If there is in fact evidence for this claim, it should be cited; otherwise I’d suggest deleting this point, or limiting its scope to the people of Carthage and Cádiz. (The only citation given before the end of this paragraph is to my own book, which certainly doesn’t say more than that – and should be Quinn 2018, not 2019, as throughout!)

Following this comment, we modified the text at the end of the second paragraph:

L132-137: “Hence, this use of the term “Punic” does not put into question the cultural links between the Phoenician people of the Levant and the Punic people of the central and western Mediterranean. The Levantine roots of Punic people are evident from their shared language and culture and the fact that inhabitants of Carthage saw themselves as the descendants of earlier Phoenicians who arrived from the East no later than the 9th century BCE (Quinn 2018)”

We also corrected the publication year for Quinn’s book throughout the manuscript.

Main article, line 135: I'd delete the phrase "leaving ample room for biases" here: the point is at least as well made without it. All historical sources contain 'bias' of one kind or another, and Phoenician sources would surely be just as 'biased' if not more so than outsiders in relation to their own societies.

We agree and have removed this phrase. Furthermore, in the following sentence, we added a clarification about the potential of ancient DNA in filling the gap of knowledge we currently have about Phoenician/Punic culture:

L139-142: "Ancient DNA provides direct information regarding genetic ancestry, a new line of complementary evidence that can help resolve open questions beyond the reach of conventional historical and archaeological data."

Main article, lines 398-401: I don't think the authors need to mention the Numidians here: they are a sub-set of a larger population of indigenous 'Libyans' (to Greek authors) or 'Afri' (to Romans) that only emerges clearly as a confederation (and then kingdom) in the late third century BCE, rather too late for the point being made here. More generally (and as this article notes in the introduction), Carthage was already a 'major population center' in the 7th century BCE (Telmini et al. 2014 (in Quinn & Vella 2014), 118-122), and there's no reason to think that there wasn't already a significant local component to the population then.

Furthermore, the connection between that point and the changing population of Sardinia is currently unclear. What this expansion of North African ancestry does coincide with is evidence for the expansion and intensification of Carthaginian imperial control overseas, beyond western Sicily (eg Quinn 2018, 87-88).

However, if the authors do wish to mention the Numidians here, Horsted 2021 is inappropriate as a limited and non-academic summary of the evidence insofar as it relates to the Numidian 'army'. Better would be Brett & Fentress 1996 ('The Berbers') with updates in Mattingly 2023 ('Between Sahara and Sea'). For the more general indigenous population of North Africa, which I suggest as a more prudent reference point, Mattingly 2023 alone would now be sufficient.

The primary purpose of the statement about Numidians was to provide a specific example of an indigenous North African population documented in the literature that had integrated with Carthaginians. We did not mean to imply that the particular case of integration with Numidians is directly related to the expansion of North African ancestry in Punic settlements in North Africa and outside of North Africa, such as in Sardinia. Following the reviewer's comment, we now refer to Mattingly (2023) for this, and we also edited this entire discussion paragraph for comprehensibility. In particular, we now clarify that the appearance of North African ancestry in Sardinia and Iberia around 400 BCE was likely facilitated by the expansion of Carthaginian control, which started before that time. See L461-474 in the revised manuscript.

Supplementary Fig. 1: I couldn't see the data for Kerkouane plotted on the 'North Africa' map, only that for Carthage.

Thank you for spotting this error. We have now updated Supplementary Fig. 1 and added the 27 Kerkouane genomes we left out of the original figure to the bottom-left panel.

Supplementary Section 1, p1 (and recurring): there are a number of mentions by of 'Sicanian' people/ancestry in Sicily, which are I think unnecessary: 'Sicanian' is not a clear historical/factual category, but a Greek label for some of the people living in western Sicily, usually understood to be the original population of the island. These people themselves exhibit no very clear ethnic (in the sense of self-identification as a community), political, or even cultural differentiation from other indigenous Sicilians (unlike, for instance, 'Elymians' and 'Sikels' who have left some evidence for distinctive languages). 'Indigenous Sicilian' would, I suggest, suffice here. (Further thinking on this topic, in case of interest: <https://crossreads.web.ox.ac.uk/article/about-arrow-shaped-alpha-and-sikel-identity>; Jonathan Prag's article on 'The Indigenous Languages of Ancient Sicily' in *Palaeohispanica* 2020.)

Thank you for raising this point. We agree that Sicani is a problematic term and have removed it from the text in Supplementary Sections 1 and 2 and from the caption of Fig. S9.

Supplementary Section 1, p2: the 'Punic' dating of the samples from Kerkouane and Carthage can be more confident. Kerkouane was established in the sixth century and abandoned in the mid-third, so any remains found in good archaeological context must date to the 'Punic' period defined here. Similarly, Carthage was destroyed in 146, and its cemeteries were not reused by the later Roman settlers, so since the remains come from an identifiable Punic-era cemetery (or at least I assume they do: the phrasing 'should have come from' (p35) is a little mysterious!) rather than the later Roman city there should be no question about relevance.

Because of these reasons, we had already associated all individuals from Kerkouane and Carthage with the "high-confidence Punic" class in all our analyses. Still, we agree that it is helpful for the reader to highlight an explicit justification for this. We have now added a statement in lines 57-59 of Supplementary Section 1 clarifying this point:

"In particular, Kerkouane was abandoned in the mid-third century BCE, and Carthage was destroyed in the mid-second century BCE, and its cemeteries were not reused by later Roman settlers."

Site Descriptions:

Solar los Chinchurros: 'Opus signum' should be opus signinum

Thank you for spotting this typo. We have corrected it.

Birgi: what is 'a marine road'?

This term refers to a road currently submerged under water in the Mozia Lagoon. We modified the text to clarify our initially ambiguous description:

“The necropolis of Birgi, located on the northern coast of the Stagnone of Marsala, was connected to the island of Mozia by a road (that is now submerged) ending at the North Gate.”

Lilybaeum: it is only in the third entry, for Corso Gramsci, that the relationship between Lilybaeum and other ‘Punic’ sites is explained; I suggest moving that to the first description of the site.

Following this comment, we moved the description of the Corso Gramsci necropolis to precede the other two.

Selinunte: the historical summary here fails to clarify the imposition of Carthaginian rule on this (originally) Greek-speaking city (in 409 BCE), and it also fails to clarify which of the cemeteries described provided the relevant data: the basis of the dating of the sample to the relatively brief and intermittent period of Carthaginian control of the city (409-c. 250 BCE) is therefore unclear.

We modified the site description for Selinunte to clarify its Greek origins and relation with Carthage. We also focused the cemetery description on Manuzza, from which all individuals sampled for this study originate. This cemetery was indeed active in the relevant time frame (between the second quarter and the second half of the 4th century BCE).

Carthage: ‘Latin’ sources should be ‘Greco-Latin’, so as to include the important evidence of Timaios. NB ‘The’ Tophet. And ‘proof’ seems a strong word to use here.

We updated “Latin” to the more accurate “Greco-Latin”, “proof” to “evidence” and “Tophet” to “The Tophet”.

Finally, I would note one issue with the clarity of the framing of the project at the beginning: the explanation of the term ‘Punic’ in the Introduction. As the authors note, this is a Roman word (in fact a simple transliteration of the Greek ‘Phoenician’, as Latin long lacked aspiration: Prag, Papers of the British School at Rome, 2006). It is indeed often used by modern historians and archaeologists (though not consistently by Romans) to refer to Phoenician-speaking communities in the western Mediterranean, at least from the sixth century on, on the (only slightly anachronistic) grounds that that this is a decisive moment in the process of Carthaginian expansion over its neighbours. And I have no objection to the adoption of this term here to point to the same chronological and cultural boundaries: it is a neat modern solution to the problem of a fuzzy ancient set.

I do however think that the meaning of ‘Punic’ remains unclear here in significant ways, especially as the sites chosen range from certain or very probable Levantine foundations (eg Motya, Carthage, Cádiz), through certain or probable Carthaginian colonies (eg Ibiza, Kerkouane), to settlements whose origins remain obscure (eg Malaga, Villaricos, Villamar). Have the sites chosen for the study been “classified” as ‘Punic’ because the Phoenician language was spoken there? Or does ‘Punic’ in this article point to a shared material culture with Carthage and other sites with (more) definite origins in Levantine settlement? Or does it represent a judgement that these particular sites had fallen under Carthaginian imperial

control (or, more vaguely, 'influence')? I think any of these definitions could work with the sites chosen (though there might be questionable cases in each case), but it would be useful to know which - or how many - were actually used to construct this corpus.

Thank you for this insightful comment. In our introduction, we added a working definition of "Punic" for our study, using a combination of criteria (geography, chronology, and material culture):

L125-130: "Here, we adopt this term to refer to populations in archaeological sites in the central and western Mediterranean associated with material culture of Phoenician origin between the late 6th and 2nd centuries BCE. This was a time when the direct influence of the Levantine city-states that founded many of these sites was replaced by Carthaginian influence and before the transition to Roman control."

Our use of "Punic" here contrasts the Phoenician people who lived in the central and western Mediterranean after 600 BCE with those who lived in the Levant. This distinction, mainly based on geography and time, helps evaluate the difference between patterns of ancestry we observe in these two groups. As noted above, we now provide an explicit definition in the introduction. Furthermore, we modified the text throughout to avoid associating the label "Punic" with sites or settlements. We also added a note at the beginning of the **Data set assembly** section clarifying that Punic individuals in our data set were sampled from a wide variety of sites of heterogeneous types, as indicated by the reviewer:

L180-185: "Sampled sites for which we generated genome-wide data cover diverse cultural, temporal, and geographic contexts. They include the Levantine Phoenician settlement of Akhziv, large urban settlements with probable Levantine foundations, such as Cadiz, Motya, and Carthage, and sites acting primarily as Carthaginian colonies, such as Villaricos or the rural settlement Kerkouane (**Figure 1a**)."

## Referee #2 (Remarks to the Author):

### General Comment

This is an interesting and well-structured study. The authors have applied relatively standard analyses to a set of 210 ancient individuals, mostly from Phoenician and Punic sites. The main findings are

1. Punic peoples derive their ancestry mostly from an unknown Sicilian/Aegean source with little detectable input from the Levant. I believe a previous paper already showed as much for a Punic population from a Carthaginian town (Moots et al. 2023). This study comes no closer to identifying the origins of this Sicilian/Aegean ancestry or explaining how Phoenician culture spread without any detectable Levantine gene flow. Genomes from the eastern Mediterranean and pre-600 BC are required to resolve this.

Moots et al (2023) observed a similar distribution of ancestries in their twelve sampled individuals from four tombs in Kerkouane, a rural settlement near Carthage. With the data available in that study, there was no evidence to support the conclusion that this pattern generalizes to a transcontinental culture. Jointly analyzing an order of magnitude more individuals from a dozen more sites across diverse archaeological and geographical contexts allows us to examine similarities and differences between key Phoenician and Punic sites throughout the Mediterranean and to make general statements (see response to comments (a) and (1) below). In our revised manuscript, we explicitly credit the important contributions of Moots et al (2023) while clarifying how our study adds value.

2. North African ancestry is widespread in Punic populations and appears to have been incorporated sporadically, illustrating the important influence of Carthage. I believe this finding is more novel than the first, although it is not clear from lines 142-143 whether North African ancestry has previously been identified in Punic populations outside North Africa.

Marcus et al. (2020) reported evidence of North African ancestry in six sampled individuals from Villamar, Sardinia (which we also reanalyzed here). We now clarify this in the modified version of the 3rd paragraph of the introduction (L153-155). However, since this is an atypical Sardinian Punic site (not coastal and likely linked to a military incursion), one cannot make a generalizable conclusion from this finding. The number of analyzed sites and samples in our study revealed a shared gradient of North African ancestry in several key Punic regions outside North Africa. In our expanded discussion of previous work, we clarified the context of the findings of Marcus et al. (2020).

3. Genetic diversity is high in Punic populations, mainly due to varying amounts of North African ancestry.

We expanded our analysis in the revised manuscript and now show that the increased genetic diversity is not only due to different proportions of North African ancestry but also to variations in other ancestry components (see newly added Fig. S6 and response to comment 2B below).

From this, claims are made about the cosmopolitan nature of the society, its openness to foreigners and the amount of continuous gene flow that occurred across the Mediterranean during the period.

We revised our manuscript to discuss these interpretations more nuancedly (see the detailed response to comments 2B and 2D below).

4. Some Punic communities may also have tended towards endogamy and closed mating networks.

I have no real criticisms of the technical approaches used, which are robust and appropriate.

My main concerns are

a) The novelty and significance of the findings (particularly number 1), given that multiple papers have previously described Phoenician and Punic ancestry in the Levant, North Africa and the western Mediterranean.

Previous ancient DNA studies of Phoenician and Punic individuals were limited to a small number of individuals sampled from a limited set of locations, typically from a single specific archeological site. As a result, no general statements could be made about the ancestry of Phoenician and Punic people. For example, while the study of the individuals from the rural site Kerkouane by Moots et al. (2023) did not see evidence of “Eastern” ancestry, the analysis of individuals from Ibiza (Zalloua et al. 2018) and Sardinia (Marcus et al. 2020) explicitly suggested that there was possible evidence of “Eastern” ancestry putatively linked to Levantine sources, which they could not confidently resolve. Our new data and analysis enable us to conclude that there was no significant Levantine ancestry in these previously published individuals and ~80 newly sequenced Punic individuals from many more settlements and key locations not previously sampled, like the Iberian peninsula and Sicily.

In addition, our extensive data set enables us to compare patterns of ancestry observed in different sites and regions and document a high degree of interconnectedness throughout the Punic world. We revised the relevant parts of the introduction and discussion sections to expand the description of previous ancient DNA studies and clarify the novel observations in our study (see response to comment 1 below).

A number of the conclusions also seem to rely heavily on archaeological and historical data without explicitly saying as much. For example, it is already well-established that the Phoenicians had a cosmopolitan culture and unprecedented levels of maritime mobility over centuries – whether there is strong genetic evidence for this is another matter.

Conventional archaeological and historical evidence cannot decisively answer questions of human mobility. For instance, the lack of genetic links of Punic people to the Levant, which we document here with ancient DNA, is arguably unexpected in light of the linguistic and

cultural connections and historical founding myths. Therefore, we believe that our systematic ancient DNA study adds a valuable new line of evidence, and we explain why our study adds value more explicitly in the revised version of the manuscript.

b) Following from the previous point, some of the authors' claims seem a little dubious on the basis of the genetic data alone. I think more nuanced discussion is required in parts.

We understand that most of this critique stems from the conflation of genetic diversity and cosmopolitanism. We agree with the reviewer that it can be misleading to conclude the cosmopolitan nature of a population from genetic diversity alone. In the revised version of the manuscript, we address this issue more nuancedly (see response to comment 2B below).

I outline my specific issues below

#### 1. Reference to prior work.

The introduction needs to provide more detail on what is already known from ancient DNA studies of the Phoenician and Punic peoples. For example, Phoenician populations of the Levant have been well-characterised (e.g. Haber et al. 2020), but these studies are only cited in the results section as sources of comparative data. Four other studies of Punic populations are mentioned in the introduction, but their findings are discussed in a very vague manner ("contributions from various ancestry sources") and then immediately written off due to sample size. It is hard to assess the novelty of the authors' work without proper context. These studies should also be appropriately cited in the discussion (e.g. lines 361-364).

We expanded the third paragraph in the introduction to provide a more complete description of previous findings from these studies (L142-158 in the revised version). In particular, we now mention the Iron Age individuals sampled from Beirut by Haber et al. (2020) and explicitly cite their findings:

L146-149: *"Whole-genome analysis of five Bronze Age individuals from Sidon (Haber et al. 2017) and twelve Iron Age individuals from Beirut (Haber et al. 2020) indicates that Phoenicians in the Levant were genetically similar to other Canaanite people from the region."*

We now also plot these twelve IA individuals from Beirut in Fig 1 and label them as previously published Phoenician individuals. Furthermore, we provide more details about the ancestry patterns found in Sardinia (Marcus et al. 2020) and Kerkouane (Moots et al. 2023). We end this paragraph by clarifying that these studies obtain key insights but cannot reach general conclusions regarding the ancestry of Phoenician and Punic people due to (1) the small number of analyzed sites, (2) the lack of coverage for key regions such as Sicily and Iberia (including a critical sampling gap of influential settlements with early Phoenician foundations such as Motya and Cadiz), and (3) the apparent complex mixture of ancestries observed in the sampled individuals.

We address previous ancient DNA studies more explicitly in the revised discussion. In the first paragraph of the discussion (L361-364 in the original version, now L403-411), we discuss in detail the findings regarding Eastern ancestry in previously published Punic genomes and clarify the new contribution of our study (that there is almost none of it despite a very large sampling from diverse contexts). In the fourth paragraph of the revised discussion (L457-474), we describe how our study confirmed previous hypotheses about “European” ancestry in Punic populations from North Africa and connect this to our findings of Carthage and its surrounding Punic settlements as a center of population admixture.

## 2. Genetic evidence for the exceptionally diverse and cosmopolitan nature of Punic societies

The authors claim in their abstract that the Punic people “were genetically exceptionally diverse relative to earlier Mediterranean people” and “the first genetically heterogeneous metapopulation of the ancient world”. They also argue for high degrees of mobility and cosmopolitanism, with new people being continuously incorporated into Punic communities over centuries. These are strong claims and ones that I am not convinced by for a number of reasons detailed below.

To substantiate our claims, we introduced several additional analyses and clarified some of the statements; we thank the reviewer for pushing us to provide further justification for these statements, which we believe has strengthened the study. See the detailed response below.

A) Lack of appropriate comparative populations. The authors appear to have very few earlier Mediterranean populations to compare to, especially from Italy and the Aegean. In Fig 3b, it seems there are only two populations dating to the millennium before the Phoenician sites, one from the Levant and one from Central Europe. The Y chromosome plot (3a) also has very few data points during this period. Without having a genetic picture of the Mediterranean prior to Phoenician expansion it is hard to assess their impact on diversity.

Our original manuscript limited our comparison to sites with a minimum number of individuals sequenced with our minimum threshold of aDNA quality. However, applying this strict criterion limited our ability to compare Punic sites with appropriate comparative earlier sites. To address this gap, we added substantially more groups to the revised analysis in Fig. 3:

- 1) As suggested by the reviewer, we now also include Aegean sites (located in modern-day Turkey and Greece). This inclusion added substantially more Mediterranean context, especially from the second millennium BCE.
- 2) We added the Mediterranean BA and IA groups we use for context elsewhere in our diversity analysis (e.g., the PCA of Fig. 1), treating each group as a virtual site.

This revised analysis shows that the pattern we initially described still holds. These newly added groups have lower diversity (both Y and PCA diversity) than we document in most Punic sites, as observed in the revised version of Figure 3. This new analysis substantially strengthens our conclusions.

B) What is the genetic signature of cosmopolitanism? The authors seem to conflate genetic diversity with cosmopolitanism. The logic appears to be the more genetically diverse a population is, the more culturally diverse/open it is, which allows the authors to claim the Punic population had an “exceptionally cosmopolitan culture” relative to comparative populations (line 278).

I can understand the authors’ line of reasoning, but it doesn’t sit quite well with me. For example, I don’t think you could argue that a Viking port was less cosmopolitan or “open to foreigners” than a Phoenician port or an Avar settlement (steppe nomads), even though I’d wager genetic diversity would be much lower. The geographical context also matters. If a highly mobile culture open to trade and outside influences emerges at a continental periphery, the impact on genetic diversity is going to be much less than if it emerges at a continental crossroads. In the case of the author’s dataset, it is admixture with North Africa that really inflates the 2D PCA diversity.

But cosmopolitanism is a cultural phenomenon and I’m not sure the genetic distance between the admixing populations is that relevant. For this reason, to use mostly Central European sites as comparative populations (Fig. 3) does not seem completely fair.

For a word that is used in the title of the paper, I would like to see a more nuanced discussion of cosmopolitanism and how it can be inferred from the archaeological and genetic record.

We agree that the term “cosmopolitanism” can be confusing without contextualization, so we have replaced it in the title with “exceptionally diverse”. However, we do think that our analysis provides evidence for a transformation in population structure that was unprecedented in ancient history and that is a genetic manifestation of the cosmopolitanism of the Phoenician-Punic world, so we have chosen to keep the use of the word “cosmopolitanism” in the main body of the text while providing more precision and nuance about our meaning when we do so. Thus, we now explicitly define the sense in which we use cosmopolitanism in the revised version:

L302-303: “In this study, we define a cosmopolitan population as one that is the product of the ongoing mixing of people of diverse cultural, geographic, and genetic backgrounds.”

This explicit definition now allows us to support our claim of cosmopolitanism more clearly. First, we modified the analysis of genetic diversity in Fig. 3 to show that even without the contribution of North African ancestry, the Punic population was exceptionally diverse (see newly added Supp. Fig. S6 and discussion in Supplementary Information S4). As explained above, we also added Bronze and Iron Age Mediterranean populations in the comparison of Fig 3, which revealed that location also does not explain the high genetic diversity. Therefore, the high genetic diversity we document in Punic individuals is genuinely exceptional and not an artifact of location alone. Furthermore, in Fig S7, we give examples of two reconstructed pedigrees exhibiting high genetic diversity (within each family). These direct examples suggest that ancestry mixing was ongoing in Punic communities and not just

a result of an earlier admixture of a central Mediterranean population and an indigenous North African population.

The results section discussing cosmopolitanism has been extensively edited (L301-351), and in the abstract, we now also unpack the main findings related to cosmopolitanism more clearly by adding a sentence:

L100-104: “The Punic sites we analyzed were genetically exceptionally diverse, and showed evidence of ongoing mixing of different ancestries and mobility between sites. These findings document Punic people as the first genetically heterogeneous metapopulation of the ancient Mediterranean, establishing a pattern that would appear in the later cosmopolitan cultures of Rome and Byzantium.”

C) Evidence from ROH. ROH provides a means to assess the openness of a community's mating networks. However, the ROH profiles do not seem particularly informative in this case as they are not discussed in the context of other populations. Is 44/73 individuals with no ROH >4cM to be expected for an “exceptionally diverse” and “genetically open” population. Looking at Figure S6b, it seems many Bronze and Iron Mediterranean individuals have no detectable ROH. Are the Punic and Phoenician profiles significantly different? If anything, it seems the Punic communities have a higher frequency of endogamy. The authors do discuss endogamous sub-communities but fail to reconcile this behaviour with their vision of a culture “open to incorporating foreigners into their communities”. Again, I think more nuanced discussion is required here.

I'm also unsure how to interpret the effective population size, which is a standalone value with no comparative data. And is it meaningful to provide a single  $N_e$  estimate for a “metapopulation” that encompasses a wide range of geographical regions and ancestries. Would it make more sense to estimate  $N_e$  for specific Punic communities?

We have substantially updated the description of the ROH-based results, highlighting the main observations and clarifying their interpretation in terms of patterns of endogamy. The results are now described in the first paragraph of the new section titled “Consistent practice of sporadic endogamy within Punic populations” (L354-373) and discussed in greater detail in the fifth discussion paragraph (L475-488). In short, we agree with the reviewer that the pattern of ROH observed in the dataset does not conclusively point to an exceptionally diverse population. The overall patterns of short ROH appear quite similar to what we see in many earlier populations depicted in Fig S8 (previously S6). We thus decided to focus this part of the analysis on characterizing the practice of endogamy within the Punic world and removed the ROH-based inference of  $N_e$ . We now use the low levels of ROH observed in most individuals to argue that endogamy was only sporadic and not pervasive:

L366-373: “The fact that we find parental relatedness for individuals from six different sites in all four sampled regions points to a geographically widespread practice in Punic communities of mating between close relatives. However, the overall low levels of even short ROHs 4-12 cM long observed in the remaining large majority of Punic individuals imply that this practice remained somewhat sporadic. Moreover, the general scarcity of ROHs indicates generally large recent effective population sizes and is incompatible with populations that

underwent a severe "bottleneck" (*Ringbauer, Novembre, and Steinrücken 2021; Waldman et al. 2022*)."

In the fifth discussion paragraph, we discuss this finding in light of the overall high levels of diversity and interconnectedness we find in the general Punic population:

L481-488: "While endogamy was persistent, as we found evidence for it in six different sites in all four sampled regions, it was not generally practiced, as indicated by the low levels of close parental relatedness in most individuals in our dataset (**Figure S8a**). Moreover, the two reconstructed pedigrees in Kerkouane and Tharros (**Figure S7**) provide examples of Punic extended families incorporating diverse ancestries, unlike the genetically homogenous relatives interred in tomb 774 from Villaricos. Thus, endogamy was likely practiced only by sub-communities within the general Punic population, which overall exhibited relatively high mobility and relatively free mixing"

D) Evidence for continuous gene flow. The authors claim to show that admixture from North African and Sicilian/Aegean sources was constant, with people of different ancestries continually being incorporated into Punic sites (lines 245-248; lines 285 to 295). While believable, given what we know about the Phoenicians from the archaeological and historical record, I am not convinced the genetic data can be used to support such a claim. The authors' make the assumption that Punic populations experienced random mating and thus inward migration must have been continuous to keep ancestry heterogenous. But there is no reason to assume this. Cultural barriers to random mating can persist for centuries among different communities within a society. The authors themselves later discuss the discovery of an endogamous sub-community, whose ancestry from Figure 2 appears to be relatively homogenous. To argue for continuous mobility and incorporation of foreigners into a population, I think more data is needed. Isotopic data would be very useful, as would denser sampling of single burial communities (e.g. identification of large extended families of diverse ancestries).

We do believe that the genetic data provide compelling evidence of the ongoing mixing of individuals from different ancestry backgrounds in Punic populations, and we unpack the evidence for this more clearly in the revision. In the revised version, we now provide two sources of evidence for this. The first source of evidence concerns the patterns of North African ancestry we observe in many sites. The fact that across sites in North Africa, Sardinia, and Iberia, the proportion of North African ancestry is continuously distributed across individuals from 0% to 90% (Figures S1 and S4) suggests that integration of African ancestry was a continuous and ongoing process. We now mention this in the fourth paragraph of the discussion (L466-471). The second source of evidence for ongoing mixing comes from the two pedigrees we inferred using IBD links, as suggested by the reviewer in comment F below. Unlike in the extended family shown in Fig 5, in these two pedigrees (see newly added Fig. S7), we found that reproductive interaction occurred between individuals with substantially different ancestries. This evidence suggests that Punic people of various ancestry profiles integrated and that there are no strict barriers to random mating. This observation applies to the general Punic community outside of the endogamous sub-communities demonstrated by the individuals from tomb 774, which were likely the exception and not the rule (see response to C above).

Finally, we agree with the reviewer that the findings outlined above do not necessarily imply a continuous influx of new genetic variants from many Mediterranean populations, so we removed the comments about the continuous incorporation of new ancestries from the manuscript (L245-248 and L289-295 in the original version). We also agree that densely sampling single burial communities and isotopic data would add valuable evidence for population mobility. However, dense sampling is unfortunately limited by availability, with samples originating almost exclusively from very old and incomplete excavations. Isotopic analysis would require creating local contexts (from all sites in our study) and comprehensive isotope maps of broad geographic areas (e.g., the North African coast and inland). The collection of such data is out of the scope of our current genetic study. We now suggest this approach as an outlook for future work in the second discussion paragraph (L429-433).

E) Ancestry Distributions. The authors state that “ancestry distributions” in Punic people were similar despite being variable within sites (Lines 296-298). I do not quite understand this line and no figure/table reference was supplied. I take it to mean the north African-Sicilian/Aegean gradient seen in PCA. I am unclear how this demonstrates interconnectedness. Populations can fall in the same place on PCA due to independent demographic processes. And even if a singular demographic process created this gradient, what evidence is there that this process is ongoing? (see comment on random mating above)

Following from this, if most Punic populations are very similar in terms of their “ancestry distribution”, despite being geographically widespread, how does this fit with the hypothesised “genetically open” nature of the society? For example, it seems quite possible that many Punic populations across the Mediterranean were not very open to local communities. The authors suggest this is the case for Iberia and Sardinia, but it could also be the case for Sicily. The authors do not know the source of this “Sicilian/Aegean” ancestry in Punic groups – it does not necessarily derive from local Sicilian groups. And if Punic populations derive their ancestry mostly from just two sources, can you really make the argument that this is an exceptionally cosmopolitan/culturally diverse population from the genetic data?

We appreciate the request for clarification and precision. Our key point in L296-298 of the original version (now L319-325) was simply that ancestry patterns in different sites are similar. This overlap is apparent when comparing the ancestry models of Fig 2 and the per-site distribution in the 2D PCA (Fig S1). This congruency is not limited to the gradient of North African ancestry in the PCA but applies also to distribution within the Sicilian/Aegean cluster. We now refer to these figures explicitly in that part of the text. This similarity of ancestry distributions suggests that while each Punic settlement was diverse, they did not differ much overall. We propose that a parsimonious explanation is that this results from continuous movement and interaction between settlements. It seems unlikely that such similar and diverse populations can emerge in very different geographic locations with different local backgrounds without these locations being connected throughout time. In our revised manuscript, we also report case examples for such connections between sites in the two related individuals from Kerkouane (North Africa) and Birgi (Sicily), and five additional more distantly related pairs, which we found in the more comprehensive report of IBD sharing added due to comment F below.

We agree with the reviewer that our data does not suggest that people in Punic settlements mixed with people in nearby indigenous settlements. Indeed, we did not document such integration in Sardinia and Iberia, and we have no positive evidence for this in Sicily.

F) IBD. The discovery of a pair of relatives between Sicily and Kerkouane is a really nice find, but as it is only a single observation it is hard to draw any firm conclusions about the extent of mobility across the Mediterranean, especially as these sites are actually quite close to one another.

Despite being geographically close, Sicily and Kerkouane are separated by the Mediterranean Sea. This geographic barrier maintained high levels of genetic differentiation between human populations in Sicily and North Africa before Punic expansion and also in modern groups. Thus, we believe this direct evidence of Punic mobility between Sicily and North Africa is notable despite their geographic proximity, and we now explicitly state this (L342-L346).

What do the broader patterns of IBD sharing look like? Is most sharing within or between sites? Do individuals who share IBD tend to have similar ancestry profiles?

Following this suggestion, we added a more detailed description of the substantial IBD links we found (see L326-351). We found 34 pairs of Punic individuals that shared IBD segments longer than 20 cM (see newly added Table S4 for summary and Supplementary Data 1H for a complete list of IBD segments inferred). Of the 34 pairs, 23 linked individuals from the same site (or adjacent sites like the case of Birgi and Motya). The remaining 11 pairs connected distinct sites. These sites were typically close to each other within the same geographic region (such as Malaga and Villaricos or Carthage and Kerkouane). However, in six cases, we found IBD sharing between an individual from Carthage or Kerkouane (North Africa) and an individual from Birgi, Motya, or Tharros (Sicily or Sardinia). This includes the pair of individuals depicted in Fig 4. These 11 pairs provide evidence for (distant) family relations between nearby sites, as well as sites that are separated by the Mediterranean Sea, indicating a high degree of maritime mobility between Punic communities. Furthermore, the two pedigrees in Figure S7, which were inferred using IBD sharing, show examples of close relatives with different ancestry profiles.

## Minor

-Please provide proper table contents for Supplementary.

We added a table of contents at the beginning of the revised SI.

Line 124-127: Hard to follow the logic of this sentence. Are you choosing to distinguish between “Punic” and “Phoenician” peoples because there is a genetic difference? If there was no genetic difference would you recommend that historians cease making a distinction? I think history and archaeology should guide the usage of the term “Punic”, not an ancestry profile (especially given the demonstrated diversity of these populations).

Reviewer #1 also raised this valid point. We modified the second paragraph of the introduction to explicitly clarify what we mean by “Punic” and added a definition based on material culture, geographical, and chronological context:

L125-130: “Here, we adopt this term to refer to populations in archaeological sites in the central and western Mediterranean associated with material culture of Phoenician origin between the late 6th and 2nd centuries BCE. This was a time when the direct influence of the Levantine city-states that founded many of these sites was replaced by Carthaginian influence and before the transition to Roman control.”

Line 128: Do you mean “gene flow” from Levantine populations? Genetic continuity is typically used in the context of continuity within a geographical region, not migration between regions.

Yes, we referred to the absence of gene flow here. We removed this statement from the revised version of the manuscript.

Line 144: Do you mean all studies combined only looked at 20 individuals?

Yes, we referred to a combined total. Actually, it is 21, which we fixed in the revised version. We also clarified the number of samples analyzed in each study.

Lines 281 and 349: What is meant by “extreme” or “ultra” Y haplogroup diversity? These seem like very hyperbolic terms. The Punic values are only extreme in relation to a very small dataset of mostly central European sites. One Italian site has a similarly high value.

In the revised version, we replaced “extreme” and “ultra” with “exceptional”, a term we believe is appropriate in this context. In our opinion, this description is not hyperbolic since four of the five highest Y diversity values across all periods are Punic sites (Fig. 3). As shown in Fig. S5, almost all males sampled from a single Punic site typically have differing Y haplogroups, which is remarkable. The comparison to earlier groups in the revised Fig. 3 is also now more comprehensive and includes Mediterranean locations (as described in response to comment A above).

The non-Punic Italian site the reviewer mentions is Himera (Sicily). The individuals analyzed from this site (Reitsema et al. 2022) were likely mercenaries who fought in the battles between Greeks and Carthaginians in this site in the 5th century BCE. In this specific case, the high genetic diversity is explained by the diverse origins of these mercenary soldiers, and it is still lower than the diversity we observed in all four Punic sites we analyzed in Sicily.

Where is the archaeological supplementary (site descriptions/broader context)? For this number of ancient genomes, I think it is important to provide sufficient archaeological detail.

The reviewer might have missed this, but the original submission already included an extensive archaeological supplementary section with detailed site descriptions that we had organized systematically into general location and chronology, excavation history, description of cemeteries, and relevant references. Reviewer 1 has reviewed this

supplementary and provided constructive suggestions for edits, which we addressed in the revised version.

### Reviewer #3

I am not a DNA expert, so I will confine my remarks to the radiocarbon data, which is my expertise.

The radiocarbon data is basically summarized in the supplemental Excel file S1, pages 1 and 2. The details are in page 2. Everything looks in good order to me, except:

1. Why is (Delta 14C) presented in columns K and L, when this is just  $(F-1)*1000$  and not the usual usage of Delta 14C which includes an age correction (where the age is known). I suspect this is solely because it's generated by the BATS program for the MICADAS machine, so I recommend deleting these two columns as they convey no additional information.

We note that our  $D^{14}C$  is not  $\Delta^{14}C$  (both defined in Stuiver and Polach (1977); the latter corrects for the decay since the time of collection).

However, as the columns  $D^{14}C$  are straightforwardly related to the "Fraction Modern" column in the way the reviewer described, we removed the two  $D^{14}C$  columns to avoid confusion and redundancy.

2. The last sample S-8135 is stated to be run at "CIRAM". CIRAM is a sample-preparation only laboratory and does not do  $^{14}C$  analyses, they are all run elsewhere. The information about WHERE the AMS measured was made should be included.

We have extracted the location of the accelerator mass spectrometer from the C14 report that we received and added the information on where the CIRAM AMS date was measured to our methods section:

"For the CIRAM date, the different carbon isotopes were separated using a 250 kV accelerator mass spectrometer in a joint venture with JSC Barnas."

## Referees' comments:

### Referee #2 (Remarks to the Author):

I thank the authors for their thorough response to my previous review. I raised two main issues – reference to prior work and the genetic evidence for cosmopolitanism.

The first issue has been rectified, with ample context from prior aDNA studies now provided. However, there is one thing I think should be corrected. In lines 151-153, it is said that whole genome data from 12 Punic individuals at Kerkouane “suggested that” Punic people in North Africa “might have” substantial European and North African ancestry (Moots et al. 2023). Why multiple qualifiers? The Moots study definitively demonstrated both North African and European ancestry in this population and this should be made clear.

We meant that the ancestry patterns in a dozen individuals from a single rural North African site might not be fully generalizable beyond this specific site and the timeframe of the sampled individuals. However, we agree that the previous text could be read as suggesting that the earlier work had not established the presence of such ancestry in North Africa. We have now updated the text as follows:

L139-142: However, a mitochondrial genome from Carthage (Matisoo-Smith et al. 2016) and whole-genome data from twelve individuals from the nearby rural Punic site of Kerkouane (Moots et al. 2023) showed substantial Southern European ancestry as well as indigenous North African ancestry.

In response to the second issue I raised, the authors have provided new analyses and softened their interpretations. I think this has greatly improved the study. However, a few concerns still remain.

A. Definition of cosmopolitanism: The authors agree that it is misleading to conflate genetic diversity and admixture with cosmopolitanism. However, they then go on to provide their own non-standard definition of cosmopolitanism that does just that, allowing them to then apply this term to the interpretation of their results.

Line 302: “a population that is the product of the ongoing mixing of people of diverse cultural, geographic, and genetic backgrounds”

But this is a poor definition of cosmopolitanism and is provided without citation. Indeed, many sampled ancient populations are the product of ongoing genetic/geographic/cultural mixtures, resulting in heterogeneous ancestry profiles. However, no one is calling the Neolithic or Bronze Age populations of western Europe cosmopolitan, despite there being high variability in diverse ancestries in some regions, sometimes within single sites (Olalde et al. 2018; Seguin-Orlando et al. 2021). This is because the term cosmopolitan refers primarily to a set of cultural values that are based on openness, tolerance and inclusivity. A cosmopolitan society is one that is open to people who possess many different ethnic, cultural and religious identities, and emphasises shared universal rights and values among its diverse members. It follows that cosmopolitan populations are likely to harbour diverse

and mixing ancestries. However, it does not follow that all populations that harbour diverse and mixing ancestries are cosmopolitan.

If the authors are committed to having the term “cosmopolitan” in the text, I’d ask them to not treat it as a buzzword, but instead engage with its full meaning and prior literature on the inference of cosmopolitanism in the classical world and archaeological record more broadly (e.g. urbanisation, cultural exchange, religious syncretism, rules of citizenship). This includes providing a well-accepted and cited definition of the term. Also, Lines 304-6 do not make sense - “Punic individuals were cosmopolitan in this sense ... but also genetically” - There is no such thing as a population being “genetically cosmopolitan”. Cosmopolitanism is a cultural phenomenon. Admixture between divergent populations does not equal “genetic cosmopolitanism”. Please rephrase this section.

We appreciate these comments. We think that the interesting findings of our study do not pertain to whether or not Punic people qualify as cosmopolitan in the sense the reviewer refers to. We removed the term “cosmopolitan” from the revised manuscript to avoid criticism about what that word means. Instead, we directly focus on genetic heterogeneity, ongoing mixing of diverse ancestries, and interconnectedness among different sites, which we have shown to be important features of the Punic Mediterranean.

Finally, the authors compare the Punic people to the “later cosmopolitan cultures of Rome and Byzantium” in their abstract (Line 103), but do not expand on this anywhere in the main text or archaeological supplementary, which could be useful. It is an interesting comparison, as I believe the cosmopolitan nature of Carthage (primarily economic and functional) is thought to have been more limited than that of Rome (e.g. integration of conquered peoples through citizenship), with the elites of Punic society less open to outside influence. It could be useful to consider these different manifestations of cosmopolitanism when interpreting the genetic data. An ethos of openness and “free mixing” (Line 488) need not have penetrated all strata of society, which could be a potential explanation for some seemingly contradictory genetic patterns (see next section).

As part of removing the term “cosmopolitan” from the paper, we removed the phrase “cosmopolitan cultures of Rome and Byzantium” from the abstract. This change also reflects a request from the editor to reduce abstract length.

B. Genetic support for claims of cosmopolitanism (and general exceptionalism):  
The authors still make several very strong claims that I remain uneasy about.

Firstly, to say that the Punic people are the “first genetically heterogenous metapopulation of the ancient Mediterranean” is quite sweeping statement (Line 102), as well as vague. Firstly, does the ancient Mediterranean refer to the entirety of Mediterranean prehistory or just classical antiquity? Secondly, what is meant by “heterogenous metapopulation”? This is not a term that is used often in ancient genetics and is not defined in the text. I take it to mean a set of subpopulations with some level gene flow between them and some level of heterogeneity within them (what those levels are I do not know).

The reviewer correctly interprets our use of this term. We agree we did not clearly define it. To improve clarity, we revised the last part in the abstract as specified below and removed the ambiguous term “metapopulation” throughout the revised version of the manuscript.

L97-100: People in different Punic sites across the central and western Mediterranean show similar patterns of high genetic diversity. We also detect distant genetic relationships across the Mediterranean, reflecting shared demographic processes shaping populations around the Punic world.

How can the authors claim that “heterogenous metapopulations” did not exist in the Mediterranean before the Punic people? Indeed, I would hesitate to call any sampled ancient population the first anything, given the patchy nature and Eurocentric bias of reference databases. I recommend the authors qualify this statement or remove it.

We removed this statement from the revised abstract (see response above) and now avoid implying that Punic people were necessarily the first people with a pattern of high inter-individual diversity.

Secondly, I am still not convinced the genetic findings alone are strong enough to “document a highly cosmopolitan culture” (line 426-427) and would remove this phrase. In their revision, the authors have now laid out five lines of genetic evidence for their own (flawed) definition of a cosmopolitan society under the header “One of the first cosmopolitan cultures of the ancient world”. Two of these lines of evidence relate to genetic diversity (Y chromosome and autosomal), two to mobility (similar ancestry distributions and IBD sharing) and one to ancestral diversity within a family. However, the strength of this evidence is medium at best. There are two main issues:

1. As I put forward in my original review, neither high genetic diversity, nor high mobility, are necessarily indicative of a “highly cosmopolitan culture”. For example, I think it would be an overstatement to describe the Avar as highly cosmopolitan or culturally open, despite their mobility and likely having higher 2D PCA diversity than the Punic people (Gnecchi-Ruscone et al. 2024). The authors seemed to agree that this conflation is problematic in their response letter, but it would be useful for them to acknowledge this in the main text.

We agree on reflection that defining what constitutes cosmopolitanism from only a genetic point of view is challenging, with the case of the Avars being a case that helps to highlight the issues. We do not wish our definition of “cosmopolitanism” to distract readers from the main findings, so we removed all mentions of this term from the revised manuscript.

Importantly, the authors note themselves that the genetic diversity observed in Punic populations is not due to them deriving from a large diversity of sources (which you might expect for a cosmopolitan society), but a limited set of similar or identical sources (Lines 321-322), including a divergent North African population. New analyses (Fig S6b) confirm this North African ancestry is the primary driver of the exceptional diversity levels seen at Punic sites (although this is downplayed somewhat in lines 317-318 – see comment below).

I do think the wide variance in North African ancestry in Punic populations and extended families is of great interest. However, I’m unclear if the authors are concluding that the

integration of individuals from this ancestral background was a sporadic occurrence (lines 287-289), or if it occurred quite freely (lines 422-424). Could they clarify this? In any case, it is clear the social dynamics underlying this admixture (including cosmopolitan cultural values) are still not well-defined and any interpretations drawn should be careful and considered.

We do not know the exact number of narrow source populations from which Punic genetic diversity is derived. While we treat the Sicilian-Aegean ancestry component as a single source, it is unclear whether it is attributed to a single source population or admixture with several populations from the central-eastern Mediterranean. We thus refrain from making any explicit statements regarding the number of source populations contributing to the observed diversity. North African ancestry indeed significantly contributes to genetic diversity in our dataset (see response below). Following the reviewer's points above, we revised the text to avoid social interpretations about the mixing of different ancestries that are not unambiguously demonstrated by our analyses. In particular, we removed the statements mentioned by the reviewer about sporadic or free mixing of ancestries.

2. Some observations seem to actively contradict the interpretation of cosmopolitanism (and these are interesting in their own right – societies can be closed in some ways and open in others). The Punic populations are described as highly mobile and genetically diverse, but also with persistent endogamy (line 481) and very similar ancestry distributions between sites, with little local admixture in a number of regions. These results do not paint a neat picture of a “highly cosmopolitan” (Line 427) and “free mixing” (Line 488) culture. Indeed, the results present the possibility that Punic society was relatively closed in some regions (e.g. ~50% of Iberians have ROH >50cM and little local admixture) or possibly in some social strata. As the authors provide no data on the inferred social status of the individuals sampled here (is there a reason for this stated anywhere in the text?) it is hard to make firm conclusions.

To be clear, I do not have a problem with the authors discussing how their results interact with archaeological and historical evidence for cosmopolitanism in the Punic world. What I take issue with is that lines 301-306 and lines 424-427 read as if the genetic data provides a strong independent line of evidence that Punic society was highly cosmopolitan, when the data presents a more complex picture. I worry that if geneticists are not explicit that their interpretations of the data are being shaped by prior knowledge from other fields, it can lead to circular referencing between disciplines. **I would change the header of line 301 to reflect the actual results within (diversity, mobility).** I would also provide a brief overview of the current thought on the nature of Punic cosmopolitanism in the discussion and whether the genetic results provide some support for these ideas or challenge them. When “free mixing” is discussed, it should be carefully caveated.

As mentioned above, we decided to remove the concept of “cosmopolitanism”. This addresses the reviewer's concern regarding circular referencing between disciplines. We hope that future multidisciplinary articles will directly engage with the question of cosmopolitanism (as defined by the reviewer) in Punic communities and the ancient world in general.

I make some other specific suggestions below:

1. Lines 487-488 seems to suggest that endogamy cannot co-exist with high individual mobility, which is obviously not the case. Indeed, in elite societal strata both often co-occur (look at Europe's aristocratic networks).

We removed this statement from the revised discussion. Instead, we highlighted the evidence of diverse social practices - from genetically homogenous and endogamous sub-communities to extended families with diverse ancestries (see last paragraph before the Discussion).

2. I don't think the sample size is large enough to conclude that endogamy only occurred sporadically across Punic populations (Line 353). It could have been quite frequent in some regions like Iberia. Also, I think it is speculative to conclude that "endogamy was likely practiced only by sub-communities" (Lines 487-488) and would soften this. While this could well have been the case, endogamous marriages can also occur alongside exogamous ones in the same sub-community due to different material circumstances of the individuals involved (inheritance is the big one – heirs may be more restricted in their choice of marriage partner relative to other children; couples without sons may choose a son-in-law from among their relatives)

The statement about sporadic endogamy is based on an analysis of 86 individuals from 14 sites (Extended Data Figure 8a), which we think is an adequate sample size to make a meaningful statement. In particular, we base our assessment of overall sporadic endogamy on the overall low levels of ROHs in our dataset.

It is indeed the case that we observe a higher rate of endogamous unions in sampled Iberian individuals. We revised the text to highlight this point and also removed the statement about the association of endogamy with sub-communities:

L348-355: The fact that we find cases of close parental relatedness for individuals from six different Punic sites in all four sampled regions points to a geographically widespread practice of mating between close relatives. However, the overall low levels of ROHs in the large majority of analyzed individuals imply that this practice remained sporadic in most regions (with the possible exception of Iberia; Extended Data Figure 8a), and indicates a large recent effective population size, incompatible with the hypothesis that a substantial fraction of the ancestry of Punic people derived from populations that had experienced an intense founder event (Ringbauer, Novembre, and Steinrücken 2021; Waldman et al. 2022).

3. The authors rely heavily on the words "exceptionally", "unprecedentedly" and "remarkably", when describing the genetic diversity of Punic populations. This seems a little sensational to me, as they are only exceptional with respect to a limited comparative dataset, although I appreciate the authors have done their best to include as many references as possible. But there are still many geographic gaps (e.g. almost all earlier pops are European with none from North Africa itself). I think the descriptive language could be softened a little without reducing the novelty of these results.

We think that currently sequenced groups are sufficiently comprehensive to warrant such qualitative statements. Nevertheless, we agree that removing words that can be read as sensationalist from a scientific paper is often helpful, so we removed most of these qualitative adjectives from the manuscript.

4. The new analysis (Fig S6) seems to confirm that the high diversity of Punic populations is primarily driven by North African ancestry, although I think the description of these results in lines 317-318 are a bit misleading. Only 2/6 Punic populations have 2D PCA diversity levels higher than earlier sites (Fig S6b). Is there a statistically significant difference here that allows the authors to state that “in Punic populations, diversity levels remain higher than in earlier populations even when this specific contribution is factored out”?

We revised the statement to highlight the prominent role of North African ancestry in increased within-site diversity more clearly:

L296-299: While varying North African ancestry is a primary driver of the high diversity we observe in Punic sites, Y haplogroup diversity levels in several sites remain higher than in earlier populations, even when this specific contribution is factored out (Extended Data Figure 6 and Supplementary Information S4).

Note that the analysis of Extended Data Figure 6 is conservative in entirely ignoring the contribution of individuals with North African ancestry (instead of replacing it with a less divergent ancestry source).

Also, there are near-contemporaneous Italian and Iberian populations with higher diversity in fig S6b (and similar diversity in Figure 3), which could indicate that these changes in population structure and mobility are not just a Punic phenomenon.

These sites are Tarquinia Monterozzi (Italy) and Empuries (Spain) (see Table SI6 in SI Section 6). Tarquinia mainly includes individuals dating to the Roman Republic after 200 BCE. Similarly, most genomes from the initial Greek colony of Empuries, Girona, date to after the Roman conquest of this region ca. 200 BCE. Therefore, these individuals post-date the Punic sites in this manuscript. Hence, these two sites do not challenge our claim that Punic sites were among the first with high genetic heterogeneity, a pattern that became common in later Mediterranean sites (as evident from Figure 3).

Also, the two Punic contexts with highest 2D PCA diversity in Fig S6b, Tharros and Lilybaeum/Birgi/Motya, have individuals with dates that may overlap with Roman expansion. Can I check that these were excluded from this analysis? Also were a minimum number of individuals per context used for Fig 6b, as was done for Fig. 3?

Figure 3 and Extended Figure 6 use the same set of individuals with the same filtering criteria, including the minimum number of individuals per context. The only difference between the figures is that in Extended Figure 6, we additionally filtered out Punic individuals with North African ancestry and grouped individuals from the nearby sites of Motya, Birgi, and Lilybaeum into one group (because otherwise, they would drop below the minimum number of required individuals). We updated the figure legend to clarify this.

Individuals who may have overlapped with Roman expansion (dated to 350-50 BCE) were included in the two figures. We included these samples in the analysis because many likely represent Punic context, and we did not want to discard all of them and substantially reduce the statistical signal. We added the following text to the Methods section to clarify this point:

L685-689: For both diversity measures, in Punic sites we included individuals with a date consistent with the Punic period (labeled Punic\_Early, Punic\_Late, Punic\_Late2, Punic\_NoRC in the column label PCA in Extended Data Table 1E). Including individuals dating to 350-50 calBCE and individuals without radiocarbon dates was necessary to obtain statistically meaningful sample sizes for quantifying diversity.

This sample selection does not meaningfully affect the diversity levels in most sites, such as Villaricos, Kerkouane, Carthage, or Motya. But as the reviewer hints at, as a result of the small sample sizes in Tharros and Lilybaeum after filtering out these individuals and individuals with North African ancestry, it is difficult to argue with high confidence that PCA diversity levels in these sites were elevated before 200 BCE when factoring out North African ancestry. We thus revised the statement about **Extended Figure 6** in the main text to mention only Y haplogroup diversity (see also two replies above).

5. Line290 – please be clear here that it is also quite possible that Punic populations in Sicily did not receive substantial local input. Given the widespread geographical distribution of this Aegean-Sicilian ancestry (and lack of local contribution in other regions) both scenarios have similar probabilities.

We revised the relevant text as follows to highlight this alternative scenario:

L265-267: Many individuals in Punic sites in Sicily and North Africa harbored substantial proportions of ancestry consistent with that observed in earlier people from the same geographic regions (this ancestry not necessarily being local).

6. Figure 3a Y axis should be Y haplogroups, not haplotypes. Also, it seems very arbitrary to use the first four characters of the ISOGG classification system as a cut-off (corresponds roughly to last glacial divergences). Why not use the full haplogroup label for each individual? By choosing such a threshold you will deflate diversity values in certain geographical regions where Y chromosome diversity underwent more recent homogenising events (e.g. R1b1 expansion during steppe migrations). As you are trying to demonstrate the exceptional nature of Punic Y haplotype diversity, it would be a more conservative approach to use full haplogroup labels for comparative sites. Please justify why this was not done in the text.

We updated the Y-axis label of Figure 3a and, similarly, of Extended Data Figure 6a and added the following justifying argument to the methods section:

L674-679: To quantify Y haplogroup diversity per archaeological site, we considered the first four characters of the ISOGG classification for each inferred haplogroup. We used this particular cutoff for two reasons: (1) low coverage aDNA does not consistently allow assigning Y haplogroups at more refined levels due to missing data and genotyping errors

caused by aDNA damage; (2) the specific 1240k SNP set enriched in these genome sequences do not have the resolution for several macro-haplogroups beyond this level (e.g., E1a1).

7. Pedigree in S7a is hard to understand. Are I24215 and I24194 inferred to be descendants of parents of I24494 and I24193?

We added a clarifying statement to this figure caption:

“In the Kerkouane pedigree shown in (a), individuals I24215 and I24194 are inferred to be 3rd-4th relatives of the two siblings I24494 and I24193, but the exact pedigree relationship cannot be resolved.”

8. The provided numbers of new samples generated and analysed are inconsistent. In the abstract it says 196 from Punic/Phoenician sites were analysed. (Line 86). But from the intro (Line 166) it seems only 131 individuals produced analysable data? The number 196 does not appear anywhere else in main text. I think the number used in the abstract should reflect that in the main text to avoid confusion.

We now mention the 196 individuals from Phoenician-Punic sites for which we generated sequence data for this study toward the end of the first paragraph of the **Data set assembly** section (starting at L153).

9. Line 329 – it is more appropriate to say “relationships up to the 5th-7th degree” as kinship is typically viewed as a social phenomenon.

We revised the wording to “biological relatives up to the 5-7th degree”.

10. Line 336. I think it would be better to be more explicit on the estimated number of generations that could separate two ancient individuals sharing >20cM. “Several” is quite vague.

We left this vague on purpose, as there is no singular cutoff. The more distant the relationship, the less likely it is to share a long IBD segment, and recent demography can (modestly) shift the plausible ranges (e.g., recent population bottlenecks produce more long IBD segments) when calculating 95% CIs of >20cm IBD segment coalescent ages.

11. Line 455 – This is odd phrasing. While I know it is not the authors’ intention, it could be misinterpreted as saying that the Greek and Phoenician civilisations were fuelled due to them carrying a particular biological ancestry. I would rephrase to avoid confusion.

We removed this sentence from the revised discussion, which we generally shortened to fit the journal’s formatting guidelines.

## References

Gnecchi-Ruscone, G.A., Rácz, Z., Samu, L. et al. Network of large pedigrees reveals social practices of Avar communities. *Nature* 629, 376–383 (2024). <https://doi.org/10.1038/s41586-024-07312-4>

Olalde, I., Brace, S., Allentoft, M. et al. The Beaker phenomenon and the genomic transformation of northwest Europe. *Nature* 555, 190–196 (2018). <https://doi.org/10.1038/nature25738>

Seguin-Orlando, A. et al. Heterogeneous Hunter-Gatherer and Steppe-Related Ancestries in Late Neolithic and Bell Beaker Genomes from Present-Day France. *Curr. Biol.* 31, 1072–1083.e10680 (2021).

Referee #2 (Remarks on code availability):

NA

### Referee #3 (Remarks to the Author):

They said in reply to my comment:

2. The last sample S-8135 is stated to be run at "CIRAM". CIRAM is a sample-preparation only laboratory and does not do  $^{14}\text{C}$  analyses, they are all run elsewhere. The information about WHERE the AMS measured was made should be included. We have extracted the location of the accelerator mass spectrometer from the C14 report that we received and added the information on where the CIRAM AMS date was measured to our methods section: "For the CIRAM date, the different carbon isotopes were separated using a 250 kV accelerator mass spectrometer in a joint venture with JSC Barnas."

Well BARNAS ([barnas.lt](http://barnas.lt)) doesn't do AMS measurements either. They do sample preparation. So, it was run somewhere else. My guess is Vilnius or Poznan.

We contacted CIRAM, who informed us that the C14 dates were produced by an AMS in Vilnius, Lithuania, as the reviewer had correctly guessed. We added this fact to our Method section:

L512-514: For the CIRAM date, the different carbon isotopes were separated in a joint venture with JSC Barnas using a 250 kV accelerator mass spectrometer from FTMC in Vilnius, Lithuania.

#### Referee #4 (Remarks to the Author):

I am leaving the assessment of the scientific methods and results to those experts.

Like the first reviewer, I am certain that this article will be of interest to people working on Punic and Phoenician topics. It is significant for its sample size and draws some exciting conclusions about the movement of and intermarriage among populations in specific Mediterranean regions during the first millennium BCE. I enjoyed reading about the genetic work very much.

I am less sanguine than the first reviewer concerning the historical and ethno-cultural framing and its reliance on archaeological and linguistic evidence. The updated text in L134-137, for example, does not strike me as clarifying the working definition of “Punic”, as no evidence is offered of the claimed “shared language and culture” for the selected sites in the article. The site summaries, while appreciated, add little more evidence of the purported connections. Later in the same sentence, we read: “inhabitants of Carthage saw themselves as the descendants of earlier Phoenicians who arrived from the East no later than the 9th century BCE”, which is not a central argument of the only supplied reference, Quinn 2018.

While the authors can certainly revise this sentence, it is a symptom of larger (but correctable) difficulties with their methodology, which begins with a definition of “Punic” that is not very rigorous. Punic archaeological and linguistic data are imperfect, and I do not wish to suggest that an article dedicated to new genetic research should attempt to work out serious problems of other fields. Yet the limitations of the evidence need to be accounted for, and, more critically, caution must be taken when moving from genetic data to the discussion of identity and related social concepts. I agree with the authors that genetic data will be helpful in ways that the historical record and archaeological material are not, but that is because they are not the same kinds of evidence. Conflating them causes problems, as when the authors suggest that “Punic people” have “no genetic link to Levantine Phoenicians” (L226-227) after defining them as Phoenicians who travelled north and west in the first millennium BCE (L80-83). By comparison, the Moots et al. study of 2023 is admirably cautious about the relationships between the geographic distribution of people, their genetic makeup, and ethnic/cultural identities.

I encourage the authors of the present study to be similarly careful when revising their work. I recommend two specific steps for the framework: 1) offer a clear and consistent working definition of “Punic” for the purposes of this study, making it clear that “Punic” is a historically bounded geographical and cultural label; and 2) anticipate criticism of that definition by addressing how the difficulty of defining a “Punic person” might impact the conclusions drawn. **Most of all, I encourage the authors to reconsider how accurate and productive it is to assign a “Punic” label to the burials or genetic material of individuals. I find it more persuasive to restrict that language to discussion of the selected sites, namely, sites “traditionally identified as Punic” in certain historical periods.**

We fully agree with the reviewer that labeling individuals and sites as “Punic” oversimplifies the complexities of Phoenician culture and population structure. At the same time, we need a term for these geographically diverse sites in the central and western Mediterranean, which are united by some aspects of material culture and Carthaginian hegemony. We therefore

think it is appropriate to define and use the term “Punic” while also problematizing it appropriately, which we attempted to improve in our updated manuscript. We substantially revised the text in the 2nd introduction paragraph:

L113-129: Many historians and archaeologists use the Roman term “Punic” to refer to culturally Phoenician communities in the West Mediterranean that were associated with or even ruled by Carthage after the mid-6th century BCE (Moscati 1988; van Dommelen and Bellard 2008; Doak and López-Ruiz 2019; Roppa, Botto, and Van Dommelen 2021). While problems of such a simplifying label have been highlighted (Prag 2014), we follow the recent Handbook of the Phoenician and Punic Mediterranean (López-Ruiz and Doak 2019) and use “Punic” to refer to sites in the central and western Mediterranean associated with material culture with Phoenician antecedents or connotations between the late 6th and 2nd centuries BCE, a time frame of Carthaginian hegemony in this region. We also use the term to refer to the people buried at these sites. However, we caution that by labeling sites and people as “Punic” we do not make any claim of a homogeneous culture or ethnicity. Moreover, our distinction between Phoenician and Punic sites does not question the Levantine cultural origins of their inhabitants, nor does it address the question of whether the founders of Phoenician-Punic sites such as Carthage and Cádiz were Levantine (J. Quinn 2018); we do not have the early samples from these sites needed to address these issues. We use “Punic” in this paper simply as a means to refer to a group of sites and their inhabitants as defined above, for which our analysis finds shared genetic signatures.

Furthermore, in the revised version, we refrain from referring to individuals as “Punic” throughout most of the text. Only in a few instances, for brevity, do we use “Punic individuals” as shorthand for “individuals from Punic sites” as discussed in the definition above. We believe that these updates address the reviewer’s concerns and also the specific points they raised regarding the following parts of the previous manuscript:

- L134-137: We revised the text to discuss shared culture more carefully. We kept the note about the Levantine foundations of Carthage because we think it is helpful, even if it is not the main argument in Quinn 2018 (who herself approvingly discussed this citation in the first round of Review).
- L80-83: We removed this sentence from the shortened abstract to avoid implying that Phoenicians from the east “became” Punic people in the west.

**Last, I do not think the discussion of cosmopolitanism works**, as it has nothing to do with genetics so far as I know and there are many examples in the first millennium BCE Mediterranean of shared social behaviors independent of biological mixture.

We agree on reflection that cosmopolitanism is challenging to define from genetic results alone (see also reply to reviewer #2). Therefore, we have removed this term from the revised manuscript and now focus on highlighting genetic heterogeneity and interconnectedness.

Specific suggestions:

L2: “few genetic links” vs L226-227 “no genetic links”. Which is it?

Our models did not detect genetic links with the Levant, which leaves room for a small fraction below our detection threshold that we cannot rule out. To avoid the resulting confusion, we harmonized our text to “few links” or “little genetic contribution”.

L91: “archaeological, historical, linguistic, and religious links” is vague in a few ways. I think instead of “archaeological” the authors mean “material culture”. But what material culture in particular? Can the authors offer some examples? L127 is also unspecific. L135 is commented on above.

We agree that our use of the term “archaeological” in the abstract was ambiguous. We revised the text for clarity:

L89-92: Levantine Phoenicians made little genetic contribution to people living in Punic settlements in the central and western Mediterranean between the 6th and 2nd centuries BCE, despite abundant archaeological evidence of strong cultural, historical, linguistic, and religious links.

We stress that it is archaeological evidence that supports the cultural, religious and linguistic links, i.e., the material remains of temples, burials, pots, and the like, as well as inscriptions - as there are no Phoenician texts preserved in manuscript traditions like Greek and Latin.

We revised the text in L127 and L135 for clarity (see response above).

L319: a place where “individuals” are called “Punic”. I recommend avoiding such assertions.

We revised this sentence:

L300-302: “Third, our genetic data document that the individual ancestry variation was high within Punic sites, but this variation was overall similar between different regions ...”

Generally, as mentioned in our response above, we mostly refrain from referring to individuals as “Punic” in the revised manuscript.

L389-390 and Figure 5a: mention and illustration of this unique plaque is perplexing, especially as the only example of material culture illustrated in the article. I’d take it out.

We highlight this plaque because it is part of the particular case example we discuss in detail in the main text (tomb 774 in Villaricos). The original excavator referred to it as a “Greek” (Astruc 1951), which makes it noteworthy given the Aegean-like ancestry we infer for all sampled individuals in this tomb.

L401 and L410: I do follow “Canaanite Phoenicians from the Levant” or “Canaanite-Phoenician culture”. I don’t think “Canaanites” should be mentioned at all in this period. “Levantine Phoenician” is better and maybe “Phoenician” is enough, depending on how the authors clean up the framework and general use of these cultural terms.

We removed “Canaanite” from “Canaanite-Phoenician” when referring to Iron Age Phoenician culture, but we kept it when referring to the full cultural continuum starting in the Levantine Bronze Age.

L454-456: reference to a “traditional view” about Greek-Phoenician competition is not very developed or supported with references and is not, to my mind, disproven by the new genetic evidence. Maybe it can be dropped?

We removed this sentence from the revised version (see also response to Reviewer #2).

In general, we thank the new reviewer for their excellent comments. They have improved our revised manuscript and helped to pre-empt some critical responses.

## Point-by-Point Responses to Referees' comments

### Referee #2 (Remarks to the Author):

Thanks to the authors for their considered response to my last review. I have only a few small comments remaining:

Y chromosome diversity

L296-298: “While varying North African ancestry is a primary driver of the high diversity we observe in Punic sites, Y haplogroup diversity levels in several sites remain higher than in earlier populations, even when this specific contribution is factored out (Extended Data Figure 6 and Supplementary Information S4)”

I note that the two sites that retain highest Y chromosome diversity after North African ancestry is controlled for are both in North Africa (Kerkouane, Carthage). I understand individuals were filtered by autosomal ancestry, but could this high Y chromosome diversity be due to the presence of North African haplogroups? If so, the above lines are a bit misleading.

The two sites with the highest Y haplotype diversity in Extended Data Figure 6 are Tharros (Sardinia) and Kerkouane, not Kerkouane and Carthage. As stated in the caption of Extended Data Figure 6, individuals in this Y haplogroup analysis were filtered based on their Y haplogroups and not autosomal ancestry:

“excluding the three Punic individuals (from Kerkouane, Villaricos and Selinunte) with distinct North African Y haplogroups E1a and L”

We note that none of the other haplogroups observed in our dataset (see **Extended Data Fig 5**) has specific associations with North Africa.

To further clarify this point in the figure, we modified the legend of Extended Data Fig. 6 to explicitly annotate “Filtered for North African ancestry **or Y haplogroup**”.

L670-677: In my last review, I queried why only the first four characters of the ISOGG classification system were used for Y chromosome diversity estimates. I was not quite satisfied with the answer. I take the authors point that not all samples can be placed further downstream of a four letter clade due to low coverage or absence from the 1240k panel. But why remove information for those that are able to be confidently placed in a haplogroup further downstream? What harm is there in retaining information where it is available? Could the authors please justify this decision and flag to the reader the potential issues in interpretation that might arise

from truncating haplogroup information to pre-Holocene divergences.

The complete Y haplotype assignment information for all sampled males is available in Supplementary Table 3 (Column Z), so we are not discarding any information from the publication. However, obtaining unbiased estimates of Y haplotype diversity (as done in Figure 3 & Extended Data Figure 6) requires applying a uniform cutoff for ISOGG resolution across the entire sample set. We justify this specific cutoff in the Methods:

L795-800: “To quantify Y haplogroup diversity per archaeological site, we considered the first three characters of the ISOGG classification for each inferred haplogroup. We used this particular cutoff for two reasons: (1) low coverage aDNA does not consistently allow assigning Y haplogroups at more refined levels due to missing data and genotyping errors caused by aDNA damage; (2) the specific 1240k SNP set enriched in these genome sequences do have little resolution for several macro-haplogroups beyond this level (e.g., I1).”

### Generational Distances

L316-317: The authors state that individuals who share >20cM in IBD share a common ancestor within the last “several” generations. In my previous review, I asked them to be more specific, but in their response they tell me they used this term to be purposefully vague. I do not think this is appropriate for a scientific journal. If they are not able to provide an estimated range of generations that might separate these individuals then they should not use “several” as a placeholder, as that term is often taken to mean ~3-7. It is possible other readers might interpret “several” in a different manner, which makes it even more problematic.

As the authors say themselves, estimating the minimum or maximum genealogical distance between individuals based on IBD is tricky, due to stochasticity in recombination and variability in background demography. Indeed, it seems to me that the average length of shared segments (or max segment length) would be more informative for estimating the maximum genealogical distance, rather than total IBD shared (which might be in quite short segments).

Please be clearer about the uncertainty here and remove the word “several”.

Following this comment, we modified the criterion for significant IBD segment sharing from “total IBD length > 20cM” to “an IBD segment of length >16cM”. This criterion does not change our underlying results (see paragraph below) but can more readily be interpreted in terms of the generations separating two individuals, which we now describe in the results and methods:

L308-311: "...we document multiple pairs of genealogical relatives separated by the Mediterranean Sea. We identify 31 pairs of individuals who share an IBD segment of length >16 cM, which indicates a shared ancestor likely within the last 20 generations (**Supplementary Table 8**; see Methods)."

L821-831: "To infer degrees of relatedness, we compared inferred IBD segments >12cM long to IBD observed in simulated genomes with various degrees of relatedness when using the same filtering as when running *ancIBD*, as described in <sup>58</sup>. We considered a pair of individuals to be significantly related if they shared an IBD segment of length >16 cM. Two individuals with a shared segment 16 cM long have a common ancestor within 20 generations with high probability. Indeed, assuming a panmictic population of diploid individuals with constant size  $N_e = 1,000$ , 95% of such IBD segments originate from a common ancestor within up to 19.3 generations ago, with a median of 8.0 generations ago, calculated as in Note S4 of <sup>29</sup>. Varying  $N_e$  only has a minor effect on these numbers, and longer IBD segments originate from even more recent ancestors."

Importantly, this modification of the criterion for IBD sharing does not change the underlying results. Out of the 34 pairs that satisfied the previous criterion, 26 also satisfy the modified one, and five pairs satisfy the new criterion and not the previous one. Notably, there are still ten pairs with significant IBD sharing from different sites, out of which six are from sites separated by the Mediterranean Sea, exactly as when using the previous criterion. We re-ordered the pairs in Supplementary Table 8 to reflect the modified criterion and modified the text in the results (L308-325) according to the slightly modified numbers.

#### General Comment

This is an important and timely paper that I believe it will attract a great deal of general interest. In my opinion, one of most interesting aspects is the heterogeneity of North African ancestry across regions, through time and within pedigrees, as well as the inter-site connectivity. The high genetic diversity of Punic populations relative to earlier (mostly European) populations is only of secondary interest, as it is simply a corollary of this North African admixture.

Given how the text has evolved throughout the review process, I wonder if the authors should update the structure of the section on "Genetic heterogeneity and interconnectedness" to better highlight the more exciting findings (heterogeneity within sites and pedigrees, IBD connectivity, similar patterns of variation in distinct regions), rather than getting bogged down in proving how diverse Punic populations were relative to a limited set of earlier groups.

We believe that the current description (after the first two rounds of revisions) provides a balanced report of the different results and insights. Much of this is thanks to the reviewer's valuable comments. In particular, we do not think the results of

elevated genetic diversity overshadow the other findings. Moreover, this result offers an important context, especially to readers less familiar with human genetic diversity patterns, since it directly compares genetic diversity in Punic sites to that seen in earlier (and later) European and Mediterranean sites. We therefore have chosen not to make further modifications on this point.

#### Referee #4 (Remarks to the Author):

I think the authors have addressed my comments and suggestions well. The modified framing makes their argument much more persuasive. They have refrained from referring to individuals or burials as “Punic” as much as possible and made the good decision to omit the discussion of genetic cosmopolitanism. They acknowledge how the term “Punic” is simultaneously difficult and useful so long as it is clear how it is being used, which the authors have shown in their revisions.

We appreciate the encouraging words about our prior responses and edits.

I encourage the authors to add references to Helene Sader's work on Levantine Phoenicia throughout the text as appropriate. The authors have yet to cite Lebanese (or Syrian) archaeologists to my knowledge (bibliographical information and explanation below, re: L418-420) and have seemingly missed a key point of connectivity in burial practices.

The concise format of Nature requires us to focus our discussion on previous studies that are most relevant to our research. In particular, the work of Lebanese and Syrian archaeologists tends to focus on the Eastern Mediterranean, while our study primarily focuses on the central and western Mediterranean. Notably, comparisons between Phoenicians in the Levant and the West are made mainly by scholars working in the West – a possible but already outdated exception concerning burials would be Gras, Rouillard and Teixido (1991). Our reference to the *Oxford Handbook of the Phoenician and Punic Mediterranean* (López-Bertran 2019) is explicitly Mediterranean-wide in scope, up to date, and easily accessible; she also cites Dixon's dissertation (p.304).

*Gras, M., P. Rouillard and J. Teixidor 1991: The Phoenicians and death, Berytus 39: 127-76.*

I have a few additional comments on specific parts of the revised document:

L82-83: I caution the authors to avoid implying that “culture” “spreads” without the very human agents at the center of their study. They might instead say: “The spread of Phoenicians and their culture from the Levant transformed the Mediterranean during the first millennium BCE.”

We appreciate this comment. However, the association between the spread of culture and the spread of people has long been questioned by archaeology – e.g., Jones 1997. Therefore, we want to avoid implying that the spread of culture is necessarily facilitated by considerable movement of people, especially since one of the main findings of our study is that the spread of Phoenician culture from the

Levant did not leave a lasting Levantine genetic fingerprint. We modified the opening sentence of our manuscript to sidestep the issue:

L82-83: "The maritime Phoenician civilization from the Levant transformed the entire Mediterranean during the first millennium BCE."

*Jones, S. 1997: The Archaeology of Ethnicity. Constructing Identities in the Past and Present. London: Routledge.*

L91-92: I'll just note that the archaeological record linking Levantine and north African/western Mediterranean sites is sometimes quite weak. The simultaneous heterogeneity and connectivity observed in Punic & Phoenician sites is part of what makes your results (L393-394 and L409-428) interesting to archaeologists. (It will of course also make them crave more data from Lebanese sites, Tyre most of all.)

On a smaller scale, East-West connections can seem limited and heterogeneous, plausibly because settlement evidence remains limited on both ends of the Mediterranean. However, we believe our statement is justified at a macro level, as evidenced by hundreds of Phoenician inscriptions throughout the North African and Western Mediterranean.

L113-129: The revision here is excellent. I only call attention again to the use of Quinn 2018 without further specification. It is acceptable to use this book, as the author herself suggested, but I think the authors of the article must include page numbers, so the reader understands what part of this long and layered work they are drawing from.

We added the page numbers 25-43 to the reference (in the reference list) to guide the interested reader towards the precise section we cite.

L367-369 and Figure 5a: I take the authors' point but still find the plaque distracting as none of the Punic material culture is given this much attention.

This point is well taken. We removed the plaque depiction from Figure 5 and moved the note about it from the figure caption to the main text (L360-363).

L413: A small but important point is that the use of "local" is vague, as it obscures the phenomenon and blurs the timing of Greek (Aegean) colonization of Sicily. I think it can be deleted. I see that "local" is used often in the article and suggest looking over each case to see if a more precise word or phrase might suit.

Following the reviewer's suggestion, we replaced "local" in several places throughout the manuscript with more precise terms:

L223: we replaced 'local populations from the central and western Mediterranean ...' with 'Bronze and Iron Age populations from the central and western Mediterranean ...'

L262: we replaced 'local Iberian ancestry' with 'Bronze Age Iberian ancestry'

L404: (previously L413): we removed 'local' from 'local people with Sicilian-Aegean ancestry'

We note that some of the ambiguity behind the term 'local' is deliberate since we typically do not wish to quantify the level of "indigenusness" of people. Cultural identity and genetic descent do not necessarily coincide, as has long been argued by archaeologists and anthropologists. In particular, in long-term colonial situations, as we are examining in this study, this distinction tends to be meaningless because how many generations should people of external-colonial descent have lived 'culturally local lives' to 'overcome' their exogenous genetic ancestry and be 'counted as' indigenous? 'Local' is a bypass to avoid identifications that we cannot justify or are meaningless. But rather than referring to this as 'vague', we would call this 'ambiguity', a term with a substantial anthropological pedigree to describe the contexts under discussion here (starting with Homi Bhabha – for archaeology, see e.g. van Dommelen 2002; Silliman 2010).

van Dommelen, P. 2002: Ambiguous matters: colonialism and local identities in Punic Sardinia, in C. Lyons and J. Papadopoulos (eds), *The Archaeology of Colonialism*. (Issues & Debates). Los Angeles: Getty Research Institute, 121-47.

Silliman, S. 2010: Indigenous traces in colonial spaces: archaeologies of ambiguity, origin, and practice, *Journal of Social Archaeology* 10.1: 28-58.

L418-420: The authors refer to a "shift in burial practices". Upon re-reading, I think they mean the shift from cremation to inhumation in the 6th c. observed in central and western Mediterranean Punic sites. This is a valid point but one that suffers from too narrow of a focus. While so far as I know, a shift from cremation to inhumation is not observed in the Aegean in the 6th c., move from cremation to inhumation is attested in Levantine Phoenicia in the late 6th c., notably in elite burials of Sidon. The apparently simultaneous change might be related or unrelated. The authors can use Helene Sader's handbook to address the Levantine phenomenon quickly:

Sader, H. S. (2019). The history and archaeology of Phoenicia. SBL Press.

The reviewer's interpretation of this term is correct. We have now adapted this phrase to be more explicit and added page numbers to make the reference more precise.

L409-411: "The shift from cremation to inhumation as the preferred burial practice in the mid to late 6th century BCE is nevertheless also a cultural transition that could reflect substantial numbers of new people integrating into these communities (López-Bertran 2019, 305–306)."

This shift is a well-known phenomenon that has long been observed in Punic settlements all over the West Mediterranean. The first explicit discussion of this phenomenon is Bartoloni 1981. The reference to Sidon is interesting but needs to be seen in a broader Levantine context, where inhumation is a funerary ritual continuously present since early prehistory, while cremation is an innovation introduced in the Iron Age (see Sader 2019, ch. 5.5). It is likely no coincidence that Carthaginian burials show a similarly mixed tradition, while cremation is much more prominent in other, usually slightly later, cemeteries in the West Mediterranean. The shift to inhumation of the mid to late 6th century thus does appear to have been a specifically regional West Mediterranean phenomenon – which does not mean that connections with prominent sites such as Sidon should be ignored.

Bartoloni, P. 1981: Contributo alla cronologia delle necropoli fenicie e puniche di Sardegna, *Rivista di Studi Fenici* 9 (Supplemento): 22-24.

Time permitting, the authors would benefit from familiarity with Helen Dixon's work, because she specializes in the topic of Phoenician burial practices of the 1st millennium BCE:

Dixon, H. M. (2013) Phoenician Mortuary Practice in the Iron Age I-III (ca. 1200-ca. 300 BCE) Levantine "Homeland". Diss. Doctor of Philosophy (Near Eastern Studies) in the University of Michigan.

<https://www.proquest.com/openview/a7f62d11f7bb37be9d3e9570a6a2234f/1?pq-origsite=gscholar&cbl=18750>

Some of us are familiar with this dissertation. Unfortunately, neither the focus of this work nor the available space allows us to engage with this interesting study.