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## Supplementary information

# A genetic history of the pre-contact Caribbean

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# Supplementary Information

## A genetic history of the pre-contact Caribbean

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## Ethics Statement

*Permissions for this study*

Permissions to carry out ancient DNA analysis of the human skeletal remains in this study were documented through authorization letters and Memorandums of Understanding (MOU) signed by a custodian who assumed responsibility for the skeletal remains collected from a specific geographic region or site. The authorization letter established permission to perform ancient DNA analysis as well as radiocarbon dating, isotopic analysis, and other bioarchaeological analyses on the ancient skeletal material and stated that 1) all skeletal material was recovered during past archaeological excavations and archived for scientific analysis; 2) the institution on behalf of which the custodian signed provided explicit permission to perform the analysis carried out in this study; and 3) the institution on behalf of which the custodian signed was supportive of publishing results of this study in a scientific journal.

- **Bahamas:** Authorization letter signed by Michael Pateman (Director, Turks & Caicos National Museum); letter of permission for the export of skeletal material from The Bahamas and for ancient DNA, isotopic, and radiocarbon dating analyses signed by Keith L. Tinker (Director, The National Museum of The Bahamas);
- **Curaçao**: Authorization letter signed by Dmitri Cloose (Director, National Archaeological Anthropological Memory Management, NAAM);
- **Dominican Republic**: Authorization letter signed by Arq. Christian Martínez Villanueva (Director General. Museo del Hombre Dominicano);
- **Venezuela**: Authorization letter signed by Prof. Carlos García Sívoli (on behalf of the Instituto de Investigaciones Bioantropológicas y Arqueológicas de la Universidad de Los Andes);
- **Haiti**: Permission for the generation of full genome data using human skeletal remains was provided by the Peabody Museum of Natural History at Yale University;
- **Puerto Rico**: Permission for the generation of full genome data using human skeletal remains was provided by the Peabody Museum of Natural History at Yale University.

*Collaboration and outreach*

In this study, we test hypotheses surrounding the geographic origins and genetic affinities of ancient peoples of the Caribbean islands using paleogenomic data as one line of evidence that is useful for studying the past. Anthropologists, archaeologists, and other scholars from each geographic region included in this study played an integral role in the interpretation of data and formulation of results and were included as co-authors. We also consulted multiple other Caribbean-based stakeholders throughout the course of the project to solicit critical feedback and local perspectives. A major goal was to ensure that local narratives were considered during the process of contextualizing these data, and thus the involvement and feedback of local scholars and stakeholders was crucial.

The inferences that we make as part of this project are intended to provide new information about the genetic ancestry of the people who lived in the Caribbean prior to the colonization by Europeans

in the late 15th century and to explore the genetic contribution from the ancient peoples of this region to the genomes of present-day Caribbean people. We note that our study of genetic ancestry of people in this region should not be conflated with individual and/or community perceptions of identity, as identity cannot be defined by genetics alone. Genetic data are one form of knowledge that contributes to understanding the past, and oral traditions and other forms of Indigenous knowledge can coexist with scientific data.

*Protection of archaeological sites*

Latitude and longitude coordinates for the archaeological sites from which the skeletal remains of individuals examined were excavated are reported in Supplementary Data 1. The Society for American Archaeology's Ethical Principle #6 (https://www.saa.org/career-practice/ethics-in-professional-archaeology) states that, "An interest in preserving and protecting in situ archaeological sites must be taken into account when publishing and distributing information about their nature and location"; thus, we have provided latitude and longitude coordinates limited to two decimal degree digits in an attempt to balance protecting archaeological site integrity against publishing the geographic data needed to test genetic correlations with distance measures and meet open science replicability standards.

## SI1 - Overview of archaeological series and culture history in the Caribbean and northern South America

### The Caribbean

Similarities and differences in material culture are arranged in classification systems to identify patterns in the archaeological record of a region. Two taxonomic systems are employed for the Caribbean islands. The first and most general is an "Age" system based on the introduction of significant changes in technology. The major weakness of the Age system is its emphasis on broad categories of technological change (i.e., flaked stone, ground stone, pottery). In this regard, Ages are not time sensitive and each spans millennia. In addition, Ages do not reflect local differences in the ways these technologies were expressed. For these reasons a second system, based on the time-space periodization of internal developments, was created to capture localized changes in style ("Series"). These systems are hierarchical in that "internal" stylistic changes are subsumed under "external" technological introductions.

The "Age" system is divided into Lithic, Archaic, Ceramic, and Historic Ages (Rouse 1992). Irving Rouse, a foundational figure in Caribbean archaeology, developed the system to recognize the most significant changes in technology observed in the region. Technological change occurs in three main ways: independent invention, diffusion of ideas, or the movement of people who carry the new technology. The latter (the arrival of new immigrants from the mainland bearing new technology) was assumed when developing this nomenclature. Working within this frame of reference, the pattern of human movement(s) into the islands was reconstructed primarily through comparisons of technologies between Caribbean archaeological sites and mainland material assemblages (Wilson 2007). In this approach, technological change is viewed as the product of external influences, and the introduction of new technologies supposedly leads to the physical and/or cultural displacement of previously established practices (Rouse 1992). In reality, even if cultural practices changed, existing technology(s) usually continued in use, albeit often in different ways (Rodríguez Ramos 2010).

We begin by providing a brief description of three distinct Ages of Caribbean occupation (we do not focus in detail on the Lithic or Historic Ages in this work as we study no individuals dating to either of these time periods). These Ages began and ended at different times on different islands; therefore, we provide the earliest date for each Age. For ease of interpretation, we discuss archaeological periods and corresponding ceramic typological styles in this section and the following section using BCE/CE dates. In the main manuscript, we discuss dates using years before present (BP, taken as AD 1950 in accordance with radiocarbon calibration convention), and we use calibrated years BP (calBP) when direct radiocarbon ($^{14}C$) dates are available. We provide details about the generation and calibration of radiocarbon dates in this paper in Supplementary Information section 3.

**Lithic Age (4000 BCE)**

The Caribbean Islands were first settled by humans around 4000 BCE, although this earliest phase of human occupation is not well studied or adequately dated. The majority of sites are in Cuba, Haiti, Dominican Republic, and Puerto Rico (Pantel 1988), with evidence of sites in the Lesser Antilles and ABC islands (Aruba, Bonaire, Curaçao) as well (Napolitano et al. 2019). The Lithic Age is characterized by flaked-stone technologies most often associated with the production of large blades. It has been argued that the first colonizers of the Caribbean came from Central America/Yucatán because Antillean Casimiroid blades (Casimiroid is named for the Casimira site, Dominican Republic) resemble blades produced in Belize at about the same time (Wilson et al. 1998). However, there is not universal agreement about this geographic localization (Callaghan 2001). Little is known about these first inhabitants of the Antilles. Most of the archaeological sites are quarries where the raw material for tool manufacture was procured. It is assumed they were Paleo-Indian hunter-gatherers who lived in small bands and lacked both cultigens and pottery (Rouse 1992). In fact, all that is known is that humans arrived in the Antilles by 4000 BCE and the only surviving element of material culture is flaked-stone tools (Keegan and Hofman 2017).

**Archaic Age (3000 BCE)**

The beginning of the Archaic Age is marked by the appearance of ground-stone tools, shell implements, and the production of freehand flakes using multidirectional flaking formats (Rouse 1992). Archaeological sites from this time are distributed from Cuba in the Greater Antilles through the Leeward Islands of the northern Lesser Antilles, although none have been identified in Jamaica (Keegan 2019). The most common interpretation of this distribution is that the spread of this technology reflects a new migration of people originating from Trinidad, and that these Ortoiroid immigrants (Ortoiroid is named for sites at Ortoire, Trinidad) encountered Casimiroid peoples in the Greater Antilles and effectively ended the Lithic Age (Rouse 1992; Boomert 2013). Interestingly, no Archaic Age artifacts have been found in the Windward Islands of the southern Lesser Antilles (Keegan and Hofman 2017) even though they are widely distributed from Panama (in Central America) to Trinidad (Rodríguez Ramos 2008). There is evidence for fires at this time in this region (Siegel et al. 2015), but no evidence that humans were responsible. Either the Windward Islands were bypassed by Ortoiroid canoes, or Archaic Age practices reached the islands by independent invention within the Caribbean and diffusion among the islands.

The absence of substantial quantities of pottery at Archaic Age sites has led to the belief that the people living during this time too were hunter-gatherers who lived in small mobile bands. A key adaptation for this group was expanding their diet to include plants processed with grinding tools and a variety of marine foods, especially mollusks (Davis 2000). A problem with the Age system, especially for the Lithic and Archaic Ages, is the tendency to assume that these societies were culturally static, and that they were all isolated groups lacking both pottery and farming. However, recent investigations demonstrate that cultigens, including maize and beans, were grown from early in the Archaic Age (Pagán Jiménez 2013; Smith et al. 2018); hutia (*Capromyidae*) may have been domesticated (Colten and Worthington 2019); pottery was made and used, albeit in small quantities

and for special purposes (Rodríguez Ramos 2008); and some of the groups developed hierarchical social formations (Rivera-Collazo 2011).

Recent research has shown that Archaic Age practices persisted much longer than initially presumed. There is evidence that the Archaic inhabitants of Hispaniola prevented the early Ceramic Age inhabitants of Puerto Rico from settling on the island in large numbers for about 1,000 years. Archaic and Ceramic Age sites overlap for some time on Puerto Rico (Rodríguez Ramos 2010; Rivera-Collazo 2011) and probably other islands, and we explore the nature of interaction between Archaic- and Ceramic-associated people in this work. Archaic Age societies in Cuba have been reported to have persisted uninterrupted at the Canímar Abajo site until as late as 950 calibrated years CE (calCE; Roksandic et al. 2015) and in the Banes Archaeological Zone of northeastern Cuba until after 900 calCE (Persons 2013), and indeed previous work (Nägele et al. 2020) has reported that unadmixed Archaic-related ancestry persisted in parts of Cuba well into the Ceramic Age (a finding that we confirm in the present study as well). Autochthonous Guanahatabey and Ciboney groups appear to have remained in the western and central parts of Cuba (respectively) for such an extended period of time that the first historical European accounts describe peoples of a different language and cultural tradition than contemporaneous ceramic-using groups (Lovén 1935; Chinique de Armas et al. 2016; Keegan and Hofman 2017).

**Ceramic Age (500-200 BCE)**

The Ceramic Age is distinguished by the introduction of pottery and agriculture almost certainly by Arawak-speaking peoples from South America. Although Archaic Age peoples planted cultigens and made pottery, their practices differed substantially from those of the Early Ceramic Age (ECA). The former is highly distinct from the latter, which is why the apparent misnomer of Ceramic Age remains in use. We use it in this study to be consistent with the literature.

In what follows, we move from the external Age system to the internal "Series" taxonomy (see Rouse 1972, 1992; Curet 2004; Bérard 2019). The finest-grained unit is the "style," which is identified as a shared set of common technical and decorative "modes" (e.g., vessel shape, paste, decoration). Styles are named for the first archaeological site at which this collection of modes were observed and are therefore not necessarily the site most representative of the style. Islands that are in close proximity to each other typically have at least one local style that shares modes with a local style on another island; these are grouped into a larger taxonomic unit called a "series," which Rouse (1992) interpreted as representing "peoples or cultures." Series also were named for the first site at which the specific collection of modes was identified, and were designated with the suffix '-oid.' For example, the Cabo Rojo site, located at Punta Ostiones, Puerto Rico, is the first at which a particular style of redware pottery was observed; thus, the local style was named "Ostiones." Other local styles with similar pottery (e.g., Santa Elena in Puerto Rico, Anadel in Dominican Republic, Little River in Jamaica) were deemed similar enough to be grouped into an Ostionoid series.

We focus primarily on the four main series that Rouse (1992) identified for the Greater Antilles: Saladoid, Ostionoid, Meillacoid, and Chicoid, ordered sequentially. We also provide a brief discussion

of two cultural traditions that are contemporaneous with Saladoid (Huecoid and Saladoid/Barrancoid), as well as Lucayan Palmetto Ware (unique to the Bahama archipelago) and the Troumassoid series which is specific to the Lesser Antilles (no individuals from this part of the Caribbean are newly-reported in this study). As with changes in technology in the Age system, the development of every new series completely replaced the previous series, such that the preceding series only survived on the margins. When other archaeologists suggested that each of the series represented separate migrations from the mainland into the islands (see below), Rouse (1992) introduced the concept of subseries, denoted by the suffix "-an," to emphasize his belief that all of the ceramic series evolved from a single Ostionoid tradition. His original series were subsequently converted to Ostionoan Ostionoid, Meillacan Ostionoid, and Chican Ostionoid.

Rouse (1992) envisioned the Ceramic Age as the product of a single population expansion (migration) that began on the banks of the Orinoco River in eastern Venezuela and proceeded "island by island" through the Lesser Antilles and on to the Greater Antilles (Willey 1971:364-365). His singular tradition taxonomy is a theory that we test using the genetic data in this work. Detailed discussions of the competing models that have been proposed to explain the relationships and transitions among the Ceramic Age cultures are presented in *Caribbean before Columbus* (Keegan and Hofman 2017) and *Handbook of Caribbean Archaeology* (Keegan et al. 2013). A brief introduction to these models with specific attention to human mobility is discussed below.

*Early Ceramic Age (500-200 BCE to 900 CE): Saladoid, Huecoid, and Saladoid/Barrancoid*

The migrants of the ECA originated in lowland South America and reached the Antilles sometime between 500-200 BCE (Lathrap 1970; Rouse 1986; Keegan and Hofman 2017). The ECA is often described solely in terms of Saladoid series pottery (Saladoid is named for the Saladero site on the lower Orinoco River in eastern Venezuela) and the cultural practices inferred from Saladoid sites. The pottery exhibits distinctive complex vessel forms, white-on-red painting (W-O-R), incised designs, and zoomorphic adornos (animal-shaped appendages) that link it to the Orinoco basin in present-day Venezuela and the Guianas (Rouse 1992; Boomert 2013), a region which today is home to some Indigenous Arawak-speaking groups. The Saladoid tradition has been identified throughout the Lesser Antilles, Puerto Rico, and possibly eastern Dominican Republic, but it does not occur in Haiti, Jamaica, Cuba, the Bahama archipelago, or the ABC Islands of Aruba, Bonaire, and Curaçao. Peoples carrying this tradition may have absorbed or mixed with autochthonous Archaic populations of the islands (Wilson 2007), though previously established communities remained as independent entities in some areas (Keegan and Hofman 2017; the results in the present study also provide support for the existence of Archaic-associated groups well into the Ceramic Age).

Although Saladoid was the first ECA tradition identified, two additional and contemporaneous cultural traditions have since received widespread acceptance: 1) the Huecoid pottery series (Chanlatte-Baik 2013), which has distinctive vessel shapes but lacks painted motifs, is centered on eastern Puerto Rico and nearby Vieques Island (Huecoid is named for the La Hueca site, Vieques Island, located off of eastern Puerto Rico) and occurs at archaeological sites throughout the Lesser Antilles; and 2) the

Saladoid/Barrancoid tradition, which is found is in the Windward Islands (Rouse 1992; Keegan and Hofman 2017). It is important to understand that these different traditions are identified primarily by differences in pottery styles. Small differences may also reflect local group identities subsumed by broader similarities that serve to unify widely dispersed autonomous communities. The manner in which ECA peoples came to settle the Lesser Antilles and Puerto Rico has been the subject of intense debate.

The first model was proposed prior to the general availability of radiocarbon dates and at a time when Saladoid was the only cultural tradition. The movement of Saladoid people at the onset of the ECA was proposed as a general 'stepping-stone' movement ("island by island") from South America to Trinidad, through the Lesser Antilles, and eventually into Puerto Rico (Willey 1971:364-365; Rouse 1986; Boomert 2013). There are two major problems with this model. First, humans cannot reproduce fast enough to settle every island in turn and still reach Puerto Rico at an early date (Keegan 1995). Thus, either some islands were bypassed or population expansion occurred in waves. Expansion in waves fits the proposed separate arrival of Huecoid as possibly occurring earlier than the arrival of Saladoid (Chanlatte-Baik 2013).

The second problem is that the earliest radiocarbon dates for Saladoid and Huecoid (circa 500-200 BCE) all cluster in the northern Lesser Antilles and Puerto Rico (Fitzpatrick et al. 2010; Napolitano et al. 2019). The evidence suggests that the northern Lesser Antilles and Puerto Rico were settled in a direct jump across the Caribbean Sea. Pottery styles similar to the Saladoid series are distributed along coastal South America (e.g., Huecoid exhibits similarities with the Río Guapo style), while various shell and exotic stone ornaments are similar to objects from the Isthmo-Colombian area (Rodríguez Ramos 2013). Based on material similarities, it is not clear precisely where in South America the ECA colonists originated; however, linguistic studies suggest they spoke an Arawak language, consistent with pottery styles connected to eastern Venezuela.

The first evidence for people in the Windward Islands is dated to the first centuries CE (Napolitano et al. 2019). Saladoid sites in the southern Lesser Antilles exhibit stronger affiliation with Barrancoid series pottery, which is characterized by an abundance of finely made zoomorphic adornos (Keegan and Hofman 2017). It is possible that this reflects yet another wave of colonists from South America, this time from the Orinoco delta region. Alternatively, because these later sites share W-O-R painted pottery with earlier Saladoid sites to the north, other archaeologists have argued that these cultures do not necessarily reflect a separate migration.

Saladoid pottery has been described as a "veneer," meaning that widely shared modes highlighted regional integration and masked local variability (Keegan 2004). These modes begin to disappear around 500-600 CE, especially in Puerto Rico, although they continue in use until around 900 CE on some islands (Versteeg and Schinkel 1992). In the Greater Antilles these new styles are classified as part of the Ostionoid series, while emerging styles in the Lesser Antilles are classified as Troumassoid (Ostionoid is named for sites near Punta Ostiones, western Puerto Rico, and Troumassoid is named for Troumassee site, St. Lucia). Troumassoid is considered an *in situ* regional development that did not involve the arrival of peoples from the mainland.

*Ostionoid (600-900 CE)*

Ostionoid series pottery is characterized by distinctive red surface treatments, simple lugs and rim projections, incised designs (with regional variation), and the disappearance of W-O-R painting. Ostionoid and Saladoid are conventionally interpreted as demographically continuous, in the sense that people descended from the same demographic movement (likely from the South American continent) practiced both. However, it remains difficult to differentiate between the movement of people and cultural diffusion using archaeological data alone (Keegan and Hofman 2017). The most common explanation is that Ostionoid developed from Saladoid within the islands. It is associated with a resumption of population expansion to the west following a 1,000-year period of adaptation to island conditions (Rouse 1992). In this regard it is viewed as a new wave of inter-island mobility that arrived first in Hispaniola (present-day Haiti and the Dominican Republic), then in Jamaica (with the first permanent human inhabitants of that island), and finally in Cuba (although Cuban archaeologists do not recognize Ostionoid pottery). It then moved north ~700 CE to Grand Turk in the Turks & Caicos Islands via seasonal visitors from Hispaniola (Carlson 1999).

It is noteworthy that Ostionoid pottery appears simultaneously in Puerto Rico, Hispaniola, Jamaica, and the southern Bahamas. This may reflect the rapid pace at which the culture expanded, or the limits of dating methods to confirm directionality. An alternative perspective, based on the abundance of crab claws in Saladoid sites and of mollusk shells in Ostionoid sites (or their respective absence), is that these series represent separate migrations, although no homeland was proposed for the Ostionoid (Rainey 1940). Rather than resulting from a separate migration, Keegan (2006) and Rodríguez Ramos (2005) have suggested that Ostionoid pottery is a variety of pottery types that originated during the Archaic Age, belonging to the Pre-Arawak Pottery Horizon (Rodríguez Ramos 2008). This would account for its sudden appearance, wide distribution, and rapid disappearance. In Jamaica, for example, it is thought that new immigrants bearing Meillacoid series pottery quickly replaced Ostionoid, and there is no evidence that the two interacted (Keegan 2019). In sum, the humans associated with Ostionoid pottery would have a Saladoid ancestry (Rouse 1992), an Archaic ancestry (Keegan 2006, Rodríguez Ramos 2008), or a different but unidentified ancestry (Rainey 1940). Ancient DNA data may provide the opportunity to distinguish between these hypotheses.

*Meillacoid (800-1500 CE)*

The next major material culture change during the Ceramic Age commences around 800 CE. The earliest evidence of this tradition is in central Hispaniola near the border between Haiti and the Dominican Republic. Meillacoid series pottery is characterized by narrow V-shaped incisions (often unsmoothed) combined in oblique-parallel-line motifs, crosshatching, punctations, appliqué, and constructed adornos. Meillacoid represented the third wave of inter-island cultural expansion, which Rouse (1986) ultimately traced to the original Saladoid settlement of the islands. Using subseries terminology, Meillacan belonged to the Ostionoid, and reflected the incorporation of design motifs copied from Archaic stone bowls. Meillacoid pottery spread across Hispaniola, and then diffused to Jamaica and Cuba sometime after 900-1000 CE (Rouse 1992). It is supposed to have disappeared in

Hispaniola around 1200 CE following the spread of Chicoid pottery, although it continued in Jamaica and Cuba until European colonization.

Marcio Veloz Maggiolo (1972, 1993) and Alberta Zucchi (1985) have suggested a different history of the Meillacoid tradition. According to their reports, there were other pottery-making traditions in Hispaniola that preceded Meillacoid (and Ostionoid) pottery styles by perhaps 1,000 years (Veloz Maggiolo and Ortega 1996) and are contemporaneous with Saladoid in Puerto Rico. These styles, culminating in Meillacoid, reflect a direct connection to the South American mainland. A study of pre-Columbian facial morphology suggested that people living in Hispaniola, Jamaica, and The Bahamas formed a single cluster (Ross et al. 2020). Meillacoid pottery is the one element of material culture shared across these islands, and the V-shaped incisions and appliqué in the design of the pottery are also associated with "Carib" pottery in coastal South America (Meggers et al. 1965; Lathrap 1970). It is possible that even if it does represent a movement of people, the Carib expansion could have involved the infiltration of Indigenous, Arawak-speaking communities, by small numbers of people, thus explaining the persistence of Arawak languages throughout these regions.

*Lucayan Palmetto Ware (800-1550 CE)*

Locally produced pottery in the Bahama archipelago (today comprising the Commonwealth of The Bahamas and the Turks & Caicos Islands, a British Overseas Territory) is called Palmetto Ware, which is named for the Palmetto Grove site on the island of San Salvador in The Bahamas. It is a "ware," and not a series, because it is represented by only one type. There are no additional Palmetto styles. Palmetto Ware is a redware made using local clay and calcified shells from the conch (*Lobatus gigas*). It is so different from Ostionoid and Meillacoid pottery that Rouse was unwilling to attribute it to either tradition. Most Palmetto Ware is undecorated, but when it is, it exhibits Meillacoid motifs. The recent accelerator mass spectrometry (AMS) dating of 60 Lucayan skeletons from across The Bahamas returned dates in the 900-1550 CE range (Schulting et al., *in preparation*), although it is impossible to be certain that the earliest archaeological site in a region has been identified and dated.

The earliest well-dated archaeological site in the Bahamian archipelago is the Coralie site on Grand Turk. At this site, short-term occupations spanning 700-1100 CE are associated with seasonal procurement of tortoises (now extinct), iguanas, fish, birds, and sea turtles (Carlson 1999). All of the pottery is Ostionoid-based, indicating that it was brought to this location from Hispaniola. As such, it does not necessarily reflect the human colonization of these islands. Nevertheless, proximity to Hispaniola (circa 150 kilometers), the Ostionoid and Meillacoid frontier model (Rouse 1986), settlement patterns, and the civic-ceremonial site on Middle Caicos (MC-6) have been interpreted as supporting evidence for the conclusion that the earliest inhabitants of the archipelago arrived from Hispaniola (Sears and Sullivan 1978; Keegan 1992). The recent study of facial morphology provides additional support for this conclusion (Ross et al. 2020).

Historic toponyms (Granberry 1991), a few early radiocarbon dates in the 700-900 CE range from the central Bahamas (i.e., San Salvador, New Providence, and Eleuthera), and certain pottery

characteristics were used to propose that Cuba was the original source of migrants to The Bahamas (Sears and Sullivan 1978; Berman and Gnivecki 1995). This Cuban connection was based on the assumption that the Ceramic Age reached Cuba by 700 CE. Local, "pre-Arawak" pottery was made in Cuba for at least 2000 years (Ulloa Hung and Valcárcel Rojas 2002), but Ceramic Age pottery did not arrive until after 900 CE (Persons 2013; Valcárcel Rojas 2002). Thus, if people from Cuba colonized The Bahamas, the colonists were likely of Archaic ancestry. The data presented in this paper do not support a biological connection between Ceramic Age peoples from The Bahamas and Archaic inhabitants of Cuba, but suggest shared affinity between ceramic users from Cuba and The Bahamas (Supplementary Information section 8).

*Chicoid (1200-1450 CE)*

Around 1200 CE, a new pottery series emerged in eastern Dominican Republic. Chicoid (Chicoid is named for the Boca Chica site in the southeastern Dominican Republic) series pottery has complicated vessel shapes with fine, hard, and smoothed surfaces. Elaborate motifs including continuous scrolls, lines ending in dots, flat and prismatic lugs, and modelled anthropomorphic ("bat face") lugs facing each other above the rim. The pottery is completely different from Meillacoid and Ostionoid and appears to be a local development. Chicoid pottery is associated with the contact-era "Classic Taínos" (Rouse 1992; Keegan et al. 2013). It has been found at archaeological sites across the entire island of Hispaniola. In Rouse's (1992) classification it completely replaced Meillacoid pottery on the island, but new evidence suggests that both were in use until European contact (Keegan and Hofman 2017). To the east, Chicoid pottery occurs at outposts in Puerto Rico and in the Lesser Antilles (Rouse 1992; Hoogland and Hofman 1999). To date, it has not been identified in Jamaica, its motifs were copied by local artists in Cuba, and tradewares of this style are found on some islands, including The Bahamas, although the nature of this contact is poorly understood.

*Lesser Antilles*

*Troumassoid (900-1550 CE)*

Troumassoid series pottery developed out of Saladoid traditions beginning around 900 CE. In the northern Lesser Antilles (Leeward Islands) it exhibits, over time, stronger affiliations with Puerto Rican Ostionoid and is classified as Mamoran Troumassoid (named for the Mamora Bay site on Antigua). The southern Lesser Antilles (Windward Islands) in contrast show stronger connections to mainland South America. The pottery in these islands is classified as Suazan Troumassoid (named for the Savanne Suazey site on Grenada). There is debate concerning whether or not Suazan (formerly Suazoid) pottery was manufactured by the "Island Caribs" (Allaire 1996; Bullen 1964; Rouse 1992). Current thinking attributes to them only Cayo style pottery, which first appeared in the islands around 1250 CE (Boomert 1986). Nevertheless, a possible relationship with Suazan pottery is not well defined. The Kalinago (formerly known as Island Caribs) spoke a predominantly Arawak language, and, as noted, shared numerous cultural characteristics with the modern Arawak-speaking Palikur of the Guiana coast.

The 16 individuals that comprise our *LesserAntilles_Ceramic subclade includes 4 individuals from the Anse à la Gourde site (Guadeloupe) and 12 individuals from the Lavoutte site (Saint Lucia) first reported in Nägele et al. 2020 and co-analyzed in this study. The cultural materials at Anse à la Gourde represent long-term, *in situ*, post-Saladoid cultural development. The dividing line between the Leeward and Windward Islands occurs at Guadeloupe, and the pottery from the site has affiliations to both Mamoran and Suazan subseries (Keegan and Hofman 2017: 216-221). The site predates any evidence for Carib intrusion from South America, although Kalinago reportedly inhabited Guadeloupe when Columbus arrived at the island in 1493. The Lavoutte site has primarily Suazan pottery (originally classified as Micoid, and named for a site in the town of Micoud, Saint Lucia). The 52 burials from this site exhibit complex and varied mortuary practices dating to between the 12$^{th}$ and 15$^{th}$ centuries (Hofman et al. 2012). Following Bullen and Bullen (1970), Nägele et al. suggest that the site was a Carib ceremonial center.

**Historic Age (1492 CE)**

It has sometimes been assumed that European colonization led to the rapid and total disappearance of Indigenous Caribbean peoples. However, the survival and continuing legacy of this group is widely recognized today, in part through the study of genetic ancestry (Martínez-Cruzado 2010, 2013, Schroeder et al. 2018, Nieves-Colón et al. 2020). Newly generated data in this study confirm the persistence of pre-contact Indigenous ancestry in present-day admixed people of the Caribbean (Supplementary Information section 15).

**Northern South America and the Arawak Diaspora**

The archaeological record of South America was originally described in much the same way as that described for the Caribbean Islands. An initial "Paleo-Indian Period" (Lithic Age) was followed by a "Meso-Indian Period" (Archaic Age), then a "Neo-Indian Period" (Ceramic Age), and finally the "Historic Period" (Willey 1971). The coastal zone, stretching from western Venezuela to Brazil, was initially occupied by autonomous communities whose economy was based on gathering plants, fishing, and collecting marine mollusks for thousands of years. Over time, some adopted the use of pottery to a limited degree, and some may have practiced small-scale incipient farming. Cultural practices reflected local conditions, and major cultural changes were not apparent until "Tropical Forest" communities began to expand along interior river valleys and out onto the coast. The degree to which these Indigenous communities were displaced or assimilated is a question of local history that remains unclear.

Tropical Forest societies began to develop sometime before 2000 BCE near the confluence of the Amazon, Madeira, and Negro Rivers (Lathrap 1970). Their economy was based on the cultivation of bitter manioc, which fueled a rapid increase in population that led to expansion along the major rivers' drainages and then out to the coast. An abundance of ceramics is associated with the development of these societies. Lathrap (1970) proposed a single ceramic homeland, which affiliates all of the subsequent changes in ceramic styles to common ancestry (Willey 1971). A significant boundary in the ceramic series of the Northwest quadrant of Amazonia occurs at the middle Orinoco

(Rouse and Cruxent 1963). To the west, Tocuyanoid is the earliest series, and forms the foundation on which later ceramic series developed.

The Santa Ana style identified at the Las Locas site (for which ancient DNA data are reported here) is an early expression of expansion into the Quíbor Valley (around 500 BCE), which appears to be related to the Dabajuroid series expansion that spread north to the central coast and then west to Lake Maracaibo and out to the islands of Aruba, Bonaire, Curaçao and the Las Aves Archipelagos (Haviser 1987; Oliver 1997; Antczak and Antczak 2015; Antczak et al. 2017). The Dabajuroid expansion is juxtaposed to the Barrancoid expansion to the east, although whether or not they share genetic ancestry has not been addressed previously. The Dabajuroid series and the Tierroid series (Lara) are associated with the historical Caquetio, a people large in number and widespread as far south as the Barinas plains and Rio Cojedes (Arcaya 1916; Oliver 1989). The Caquetio lived along rivers and streams and on the plains where suitable agricultural soils were available. They had a versatile diet, including agricultural production, hunting of game, and exploiting marine resources such as reef fish and shellfish. When living in locations without access to maritime food sources, they would trade crops and game for fish with other peoples (see Federmann 1557). Caquetio settlements on Curaçao are located on low hills at about 500 meters distance from a bay with sea access, close to soils suitable for agriculture and gullies where rainwater comes together after heavy rainfall (Haviser 1987). The Caquetio were polygamous, or at least their caciques were (Federmann 1557; Oliver 1989: 280). Federmann (1557) describes a Caquetio household as consisting of a man with his wife and children. A daughter of cacique Manaure went to live with her cacique husband after marriage, suggesting patrilocality (Oliver 1989: 281).

To the east, linguistic evidence suggests that around 1000 BCE, Arawak communities began to move rapidly through the Negro and Orinoco floodplains and onto the Caribbean and Guiana coasts (Heckenberger 2002; Zucchi 2002). The center of dispersion is not certain, but appears to be the "ceramic homeland" in the Northwest Amazon (Lathrap 1970; Rouse 1992). The first wave of this expansion is characterized by the painted motifs of the Saladoid series, which spread downriver from the middle Orinoco to the eastern Caribbean coast of Venezuela, the Guianas, and then into the Antilles. It was soon followed by a Barrancoid expansion into the same areas, but which differs from Saladoid in its elaborate incised motifs and relative absence of painting (Lathrap 1970).

The early Arawak expansion, including the colonists who migrated into the Antilles, shared a system of meaning and continuity in the broader cultural pattern. Summarizing Heckenberger (2002), Arawak speakers reproduce a *habitus* predisposed to perpetuate an ethos of settled village life, commonly coupled with large, fixed populations, fairly intensive subsistence economies, and landscape alteration (rather than mobility and low impact); institutional social ranking based on bloodline and birth order; and regional integration (particularly coupled with a social preoccupation with exchange and a cultural aesthetic that places great symbolic value upon foreign things) and a foreign policy commonly characterized by accommodation and acculturation of outsiders. This description is best suited to the Piapoco and their Arawak-speaking neighbors who share many additional cultural similarities with Indigenous Caribbean communities (we use the population names most commonly

found in scientific literature for discussions of modern Indigenous populations in this work but see Supplementary Data 5 for population self-denominations).

**Modern Arawak Language and Culture**

Anthropologists have long recognized that there is no necessary correlation between ancestry and culture (Boas 1940). An excellent example is a study of material culture along 700 kilometers of coastal New Guinea encompassing 55 languages and belonging to at least eight major language families. This study revealed that similarities and differences in material culture were better predicted by geographical than by linguistic affiliation (Welsch et al. 1992). Indigenous societies in South America exhibit similar linguistic diversity, with at least three major language families (i.e., Arawakan, Cariban, and Tupian) and hundreds of individual languages. The diversity of languages many have developed among autonomous lineage-based villages when population densities were low (Steward and Faron 1959). As groups associated with the different language families expanded, it has been hypothesized that they produced "multiethnic and multilingual regional sociopolitical systems" (Heckenberger and Neves 2009). A similar process could explain why in Papua New Guinea groups more closely resembled their neighbors who spoke different languages than they resemble more remote speakers of the same language. Moreover, the high degree of mobility and exogamous marriage contributed to widely shared cultural practices (Lowie 1948a). The practice of lineage and clan exogamy contributed to biological connectivity between communities, which suggests that neighbors should exhibit a closer genetic relationship irrespective of the language that they speak.

Language families continue to be used as an organizing principle for the recognition of ethnic communities, including in the Caribbean (e.g., "Arawak speakers"), and shared languages do establish cultural linkages between groups of people and raise the possibility of substantial amounts of shared ancestry as language shifts in pre-state societies are usually propelled by movements of people (Bellwood 2001). In light of examples like the study from New Guinea, however, it is incumbent on investigators to demonstrate empirically the degree to which genetic, linguistic, and material culture classifications are correlated. Confusion can also arise from the use of similar names in different taxonomies and from changes or differences in interpretation. For example, it was initially assumed that the "Caribs" or "Island Caribs" of the Lesser Antilles (today called 'Kalinago') spoke a Cariban language (reviewed in Rouse 1992: 21-22), but detailed linguistic analysis has demonstrated that they actually spoke an Arawak-based language (Granberry and Vescelius 2004). As Salzano and colleagues caution (2005: S126): "Analogies between linguistic and genetic variability should be performed with caution."

In light of the fact that it cannot be assumed that shared language necessarily expresses a close genetic relationship, we considered the results of the present study which show that by ADMIXTURE, *TreeMix* and *qpAdm* analyses (albeit not replicated with $f_4$-statistics), the main group of individuals from the Ceramic Age in the ancient Caribbean showed greatest affinity with present-day Indigenous South Americans who speak Arawak languages (see Extended Data Fig. 3 in the main manuscript; we note that Ceramic-associated Caribbean individuals studied in this work also how some affinity to

Cariban and Tupian speakers, see Fig. 2b in main manuscript). This finding suggests that the spread of ceramic-related ancestry could have been plausibly carried out at least in part by Arawak speakers, as Arawak languages were widely spoken in the pre-contact Caribbean. Arawak languages are known to be concentrated in populations to the north of the Amazon River, while Tupi speakers are concentrated in the south, and Carib-speaking groups cluster along the coast (Salzano et al. 2005; Walker et al. 2012).

In northern South America, Arawak-speaking Indigenous groups tend to focus on controlling riverine habitats distinguishing them from their interfluvial upland neighbors who spoke Cariban and other languages (Steward and Faron 1959). The largest number of Arawak languages are recorded in the Northwest Amazon, which led to the conclusion that this was likely the Arawak homeland, and would be consistent with the interpretation that the Arawak-speakers expanded down the Amazon and Orinoco Rivers (Heckenberger 2002). However, it also is possible that the demographic catastrophe that resulted from European conquests led to migrations upriver to the point where further westward progress was impeded by the Andean foothills and the absence of navigable rivers, and thus the resulting distribution of Indigenous languages today might be qualitatively different from the pre-contact one (Lathrap 1970).

Today, some Arawak-speakers live in close geographical proximity to areas from which the initial Ceramic Age colonists of the Caribbean Islands likely originated more than two millennia ago. Some also live in western Venezuela, which is significant because it is where V-shaped incised and appliqué pottery motifs from the west (Araquinoid and Dabajuroid series) abut U-shaped incised and painted motifs (Barrancoid and Saladoid series, respectively) from the east (Zucchi 2002). Dabajuroid series pottery is relevant to this study as it is the only ceramic tradition identified for Curaçao, where we infer about two-thirds ancestry from the main Caribbean Ceramic genetic cluster, and Meillacoid pottery from the western Greater Antilles and The Bahamas has been affiliated to V-shaped incised and appliqué pottery traditions (Zucchi 1985; Ross et al. 2020). In what follows, we specifically discuss similarities between material culture of Caribbean islanders as inferred from archaeological evidence, and ethnographic information for the two genotyped Arawak-speaking groups to which they show strongest affinity according to *TreeMix* and *qpAdm* models (Supplementary Information sections 8 and 9).

*Piapoco*

Ethnographic research indicates that the Piapoco (Tsase) occupied the middle course of the Guaviare River, María River, and Cuinada River in the vicinity of Lake Maracaibo in western Venezuela and eastern Colombia. Gregorio Hernandez de Alba (1948) describes cultural practices in this region with a particular focus on the Achagua and includes the Piapoco in this description. Most of these features also are found in the Caribbean Islands (Rouse 1992; Keegan and Hofman 2017). The communities in this region were palisaded villages built to protect the inhabitants from raids by Carib communities of the interior uplands. The palisade contained large communal dwellings (up to 500 people) as well as a special men's house. Dugout canoes were an important mode of transportation. Gardens were

prepared by men and cultivated by women. Maize (*Zea mays*) along with sweet and bitter manioc (*Manihot esculenta*) were the most important cultigens. Pottery was used for food preparation and storage, and calabashes for water. They produced baskets and woven matts, made cotton fishing nets and hammocks, and short women's skirts were the only article of clothing. Objects of wood included stools and large hollow-log drums. The most important ornaments were shell beads, which on occasion were sacrificed to mark successful fishing (see Carlson 1993 for a similar practice at GT-2 on Grand Turk, Turks & Caicos Islands). They painted their faces for luck in hunting.

Social organization is characterized as patrilineal sibs (i.e., clans), and individuals "had to go to distant villages to marry" (Hernandez de Alba 1948: 404). Polygyny was practiced, and every village had a chief. They fought with neighboring Caribs and the bow and arrow and a war club (*macana*) were the main weapons. Shaman were healers who extracted an object from the patient and were intermediaries with the spirit world. They inhaled a powdered drug, using crossed bird bones, to facilitate divination. The narcotic is identified as *Piptadenia* sp., which was called *cohoba* in the Greater Antilles. Of note is that the use of *Piptadenia* may have been restricted to Western and Northwest Amazonia (Cooper 1948: 537). Frogs symbolized the "lords of the water" (Hernandez de Alba 1948: 410). They did not produce idols, but instead represented the spirit world with masks.

*Palikur*

The Arawak-speaking Palikur, or Pa'ikwené, inhabit the northeast coast along the border between Brazilian and French Guiana, along the Oyapack and Vaçá River drainage. They express a hereditary enmity with the neighboring Carib-speaking Galibi, although the Carib and Arawak tribes of the Guianas share many features (Gillin 1948). Their historic homeland of Amapá, between the Amazon and Oyapock Rivers, was in the past characterized by "a profusion of diverse of ethnic groups, clans, and languages (Arawak, Carib, Tupi) out of which there seems to have developed a unified (though not homogeneous) culture, entailing peaceful and interdependent relations and interethnic trade, festivities, and marriage (Passes 2002:178). Their territory is swampland with small clusters of beehive huts (with walls and roof merged) constructed on forested islands. Movement between these clusters is hampered by swampy conditions, especially during the midsummer rainy season, so log causeways are built across the muddy terrain (Lowie 1948a). They make a crude pottery that is less refined than the urns they once made. These urns were used for burials, and each moiety had its own cemetery. They are known for the realistic representation of turtles on clay vessels. Their material culture is not distinctive, but they share the use of wooden shields and fall traps for capturing game with the Arawak-speaking Island Caribs (Cooper 1948; Métraux 1948a). They also used the bow and arrow. Their sides of their canoes may have been built up with planks. They were propelled with poles or paddled with "crutch handle" elongated leaf-shaped blade paddles (Gillin 1948: 837-838).

Each of the original 18 clans had their own territory and spoke their own language. The remaining seven or eight Pa'ikwené clans are the product of accretion and consolidation. A process that began in the 17$^{th}$ century with elements of depleted groups progressively absorbed into Pa'ikwené clans, including the Carib-speaking Paragoto (Passes 2002). Every village had a headman or "chief" whose

authority was based on consensus. His main task was greeting visitors and representing the community. Social organization is characterized as moieties divided into seven patrilineal clans. However, it is possible that they originally were matrilineal and that current practices developed in relation to neighboring groups. Marriage distances are short, women exert considerable authority (even with regard to a husband's personal possessions; Lowie 1948c: 356), and residence is described as both uxorilocal (residing with the wife's family) and ambilocal (residing with the parents who offer the best accommodation). Their moieties are agamic, meaning there are no restrictions on inter-clan marriage. Like the Carib, premarital license is accepted, although the Palikur are more strictly monogamous than neighboring tribes. Boys experienced scarification and flagellation during puberty rites, after which they wore cotton bands on the arms and legs (Metraux 1948b: 377). The later practice is described for the Island Carib. Shamans were responsible for healing the sick and could serve as "master of ceremonies" during feasts (Lowie 1948a). Like the Island Carib they celebrated the recovery of the sick person with a feast; and like the Taíno, made food offerings to the spirits (Métraux 1948c:578). There is no mention of drug use other than tobacco.

While the convergence of genetics, linguistics, archaeology and ethnography connecting Arawak-speakers of the Caribbean islands to Arawak-speakers in northern South America is striking, our $f_4$-statistic analysis failed to replicate a specific association to Arawak-speakers. However, support for that inference comes from ADMIXTURE, *TreeMix*, and $f_4$-statistics-based *qpAdm* analyses. It is possible that the inconsistent genetic signal reflects admixture and complexity in the history both of present-day Arawak speakers on the South American mainland, and in the people who settled the Caribbean during the Ceramic Age. Thus, there is also some evidence in our data for there not being a one-to-one relationship between genetics and archaeological style.

## SI2- Archaeological site information for 174 newly-reported individuals

**THE BAHAMAS:**

The Bahama archipelago (today comprising the Commonwealth of The Bahamas and the Turks & Caicos Islands, a British Overseas Territory) is located in the Atlantic Ocean extending south from Florida to Hispaniola (over 1,000 kilometers). It consists of over 700 islands and cays. The archipelago comprises a land area of approximately 14,000 kilometers$^2$ laid out in a northwest to southeast direction. The elevation throughout the archipelago is less than 60 meters above sea level and, in most places, no more than 20 meters above sea levels. The islands are the result of coral reefs, which became dry land when the sea level dropped hundreds of centuries ago. The islands are mostly flat with miles of white and pink sandy beaches. Solution features in the limestone have created a karstic landscape with sharp pinnacles, crevices, caves, and sinkholes. Additionally, there are no freshwater rivers located in any of the islands of the archipelago (Sealey 1995).

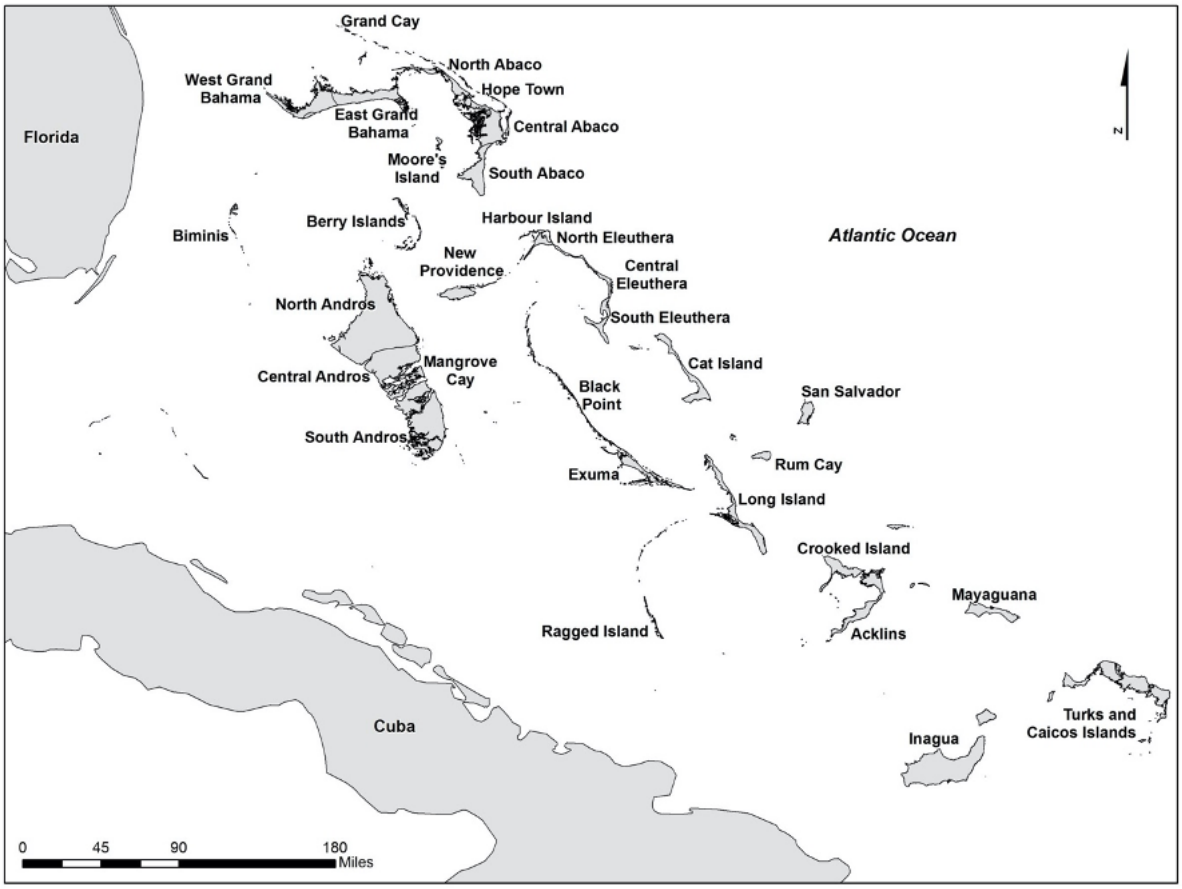



**Figure S1. Map of the Bahama archipelago.** Map by William F. Keegan, created using Adobe Illustrator.

The first skeletal research in The Bahamas was conducted by Brooks (1888), describing three crania found in caves throughout the islands. Caves played a vital role in Taíno lifestyle and spiritual beliefs,

and as such, they also played an essential role in the lives of Lucayans. Therefore, it should be no surprise that caves represent a significant aspect of the archaeological record. These caves exist in two forms, wet (including blue holes and caves with a direct connection to the water table) and dry; they contain a variety of artifacts which have not been preserved at open sites such as human burials, petroglyphs and pictographs, faunal and botanical remains, and a variety of wooden artifacts (examples in De Booy 1913; Rainey 1934; Hoffman Jr. 1973; Granberry 1978; Keegan 1982; Pateman 2007; Pateman and Keegan 2019). The majority of Lucayan burials are known from caves throughout the archipelago.

The human remains from The Bahamas included in this study were curated at either the Yale Peabody Museum of Natural History (Peabody Museum) or at the National Museum of The Bahamas. All of the human remains in the Peabody Museum from The Bahamas used in this study were collected in 1934 by Froelich G. Rainey. In January 1934, Rainey visited The Bahamas to locate and excavate archaeological sites and examine cultural materials found by locals on many islands (Rainey 1934). Rainey recovered skeletons from thirteen dry cave sites from the Abaco Islands, Eleuthera, San Salvador, Rum Cay, Long Island, and Crooked Island (Rainey 1934). Although Rainey referred to the survey and excavations in The Bahamas in his field diary, he did not write a report about the excavations or provide detailed field notes of the recoveries (Granberry 1978) and therefore the context and antiquity of the remains are not well documented. Some of the locations where human remains were recovered are now lost. The remains of some individuals were found by cave earth diggers and thrown on the outside of the caves. Similarly, numerous human skeletal remains have been collected from wet caves by cave divers and explorers who had little regard for proper archaeological methods and protocols (Pateman and Keegan 2019). Previous skeletal studies have shown a population high in dental disease as well as multiple traumas, either the result of accidents or violence (Pateman 2007; Schaffer 2015) and a relatively young population at death (Pateman and Keegan 2019). The Bahamian human remains from the Peabody Museum have been the subject of several publications and are most systematically described by Schaffer (2015).

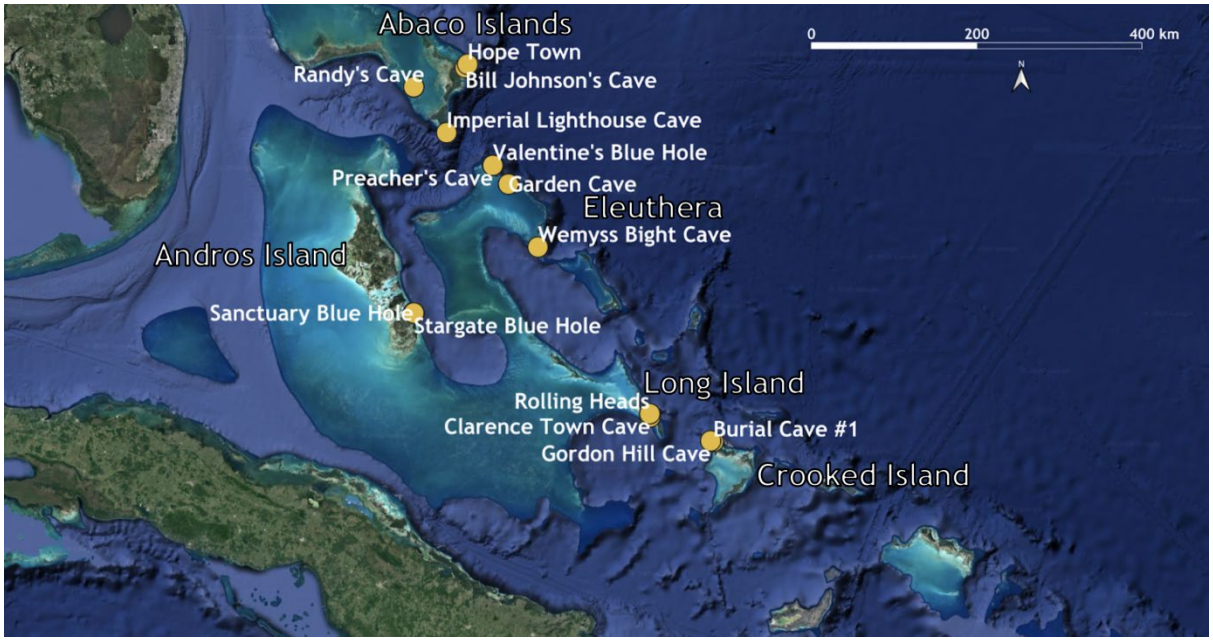



**Figure S2: Location of 14 archaeological sites in The Bahamas included in this study.** Map created using QGIS Geographic Information System v3.6 (http://qgis.org); basemaps from Google Earth.

**The Abaco Islands**

The Abaco Islands lie in the northern Bahamas and include the main islands of Great Abaco and Little Abaco along with numerous cays. The climate of these islands consists mainly of tropical marine wet and dry climatic zones (Sealey 1995). Walker's Cay of the Abacos is the northernmost point of the Bahama archipelago. The combined landmass of the Abacos is approximately 2,009 kilometers$^2$. Caribbean pine (*Pinus caribaea*) dominates the modern vegetation. Research into the paleo-environment showed that the landscape consisted of lush tropical forests (Steadman et al. 2007). Hurricane Dorian (2019) razed the Abaco Islands, flooding the main island and irrevocably changing the coastline due to erosion. There are four burial locations in the Abaco Islands, with four individuals (one from each location) included in this study.

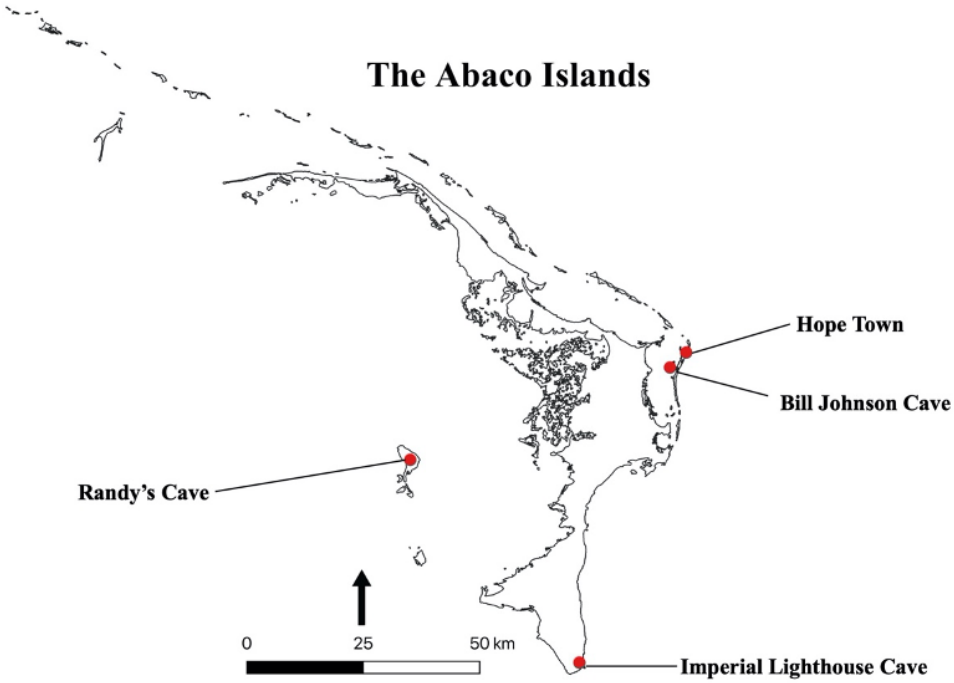



**Figure S3: Map of the Abaco Islands with burial locations included in the study.** Map created using QGIS Geographic Information System v3.14 (http://qgis.org); basemaps from The Nature Conservancy (2020).

**Bill Johnson's Cave, Lubber's Quarters**

Bill Johnson's Cave (AB-10) is on the southeast side of a cay known as Lubbers Quarters, which is situated southwest of Elbow Cay, facing Tilloo Cut to the east. It is 10 meters due north of the house owned by Bill Johnson. This flank margin cave is a large single chamber situated in the south flank of a sheer cliff face with a narrow single cave passage that penetrates the cliff approximately 15 meters into the hillside. The cave's entrance is about 25 meters west of the shoreline and 4 meters above sea level. The entrance chamber is 3 meters high and runs 10 meters along the cliff face. The cave entrance is partly filled with yard debris on the west side of the chamber, but otherwise there is almost no sediment in the interior passages. Other than a small section of flowstone, there is no evidence of bats or guano. Numerous broken conch shells, many with small round holes (Lucayan-modified), are scattered downslope of the cave's entrance chamber. Approximately 3 meters inside the eastern dripline, Nancy Albury collected a small sample from dry sediment, including an iguana (*Cyclura* sp.) jawbone, bone fragments from fish, bat, tortoise, and human bone fragments.

**Hope Town**

There is not much known about the provenience of this individual. Found by residents in Hope Town, Elbow Cay during excavation for a water cistern, this individual was collected by amateur archaeologists in 1990. The remains from Hope Town represent the first, non-cave human burial found in The Bahamas. They consist of a nearly complete female individual, estimated to be 21 to 25 years

old. Artifacts were found with the burial, but it is challenging to say if they reflect a funerary offering or a separate deposit. Objects include a large limestone fragment (possible pounding stone), five Palmetto Ware sherds, one worked conch tool, parrotfish bones, fire-cracked rock, turtle bones, and various shell fragments. A detailed archaeological report is lacking.

**Imperial Lighthouse Cave**

Rainey (1934) investigated an ocean sinkhole on the southern point of Abaco approximately 1.6 kilometers from the Hole in the Wall Imperial Lighthouse. At the site now known as Imperial Lighthouse Cave (AB-072) he collected cranial and postcranial bones of a child, aged 5 to 10, along with five undecorated Meillacoid sherds, fish bones, conch shells, charcoal, bird bones, and hutia bones (Keegan 1982). Rainey's notes do not provide further information.

**Randy's Cave**

Randy's Blue Hole (AB-55) is located on Moore's Island, southwest of Great Abaco Island, approximately 550 meters northeast of the nearest mangrove shoreline. The small blue hole is an eroded subsurface feature with geologic characteristics of a flooded vertical pit cave, having a subaerial diameter of three to five meters. Surface water lies three meters below ground level with a small subsurface chamber extending laterally 15 meters to a depth of eight meters. Brian Kakuk and Kenneth Broad conducted a SCUBA dive in July 2007. They discovered a partial human skull of a Lucayan, one human molar, a hutia sacrum, and of tortoise bone fragments. Subsequent trips by Albury noted that bulldozers infilled the blue hole during the clearing and construction of a nearby athletic field.

**Andros Island**

Although Andros physically comprises multiple islands, politically, it is considered one island. Andros is located in the central Bahamas and consists of the main islands of North Andros, Mangrove Cay, and South Andros, along with numerous cays. Andros' three main islands are separated by trifurcated estuaries (tidal creeks), connecting the island's east and west coasts. Collectively the fifth largest in the West Indies, the islands are 6,000 kilometers$^2$, 167 kilometers long, and 64 kilometers at the widest point. Andros has a rare combination of marine features, ecosystems, and the world's largest collection of blue holes. It is flanked on the east by the Tongue of the Ocean, a 6,600-foot deep trench. The world's sixth longest atoll, The Andros Barrier Reef, runs for 225 kilometers, and to the west, northwest and south lies the Great Bahama Bank. Paleoclimate and vegetation studies show that during the late Holocene the environment supported a dry shrubland environment (Kjellmark 1996). Andros' higher levels of rainfall, cooler temperatures, and pine forests are very different from the other islands of the central Bahamas. Andros exhibits greater faunal and flora biodiversity than any other Bahamian island (Campbell 1978). The island's land ecosystem is hugely varied, including pineyard, scrub, hardwood coppice, saltwater marsh, rocky and sandy beaches, palm savannas, and mangroves (Randolph 1994). There are two burial locations in Andros with eight individuals included in this study.

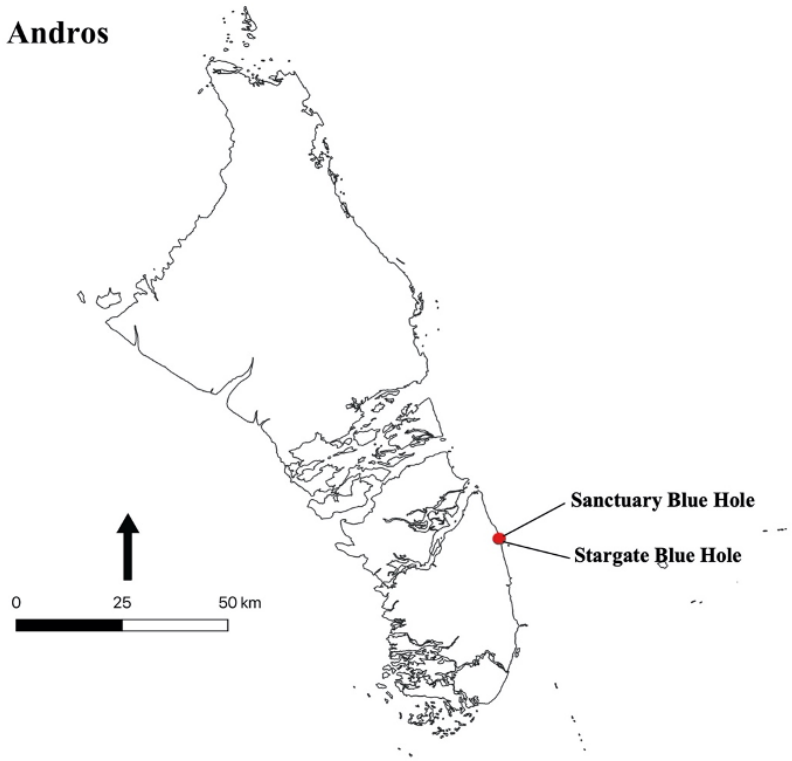



**Figure S4: Map of Andros with burial locations included in the study.** Map created using QGIS Geographic Information System v3.14 (http://qgis.org); basemaps from The Nature Conservancy (2020).

**Sanctuary Blue Hole**

Sanctuary Blue Hole (AN-12) is about 500 meters inland from the east coast of South Andros Island on the eastern side of the settlement of The Bluffs village. It is part of a significant north-south slump fracture zone paralleling the underwater escarpment separating the Tongue of the Ocean and the Great Bahama Bank. This slump fracture extends for tens of kilometers and formed as a result of glacio-eustatic sea-level changes and gravitational forces along the edge of the limestone banks (Palmer 1986a, 1986b). The entrance consists of a collapse-floored fissure extending down beneath a bedrock ledge to a pool about five meters long by two meters wide. A rift drops vertically beneath the water surface and opens out at a depth of 25 meters into a significant fissure passage that extends in both directions. Water depths in the cave do not exceed 60 meters. Between 1990 and 1991, cave diver Rob Palmer recovered 17 sets of skeletal remains. In 2009, as part of a National Geographic expedition, another individual was retrieved (Pateman and Keegan 2019). Osteological studies reveal a population that was short in stature and relatively active, probably due to subsistence activities. Indicators of health show that they had a relatively good childhood and adult health, although it appears there may have been some dietary stress (Pateman 2007). Existing radiocarbon data show a date range for the site between 1200 and 1400 CE (Pateman and Keegan 2019).

**Stargate Blue Hole**

Stargate Blue Hole (AN-13) is about 500 meters inland from the east coast of South Andros Island, on the western side of the The Bluff settlement; it is a part of the same cave system as Sanctuary Blue Hole. The cave's entrance is a partially roofed-over cavern with a vertical drop of six meters to the water level. The restricted nature of the access limits organic input, and as such, the surface water is relatively clear. Underwater, a shaft drops vertically to depths over 80 meters, while rift-like passages extend north and south. A gossamer layer of fine, brown sediment covers breakdown blocks on the floor of the cave; speleothems are present at all depths (Palmer 1986a, b). Divers recovered a ceremonial canoe from a ledge at 20 meters (the canoe is less than two meters long). The shelf also contained human remains, but these were not retrieved. During a National Geographic expedition in 2009, divers encountered two additional sets of human remains, removing only one. These remains date from 1210 to 1390 CE (Hastings et al. 2014).

**Crooked Island**

Geographically positioned southeast of Long Island, Crooked Island is 238 kilometers$^2$ and sits in the central region of a tropical moist forest zone (Stokes 1998). It is both an island and a district, the largest of a group surrounding a large shallow lagoon known as the Bight of Acklins. Crooked Island is a part of The Bahamas' cotton belt, settled by Loyalists in 1780. It suffered extreme deforestation by plantation economies that collapsed in 1825 (Craton and Saunders 1992). Crooked Island was catastrophically damaged when Hurricane Joaquin's eyewall passed over in 2015.

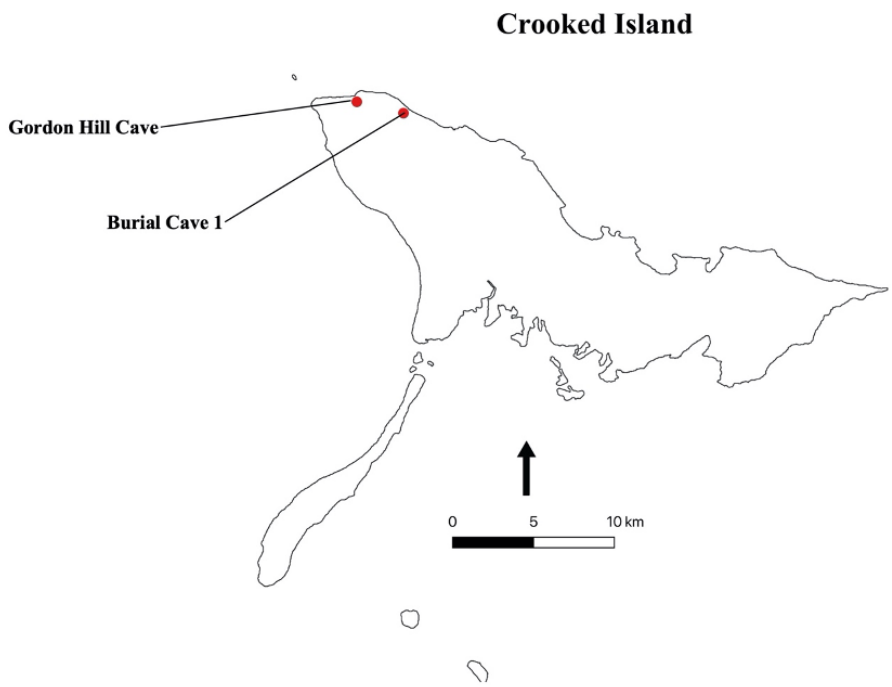



**Figure S5: Map of Crooked Island with burial locations included in the study.** Map created using QGIS Geographic Information System v3.14 (http://qgis.org); basemaps from The Nature Conservancy (2020).

**Burial Cave #1**

Rainey (1934) collected the remains of four individuals that had been excavated by cave diggers. The exact location of the site is unknown but is on the northern end of the island (Rainey 1934, Stokes 1998). Of these four individuals, one was male, one was female, and two were of undetermined sex; all were between the ages of 17 and 25 (Keegan 1982; Stokes 1998). Burial Cave #1 may be part of the cave system (Crossbed, 1702, and Owl Roost caves) recently investigated by David Steadman and Nancy Albury who recovered crocodile, hutia, and tortoise bones but do not mention human or other cultural remains (Steadman et al. 2017). The animal bones from these caves are dated between 1450-1620 CE.

**Gordon Hill Caves**

According to Rainey's diary (1934), the only site he systematically excavated was the Gordon Hill Caves (CR-020). The Gordon Hill Caves are a series of caves along a limestone bluff located on the northwest end of the island about 450 meters from the coast. Gordon Hill Caves represent Rainey's most productive excavations, both culturally and osteologically (Granberry 1978). Seven caves were excavated, four of which were partially excavated previously for cave earth. Two chambers were excavated in the Gordon Hill Burial Cave, each containing a single burial. One burial was very fragmentary, lying on its left side with the legs partly flexed on a small rock shelf on the cave floor just beneath the surface with finger bones covering the pubic region. A second burial (of disturbed context) was found in the south side of the cave where the ground surface sloped up to the entrance. This burial also lay on the rock floor of the cave. The bones of hutia and various birds were found near the burial in chamber one. Another cave, described as a residential cave, was extensively excavated. A variety of artifacts were recovered, and there was evidence of multiple fires. The material collected was described as Meillac-like (Meillacoid) and Carrier-Like (Chicoid) (Granberry 1955). These pottery styles are found as imported trade wares in The Bahamas.

**Long Island**

Long Island, located in the central Bahamas, is approximately 130 kilometers long and 6 kilometers wide with a landmass of 596 kilometers$^2$. The Tropic of Cancer splits the island, which is acclaimed for its caves and surrounded by smaller islands, bays, and inlets. Its orientation, geology, topography, and climate share commonalities with the other central islands. The island experienced catastrophic deforestation during the Loyalist Period by land clearance for cotton plantations and the harvesting of valuable hardwoods between 1783 and 1788 (Craton and Saunders 1992). Long Island's terrain varies widely, including white salt flats (salina), swamplands, sand beaches, and northern sloping and southern low hills. Hurricanes Joaquin (2015) and Dorian (2019) severely damaged the island's coastline.

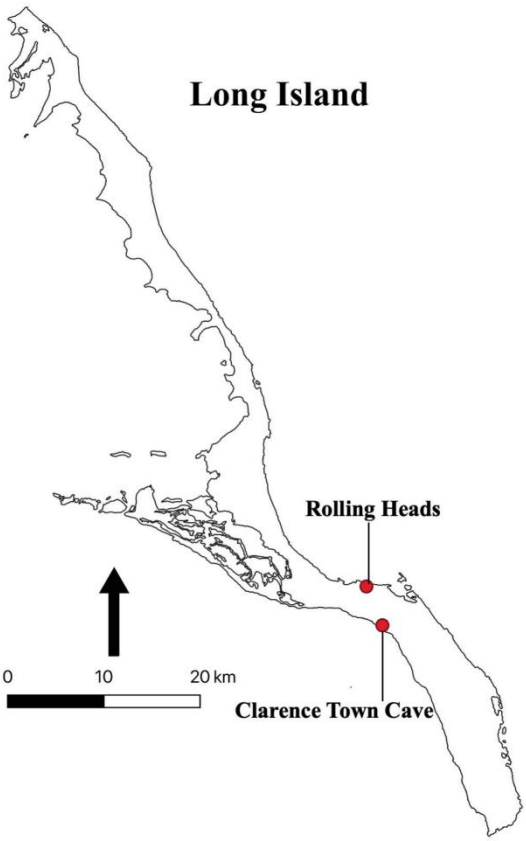



**Figure S6: Map of Long Island with burial locations included in the study.** Map created using QGIS Geographic Information System v3.14 (http://qgis.org); basemaps from The Nature Conservancy (2020).

**Clarence Town Cave**

In 1934, Rainey located and excavated one cave on southern Long Island near Clarence Town (Stokes 1998). The Clarence Town cave contained a main chamber with smaller branched compartments that showed evidence of guano excavation. The material culture found in chamber one included skeletal remains and an undescribed piece of pottery. Rainey assumed the remains were one individual, but Keegan (1982) subsequently determined the skeletal remains were those of two individuals. The first was an under 14-year-old juvenile represented by a humerus, radius, small temporal bone, and section of the ilium. The second is a female between 20 and 30 years old who is represented by a maxilla, a third molar, and part of the ilium. The adult female from chamber one was radiocarbon dated to 1175-1295 calCE (Stokes 1988).

**Rolling Heads**

After Hurricane Joaquin devastated Long Island in September 2015, residents Nick Constantakis, Nick Maillis, and Anthony Maillis found two fronto-occipital modified Lucayan skulls on Lowe's Beach. They also identified exposed human bones in two places on the dune face. Between October 17-19, 2016, Pateman and Keegan excavated three sets of human remains from what is the first multiple Lucayan burials found outside of a cave environment by archaeologists. The graves had no associated cultural

materials (Pateman and Keegan, 2019). Further testing revealed two seasonal occupation areas with associated earth ovens located 120 meters east of burial 1 (LN101A) and 20 meters west of burials 2 and 3 (LN-101B). The burials are AMS dated to between 1160-1405 calCE (2-sigma extremes) (Hanna, J.A., Keegan, W.F., Pateman, M.P., & Bloch, L. The Geomorphology of Natural and Cultural Processes on Lowe's Beach (Long Island, The Bahamas). *In Press.*).

**Eleuthera**

Eleuthera refers to the main island, its associated chain of smaller islands, including Harbour, Russel, Royal, and Windermere, and numerous cays. Eleuthera is located on the Great Bahama Bank in the central Bahamas. The main island is 180 kilometers long, little more than 1.6 kilometers at its narrowest, and has a landmass of 457.4 kilometers$^2$. The islands also were part of the Bahamas' cotton belt and faced extreme deforestation due to plantation economics. The hardwoods harvested during the Loyalist occupation included ten tons of Brasilwood sent as part of an endowment for Harvard University (Craton and Saunders 1992). Hurricane Andrew (1992) severely damaged The Eleutheras; the category five storm carried immense wind speeds, and an 18-foot tidal surge inundated the coastline. The islands' current topography differs extensively, ranging from pink sand beaches, while also including sizable ancient coral reefs, caves, and other geological features.

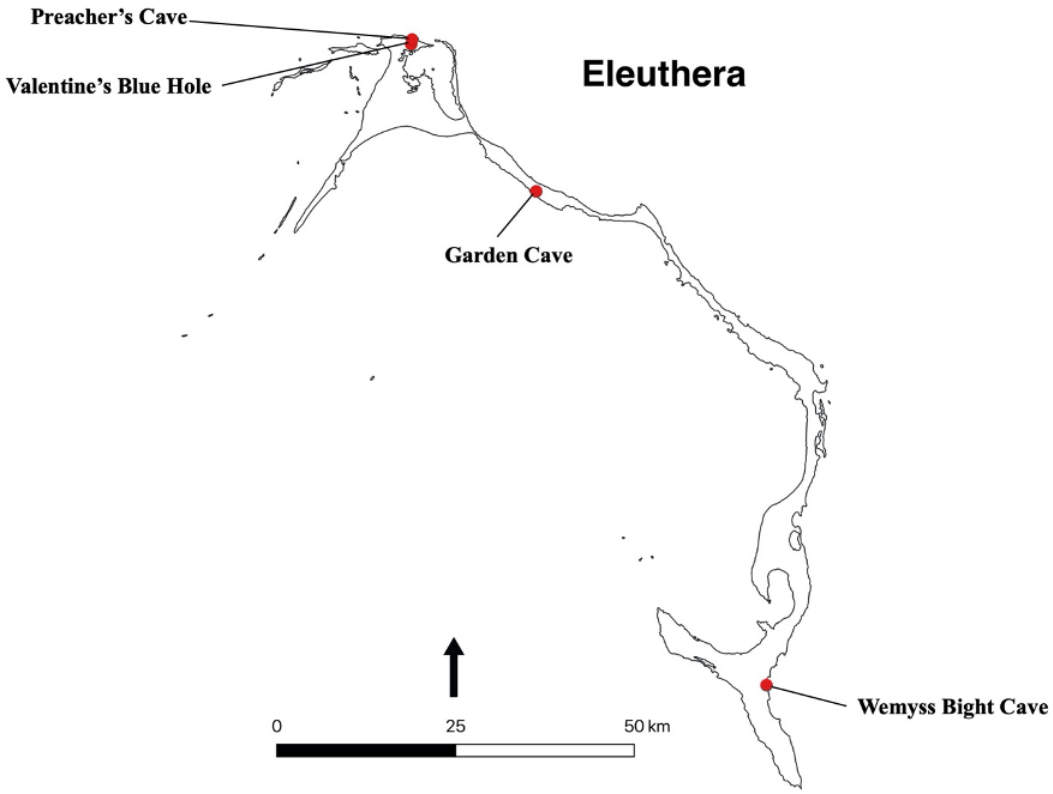



**Figure S7: Map of Eleuthera Island with burial locations included in the study.** Map created using QGIS Geographic Information System v3.14 (http://qgis.org); basemaps from The Nature Conservancy (2020).

**Garden Cave**

Garden Cave (EL-229) is a shallow flank margin cave (see Mylroie and Mylroie 2013) developed in an inland but seaward-facing cliff in the region of Hatchet Bay, Eleuthera. The cave is a short distance from the more famous Hatchet Bay Caves. Various caves riddle the entire ridgeline in the area. In 2006, officials from the National Museum of The Bahamas were made aware of the site, because of reported looting, and recovered two exposed skulls. Local eyewitnesses reported that when local explorers first discovered the site, there were 12 skulls arranged with other human bones. In July 2017, Keegan, Pateman, and Maurice White excavated the remains of at least six individuals (Keegan et al. 2018). Paleontologists Nancy Albury and David Steadman also studied the cave. They conducted excavations in the cave to recover animal bones as part of a larger project to understand the indigenous fauna of The Bahamas (Steadman et al. 2017).

**Preacher's Cave**

The Preacher's Cave site (EL-018) is on the northern coast of Eleuthera adjacent to Jean's Bay. Preacher's Cave is characteristic of a sea cave, mostly horizontal and lacking speleothems. It received its name from the original Puritan settlers who shipwrecked there in 1648 (Craton and Saunders 1992). Collected from within the cave were a total of seven Lucayan burials (Carr et al. 2006; Pateman and Keegan 2019). These burials are among the most complete archaeologically documented Lucayan burials in the Bahama archipelago. Two of the burials show evidence of binding with plaited matting, and one of the individuals has associated grave goods comprised of a triton (*Charonia variegata*) shell, 29 sunrise tellin (*Tellina radiata*) shells, red ochre, and a fishbone pin; existing radiocarbon dates show a site range for the burials of ~800 to 1250 CE (Schaffer et al. 2012).

**Valentine's Blue Hole**

“Valentine’s Cave” (EL-179; also known as “Bat Cave”) is situated about 1.7 kilometers southwest of Preachers Cave and 900 meters south of the north shoreline of Eleuthera. The cave is named for Valentine Yacht Club on Harbour Island, whose members surveyed it many years ago and left lines and markers. Approximately 20 meters south of the road, an east-west fissure is open to a large subsurface flank margin cave. The small entrance (approximately 2 meters x .5 meters) is partially filled with a ficus tree root system and opens into a large dry cave chamber ~25 meters in diameter and 3 meters high. A shallow lake (approximately 3 meters depth) fills the back of the dry chamber and a small area of the central chamber. Sediments are almost absent from the dry portion of the cave, but black organic residue and guano are interspersed between rocks and boulders, covering the bottom of the flooded portion. A roosting colony of about 200 Buffy flower bats (*Erophylla sezekorni*) was noted in February 2018. The surface saltwater is crystal clear, crisp, without hydrogen sulfide. A flooded tunnel leads away from the main chamber to the southwest reaching a maximum penetration of approximately 200 meters and maintains a depth of 3 meters. In August 1993, cave divers removed human skulls and other bones from this site. Osteological analysis of these remains reveals at least five adult individuals, four males and one female (Keegan et al. 2018). Further investigations by Nancy Albury revealed several hutia bones in the shallow cave sediments and the

shaft of a human bone, both within the flooded portion of the main chamber. Brian Kakuk collected these; the unassigned leg bones are the shaft (missing proximal and distal ends) of a juvenile.

**Wemyss Bight Cave**

Rainey located a cave two and a half miles inland from Wemyss Bite, southern Eleuthera. Upon discovering the site, excavation revealed a low cave that contained a surface burial. Upon inspection, it contained no artifacts, only scattered remains mixed with dirt and organic decay. A cranium bought from Wemyss settlement fits the mandible found during excavation (Keegan 1982). The skeletal remains represent at least two individuals. When Keegan (1982) analyzed the bones, they were determined to be male, but indistinguishable due to similarities in appearance and size. His count indicated two separate individuals represented by two left temporal bones, two right humeri, two right scapulae, one almost complete cranium and one left temporal, and one lumbar vertebra (Stokes 1998).

**CURAÇAO:**

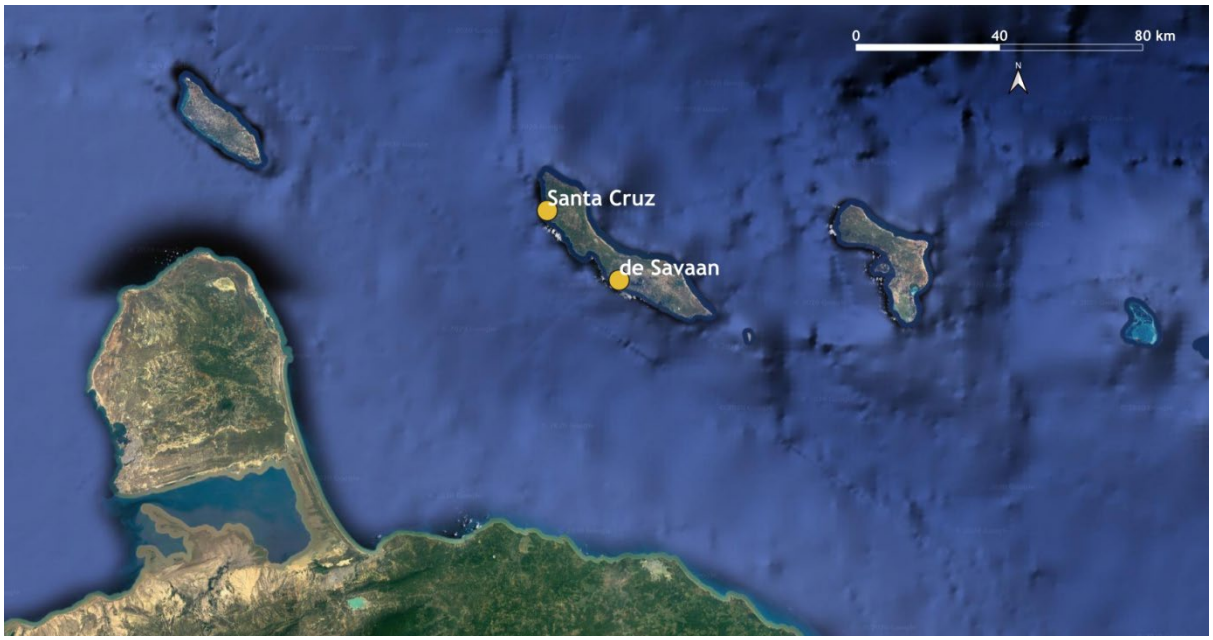



**Figure S8: Location of two archaeological sites in Curaçao included in this study.** Map created using QGIS Geographic Information System v3.6 (http://qgis.org); basemaps from Google Earth.

Curaçao is the second largest island in a chain of small islands and cays parallel to the coast of Venezuela (called the "Southern Caribbean Region"; Keegan and Hofman 2017). The island is semi-arid, with a mean annual precipitation of ~550 millimeters and a near constant average annual temperature of ~27ºC. The strong trade wind blows for almost the entire year from the northeast,

and hurricanes are infrequent. Previous work (Beers et al. 1997) recognized seven different landscape types with vegetation specific to geology and soil types.

Archaeological investigations on Curaçao were initiated by the amateur archaeologist Antonius J. van Koolwijk between 1878 and 1880 and have continued to the present. A total of 97 Amerindian sites were recorded in 1987. The earliest evidence for human presence on the island is dated between 3735-2895 BCE for the Rooi Rincon site. Jay Haviser (1987) describes the characteristics of five early sites as reflecting Archaic Age practices, with some continuation of Lithic Age technologies. The St. Michielsberg site especially has a material culture that is very similar to El Heneal on the north coast of Venezuela (see Willey 1971:366). The influence of these material cultures disappeared following the arrival of agriculture-associated Ceramic Age material culture about 1,500 years ago. The new arrivals manufactured pottery with motifs that are classified as Dabajuroid and which show direct connections to western Venezuela (Oliver 1997). This pottery has distinctive patterns of parallel incised lines, punctations, and applique. In addition, pottery painted in Dabajuroid motifs on Curaçao reflects continuity with the Second-Painted Horizon of Colombia even further west, and it is not affiliated with painted pottery to the east (i.e., Saladoid). Given that our genetic analysis models about ⅓ of the ancestry found in the studied individuals from Ceramic Age Curaçao as related to that of the individuals we have studied from Las Locas in western Venezuela (who were also a ceramic using group), it is parsimonious to identify that the arrival of Dabajuroid pottery in Curaçao about 500 CE was related to the same events that brought this ancestry component to Curaçao. In this context, it is striking that the other ~⅔ of ancestry in the Curaçao individuals we analyzed (all from ~1300 CE) can be well- modeled as coming from the Caribbean Ceramic cluster (specifically, from the *LesserAntilles_Ceramic sub-clade), suggesting that there was major gene flow from the Antilles that affected Curaçao in the centuries after the arrival of Dabajuroid pottery. An important topic for future research will be to identify the archaeological correlates of these events; see Supplementary Information section 9 for estimates of ancestry proportions in the individuals from Curaçao included in this work.

Human remains are described as scarce in Curaçao (Tacoma 1990). Haviser (1987) describes the characteristics of seven Ceramic Age sites, including the two sites from which the human remains in this study were recovered (i.e., de Savaan and Santa Cruz). The individuals analysed for this study come from two Caquetio settlements, associated archaeologically with large quantities of Dabajuroid ceramics.

**De Savaan**

The De Savaan site (C-0021) is located in south-central Curaçao about 500 meters from the northern shore of Piscadera Bay, and about 2-3 kilometers from Schottegat Bay (East) and St. Michiels Bay (West). All three bays have access to the sea in the South (Bonaire Basin). The site is located on a hilltop close to gullies where water concentrates after heavy rainfall, and soil is good for agriculture. The settlement and its agricultural lands encompass an area of at least 2-3 hectares (Haviser 1987). From 1980 on, several archaeological excavations have taken place at the site, some of which have

been published (Tacoma 1990; Haviser 1987). The finds of NAAM fieldwork in 2016 are still in the process of being analysed. In total, 14 graves have been recovered from De Savaan site. In general, individuals were buried with legs flexed and arms folded in a grave, although there is some evidence of secondary burials. In some cases, individuals were covered with inverted ceramic vessels (Haviser 1987; Kraan et al. 2016). Its context date of 1160-1500 CE and the abundant presence of Dabajuroid ceramics indicate the site was a Caquetio village.

**Santa Cruz**

The Spanish reported the Santa Cruz site (C-0004) as the village of a local Caquetio chief. The site is located on a high hill about 500 meters from the Santa Cruz Bay with access to the sea, close to gullies and with soil suitable for agriculture (Haviser 1987). The settlement encompasses about 2-3 hectares without associated agricultural plots. During two salvage excavations, one in the 1980s (Haviser 1987) and another in 2004 (currently unpublished), a total of three graves were documented, and another identified but not excavated. In general, the individuals were buried in graves with flexed arms and legs. In some cases, grave goods were identified. There was no evidence of urns. Recent research resulted in a context date of 1443-1522 CE (Kraan et al 2016).

**HISPANIOLA:**


**Figure S9: Location of 15 archaeological sites on Hispaniola included in this study.** Map created using QGIS Geographic Information System v3.6 (http://qgis.org); basemaps from Google Earth.

In much of what follows, we discuss the island of Hispaniola as separated into Haiti and the Dominican Republic using the present-day border. Here, we begin with Diale 1 in Haiti and move clockwise around the island.

**Diale 1**

The Diale 1 and Diale 2 sites are located on the western side of Ft. Liberté Bay on the northeast coast of present-day Haiti. The site is on a peninsula that juts slightly into the bay at the point where the entrance opens into the bay. It is about 5 kilometers north of the modern town of Faiton. Diale 2 is immediately to the south. It differs from Diale 1 (Meillacoid pottery) in being smaller and having Carrier style (Chicoid) pottery. The sites were first investigated by Froelich Rainey from Yale University in 1934 (Rainey 1941). Rainey dug one 18 x 2-meter trench through midden 17 at Diale 1. At the time he was assisted by Irving Rouse, then a student.

After completing excavations at other sites in 1935, Rouse directed the excavations at Diale 1. The site is described as 29 "middens," "which appear as perceptible mounds," extending in a roughly north-south direction, sloping gently upward from the shore to a low ridge (Rainey 1941: 42-43). The shore is rocky and lined with mangroves except for two small beaches around which the mounds are clustered. Rouse excavated trenches in mounds 1, 2, 5, 6, and 8. The mounds are composed of marine shells; the bones of fishes, manatee, sea turtle, and hutia; and land crabs. Cultural materials include large quantities of Meillacoid pottery, shell and stone implements, and stone and shell beads. The maximum depth of any midden was 1.5 meters, although most are less than one meter deep.

All of the human burials were excavated from mound 5, which has the most burials of any site investigated in the area (Rainey 1941: figures 1-19). The mostly primary burials are in a shell refuse layer at depths between about 25-40 cm. Most are disturbed and partially disintegrated. The remains of at least 7 individuals were collected. Burial 1 contained two skeletons, side by side; the primary burial of an adult flexed on its left side, and a youth. Burial 3 was an adult male on his back with his head pushed forward and the knees drawn up to the chest. Burial 4 was one meter from Burial 3; it is described as a small child placed on its back in a prone position with legs extended. Described as a secondary burial, the bones in Burial 5 are scattered across a one-meter square area at a shallower depth (about 16 cm below surface).

The human skeletal remains included in this study (ANTPA.000159) were acquired by Rainey in 1935 [according to the museum accession files (Diale 1 site. Section B, Trench B, Burial 2)]. Burial 2 was in mound 5, section 8, at a depth of 25-30 cm below surface, four meters from Burial 1, and eight meters from Burial 3. Burial 2 contained two skeletons with additional bones from secondary burials. A complete bowl was positioned between the skeletons close to the feet. These primary, flexed burials were disturbed and slightly disintegrated.

Our detection of admixture (16-20% Archaic-related, and the rest Ceramic-related; see Supplementary Information section 9) in both of the Diale 1 individuals is striking in light of the genetic findings in the Dominican individuals in our dataset, all dating after ~100 calCE from the same island of Hispaniola including from sites not too far to the east from Diale along its north coast. Of

these Dominican ceramic-using individuals, all but one are consistent with having no significant amount of Archaic-related admixture (the only exception is the outlier individual I16539 from La Caleta who is inferred to have ~10-14% Archaic-related admixture). These results suggest that there may have been a cline of admixture between Archaic and Ceramic-related ancestry within Hispaniola during the Ceramic Age, with larger proportions of residual Archaic-related ancestry persisting in the western part of the island (in present-day Haiti) where we have no sampling except for Diale 1 and where we do detect such admixture. An important topic for future research will be to carry out dense sampling of ancient DNA from western Hispaniola (Haiti) as well as eastern Cuba to determine the geographic, temporal, and genetic characteristics of this admixture cline and to correlate it to the archaeological evidence for interaction between east and west in this period.

**Edilio Cruz (Rancho Manuel)**

The site (RD/PR/02) is located on the northern side of the road that goes from Rancho Manuel toward Gregorio and the crossroads for Punta Rucia. The area where archaeological materials were found extends for 6000 square meters, while its height above sea level corresponds to 70 meters. The site was identified during the July 2006 survey conducted by the Italian Archaeo-Anthropological Mission in the Dominican Republic in collaboration with the Museo del Hombre Dominicano. A short excavation test was conducted on the site with the opening of a 2x2 meter trench which led to the identification of a burial. The excavation also produced faunal remains, in particular hutia and manatee, as well as a fair number of fish bones and gastropods.

The archaeological material identified during the excavation mainly includes fragments of decorated and undecorated ceramic containers. Most of them are representative of the Chicoid phase with the presence of bowls and plates characterized by handles with zoomorphic and anthropomorphic depictions according to the typical style of this phase. A smaller percentage is instead characteristic of the production of Meillacoid culture, typical of the northern coast of the island, and defined by the presence of low cups and cups decorated with plastic elements depicting zoomorphic depictions and engraved geometric patterns. In addition, the portion of the site investigated produced a good amount of *buren* fragments which are clay plates for domestic use, in particular for cooking food.

**Los Corniel (Rancho Manuel)**

The site (RD/PR/01) is located behind and towards the south of Rancho Manuel. After careful observation of the limits of dispersion of surface materials, the total extension of the site was assessed, which was approximately 1000 square meters, while the height above sea level was approximately 57.5 meters. The site was identified during the July 2006 Survey conducted by the Italian Archaeo-Anthropological Mission in the Dominican Republic in collaboration with the Museo del Hombre Dominicano. The mounds appear lined up in a double row on the crest of the hill. Twenty-two mounds were counted.

In order to understand the size of the site and above all to estimate the archaeological deposit and to distinguish exactly the occupation, a verification trench was opened in the area which showed a greater quantity of ceramics and remains of shells.

The trench followed the standard for rapid checks and measured four square meters. The remains of a burial were also identified in this trench as well as several elements that shed light on the consistency of the site. Faunal remains, fish bones and gastropods represent the maximum percentage among the finds. The ceramic fragments are characteristic of both the Chicoid and Meillacoid phases and consist of highly fragmented material referable to plates and bowls. In addition, some examples of polished stone tools such as millstones, strikers, and axes have been collected.

**Los Muertos**

The Los Muertos site (RD/PR/21) is located on the north slope of the northern cordillera. The entrance is on the southern side of the road from Rancho Manuel to Hestero Hondo. The site consists of three lines of mounds of which the northern and the southern run along the limits of the ridge. Thick vegetation covers the whole area, which includes probably 10 mounds with a size of about 20 meters in diameter and oriented in an east-west direction. A burial was excavated from a depth of 0.60 centimeters (Individual A). This burial was in a crouched position, oriented with the head to the north and facing west. Fragmentary remains of three other individuals (B, C and D) were also recovered.

**Loma Perenal (Puerto Plata)**

Archaeological research was carried out in 1998/1999 in the locality of Loma Perenal. This site is located on a plateau overlooking the alluvial valley of the Rio Bajabonico just before it flows into the bay of La Isabela. It is situated on the north coast of the Dominican Republic, a few kilometers from the town of La Isabela, in the province of Puerto Plata. This archaeologically-rich area covers the southern portion of the plateau in the direction of the river at approximately 70-80 meters above sea level, and was rich in cultural materials associated with Indigenous peoples. Excavation campaigns in 1998 and 1999 were conducted jointly by the Museo del Hombre Dominicano and the University of Rome "La Sapienza," thanks to the commitment of the Costa Foundation and with the collaboration of the Dominican Dirección Nacional de Parques.

The November/December 1998 campaign focused on Mound 3. The mound has an oval shape with a long axis oriented north-south and dimensions of approximately 16 x 12 meters. The height of its peak measured from the base varies in the southern half of the mound from 66 centimeters (compared to the south/east margin) to 124 centimeters (compared to the south/west margin).

The first layer (US 5) present on the surface consists of reddish-brown earth that is rich in humus as well as in roots, with numerous stones of different sizes variously distributed both on the surface and within the layer. During the excavation of US 5, some layers of ash appeared, light gray in color, of limited extension and with irregular edges. Three similar formations (US 9, 10, 11) were documented and removed in different locations. It does not seem that combustion took place *in situ* to create

these layers, but rather that ashes were thrown on the mound before US 5 was formed, after or during the deposition of the underlying layer. Ceramics present include both Meillacoid and Chicoid, with these contexts dated to between 900 and 1500 CE.

There are numerous lithic tools, made mostly of roughly worked stones. The majority of the tools are rather simple, but there are some more elaborate examples. Relevant materials include some personal ornaments, made from bone and shell, as well as a fragment of a spatula. It should be emphasized that the artifacts found are all Indigenous-associated, without any indication of Spanish origin. Animal bones were found (though less frequently), almost all from small rodents or birds. In addition, there are some fish vertebrae.

In May 1999, a burial was found approximately 60-80 meters to the southeast of Mound 3. Called Burial 1, it was determined to be a tomb partially disturbed by recent excavations. The excavation was therefore carried out on a disturbed context in which only a few portions of the skeleton were in the original position. The human bones were in poor condition. The rather loose soil, gray-brown in color, was full of fragmented ceramics, lithics, and shells.

**La Union**

The site of La Union was studied as part of an emergency excavation in July 1972 by the Museo del Hombre Dominicano, and consisted of a single trench (Veloz Maggiolo et al. 1972; 1973). It was determined to be the site of a community of fishermen with evidence of the presence of agriculture. It included ceramics of the Chicoid series, with very few Meillacoid ceramics, and was context dated to ~1435 CE. Twenty burials were excavated, 19 of which were associated with funerary goods. Four tombs only contained postcranial skeletal material and were devoid of skulls, consistent with a Taíno practice of keeping the skulls of ancestors in their houses (Veloz Maggiolo 1977). At the La Union site, the large volume of fishing net weights and marine gastropods (*Cittarium pica*) suggested the development of an exchange network characterized by local adaptations in which coastal communities specialized in fishing and collecting marine resources exchanged products with the more agricultural settlements of the interior (Veloz Maggiolo 1993).

**Cueva Juana**

The archaeological site was located at a karst rock spur approximately 100 meters from the east coast of the Cape of Samanà. The archaeological deposit was identified in the portion covered by the shelter and was investigated by the archaeologists of the Museo del Hombre Dominicano in the 1970s. The archaeological material identified during a recent survey is linked to the Ostionoid phase. Four human burials were discovered during the archaeological explorations, allowing us to consider this site as linked to the funeral aspects of the community.

**El Frances**

The research activities on the El Frances site (Samaná peninsula) were conducted in three excavation campaigns during 2018 and 2019 by the Italian Archaeological-Anthropology Mission in the Dominican

Republic (Sapienza University of Rome, Museo del Hombre Dominicano, and the Shelley Foundation). During these three campaigns, nine excavation areas were analysed. Both habitation and funerary areas were excavated. Seven excavated primary burials were dated between 788-874 calCE and 968-1023 calCE, and a context date of 532-584 calCE was also obtained from the site.

The site is of particular importance in the history of the development of the cultures in the eastern part of Hispaniola especially with regard to contacts with the nearby island of Puerto Rico, from which a sequence of cultural development subsequently spread throughout the eastern part of the island. The study of ceramics and radiometric dating attribute the site to an ancient phase of the island's ceramic population. This phase coincides with the introduction of the Saladoid material culture, which is found beneath a stratum containing Ostionoid materials. The Saladoid ceramics show clear morphological and stylistic characteristics that distinguish them from the original context, including surface treatment and the white-on-red paint as the main decorative element. During the last excavation, a whale vertebra was found for the first time at a site in the Dominican Republic.

**Macao, El Morro (La Altagracia)**

Punta Macao is a multi-component, but predominantly Late Ceramic Age, habitation site on the north-eastern coast of the easternmost province of La Altagracia. The site is situated on a rocky promontory called El Morro and is near the modern-day town of Macau. The site is mentioned in Las Casas' Apologética Historia, in which he claims to have visited the town and notes that a large population inhabited the area where almost 100 mounds were identified (de las Casas 1992; Olsen 2004; Veloz Maggiolo 1972; Veloz Maggiolo and Ortega 1972). Excavations at the site were undertaken by De Booy in 1915, Rainey in the 1940s, Veloz Maggiolo and Ortega in 1972, and a team from the Museo del Hombre Dominicano in 2004 (Atiles 2004; Olsen 2004; Tavarez María 2004).

The excavations conducted in 1972 showed two levels of occupation, an older Level I comprising an Ostionoid horizon, and a more recent Level II comprising a Chicoid horizon. Some evidence of Saladoid ceramics also was found at the base of the stratigraphy. The “Monticulos” are agricultural mounds, some of which contain burials, and most of which are disturbed by illegal excavations. The subsistence economy of the Level I population was mainly agricultural, supplemented by gathered plants and to a lesser extent fishing. Agriculture continued in Level II, but fishing increased compared to gathering. In general, more than 50% of marine-type foods were oysters and crabs collected from shallow water. Land snails represent 90% of the animal protein collected on land.

The skeletons included in this study come from 15 burial pits containing the remains of 26 individuals that were excavated by the Museo del Hombre Dominicano in 2004 (Atiles 2004; Olsen 2004; Tavarez María 2004). Most burial pits contained the remains of a number of individuals, who seemed to have been deposited simultaneously in primary depositions. The individuals were primarily males (n=11) and juveniles (n=8), with only six females present (Tavarez María 2004). The bodies were generally interred in small round or oval burial pits in supine positions or reclined on one side. All of the skeletons were in a flexed position, with the legs drawn up to the chest. Burial 2 was interred with a large Chicoid ceramic vessel placed upside down on the head, a practice observed in other

contemporary cemeteries. The majority of the burials were located in the southern part of the site, leading the investigators to suggest that this part of the site comprised the cemetery area. Many burials remain unexcavated in this part of the site, which has since been transformed into a golf course (Atiles 2004; Olsen 2004; Tavarez María 2004). Three radiocarbon dates, ranging from 1240±40-790±60 BP (uncalibrated) were obtained for three skeletons excavated in 2004, though it is unclear which three (Hofman et al. 2007). These dates, along with ceramic finds associated with the burials, indicate a predominantly Ostionoid and Chicoid chronology for the burials. However, some Spanish colonial ceramics (*majolica*) were found in the cemetery area of the site, perhaps indicating that the cemetery was still in use during the early contact period. The precise relationship between these ceramics and the burials is unclear (Atiles, 2004).

**El Soco**

The Boca del Soco site was excavated in 1975 and 1980 by researchers from El Museo del Hombre Dominicano. The site is located in the province of San Pedro de Macoris approximately 75 km east of Santo Domingo (Luna Calderon 1985; Veloz Maggiolo 1972, 1993). Two occupational phases are present at Boca del Soco. The first is an Ostionoid period occupation called the Margarita Phase and dating to around 700-800 CE. This occupation was excavated in 1975 with the name of El Soco I. The second phase of occupation is characterized by Chicoid ceramics (called the El Soco Phase and dating to around 1000-1500 CE). It was excavated in 1980 under the name of El Soco II. The burials are in both primary and secondary contexts and are accompanied by grave offerings such as pottery and dogs. Burial was collective during the Margarita phase.

A total of 158 individuals were recovered from Boca del Soco, 118 belonging to the Margarita Phase and 40 belonging to the El Soco Phase (Coppa et al. 1995). A high incidence of disease, along with infections, caused a high mortality rate among the sub-adult portion of the population, mainly infants in the Margarita Phase. This is in contrast to the high rate of adolescent death in the El Soco Phase (Luna Calderon 1985). In general, the Boca del Soco population was not in good health. Comparatively, the mortality of the adult population is higher than in the Juan Dolio cemetery, located about 20 kilometers away (Coppa et al. 1995). The isotopic data of El Soco, from eight individuals from the Margarita Phase and only one from the El Soco Phase, suggest that the diet was based more on terrestrial proteins than Juan Dolio's individuals (Stokes 1998).

**Juan Dolio**

Juan Dolio is a Late Ceramic Age site, located on the southern coast of the Dominican Republic, approximately 70 kilometers to the east of the capital city of Santo Domingo. The site was excavated by numerous researchers (Boyrie Moya 1960; Boyrie Moya and Cruxent 1955). It is known as one of the key sites of the "Boca Chica" (Chicoid) ceramic style in the southern and south-eastern Dominican Republic, however, as excavations at the site focused predominantly on the cemetery area, little is known about the house structures and material culture at the site. The archaeological remains appear in two different, separate humiferous layers, clearly detailing two important Indigenous occupations (Veloz Maggiolo 1972:157).

Juan Dolio was occupied as late as European contact as evidenced by the abundance of Spanish and other European pottery sherds in upper levels and in some burials (Goggin 1960; Garcia Arévalo 1978, 1990; Ortega and Fondeur 1978; Veloz Maggiolo 1993; Keehnen 2019).

Skeletal remains of 102 burial contexts were identified during excavations at Juan Dolio under the direction of Fernando Luna Calderón in 1974; however, many of these individuals comprised only a few fragmentary bones. Further analysis of the skeletal assemblage yielded an estimated minimum number of individuals of 78 persons, of which 31 were adult males, 29 were adult females, 18 were individuals of unknown sex, and 11 were juveniles (Veloz Maggiolo 1972; Drusini et al. 1987). A subsequent, more detailed study identified the skeletal remains of 108 individuals (Coppa et al. 1995). A large number of skeletal remains uncovered at the site during excavations in 1974 reportedly date predominantly to the late 15th century (Veloz Maggiolo 1972; Drusini et al. 1987).

As at Boca del Soco, cranial deformation was common (Drusini et al. 1987). In comparing the skeletal material from Boca del Soco and Juan Dolio, Coppa et al. (1995) found that living conditions improved from the early Taíno period (El Soco) to the period directly preceding European contact (Juan Dolio). The analyses conducted on the morphology and morphometry of the teeth of this necropolis in comparison with the pre-Ceramic one of Cueva Roja and with El Soco had highlighted marked differences such as different mobility patterns at the origin of the two cultural and chronological phases (Coppa et al. 1995). These differences have subsequently been confirmed on a larger number of ancient individuals and using more sophisticated multivariate statistics (Coppa et al. 2004, 2008; Cucina et al. 2003) and can be further tested using paleogenomic data.

A study on syphilis showed that this population like the others of the Ceramic Age were endemically affected by this disease, while the Archaic population of Cueva Roja shows no evidence of this pathology (Rothschild et al. 2000). Isotopic analyses for the study of diet have shown a mixture of marine and terrestrial diet with a more significant presence of the terrestrial component (Stokes 1998).

**Andrés**

The archaeological site of Andrés is situated on a narrow sand spit projecting into the Bahia de Andrés on the Caribbean Sea near the Dominican town of Boca Chica. It is about 25 kilometers east of the capital city of Santo Domingo and directly adjacent to the sugar warehouses of the Compania Azucarera Boca Chica and the adjoining Dominican village of Andrés. A first exploration of Andrés was carried out in 1922, with the excavations directed by Mr. Amado Franco Bido. The materials were studied by Dr. Narciso Alberti y Bosh, who in 1920 had also recovered many materials from the area. The entire bay appears to be a single large Taíno site, probably composed of multiple villages. In 1929, the American Museum of Natural History (AMNH) in New York City arranged a visit to the site. Construction of a new sugar warehouse during the previous year had exposed a substantial quantity of ceramics and human skeletons. Through this expedition, the AMNH obtained a collection of skulls, entire skeletons, and earthenware vessels, along with the skulls from excavations made at the cemetery directly in front of the warehouse (Krieger 1931). In 1930, new excavations were

undertaken by the Smithsonian Institution (Krieger 1931). Somewhat later, Dr. Narciso Alberti y Bosh of the National Museum of the Dominican Republic went to Boca Chica and collaborated in further excavations on the sandy beach in front of the sugar warehouse and obtained additional earthenware vessels and a large number of skulls (Alberti y Bosh1932).

The skeletons used in the present study come from materials excavated and published by Alberti y Bosh (1932). Complete vessels and pottery sherds exhibit Chicoid motifs associated with the Late Ceramic Age Taíno (Krieger 1931). The AMS dates obtained from most of the human bones are consistent with the cultural materials, however one skeleton returned a date of 2890±20 BP. This pre-Ceramic individual clearly represents an earlier occupation of the area, but there is insufficient site documentation to determine its particular context. There is, however, substantial evidence that this area continuously was exploited by humans since their initial arrival on the island (Veloz Maggiolo 1972, 1993). The specific location of the Andrés burials has been explained as reflecting limited access to sandy soils (Krieger 1931). It is therefore not surprising that individuals from different time periods were buried in the same location.

**La Caleta**

La Caleta is a multi-component habitation site with a large burial population situated in the town of La Caleta, approximately 17 kilometers east of Santo Domingo. The site was first inhabited during the Archaic Age, with radiocarbon dates indicating an early occupation around 545 BCE that continued throughout the Early and Late Ceramic Ages, with the most recent radiocarbon dates around 1280 CE (Morbán Laucer 1979; Ortega 2005). The site belongs mainly to the Boca Chica cultural horizon ("Chicoid"), but there is also evidence of Saladoid ceramics from the early Ceramic Age (Veloz Maggiolo 1972). During the excavation of the site, but not in association with the burials, European-made objects also were found (Veloz Maggiolo et al. 1976; Keehnen 2019).

Numerous excavations have been undertaken at the site over the years, uncovering at least 373 human skeletal remains (Morbán Laucer 1979). A first set of burials was excavated during the 1944-1945 campaigns (Herrera Fritot and Youmans 1946). This collection includes 14 skeletons, five of which were brought to Cuba and two to the Dartmouth College Museum in Hanover, New Hampshire. These are primary depositions in a flexed position. Further excavations were conducted in the following years (Boyrie de Moya and Herrera Fritot 1948). Most of the other burials in the collection were excavated in 1970–1971 by Chanlatte-Baik and Morbán Laucer among others (Veloz Maggiolo 1972).

Individuals were interred both as primary and secondary depositions in the midden area and other parts of the site. Primary interments consisted mainly of flexed, supine skeletons, with the legs drawn up to the chest. In a small number of cases, individuals were interred in a seated, flexed position. A large number of subadult skeletons (fetuses and infants) were recovered from the site. In one case, two juveniles pertaining to the Ostionoid occupation of the site were found buried with seven ceramic vessels of different sizes and a dog. Other grave goods include sherds of Ostionoid pottery, stone axes, shell amulets and vomiting spatulas, and the remains of marine foods. Secondary burial of

juveniles sometimes consisted of interment in a ceramic vessel. Some individuals, both adults and juveniles, were found buried with a ceramic vessel placed over their head and/or face (Morbán Laucer 1979). The skeletons used in the present study come from the excavations of 1970-1971.

**Atajadizo (La Altagracia)**

The El Atajadizo site is located near the southeastern coast at the mouth of the Yuma River. It was partially investigated in 1974 by a field party from the Museo del Hombre Dominicano.

The beginning of the ceramic period occupation in El Atajadizo probably occurred around 800 CE. Veloz Maggiolo and colleagues obtained a radiocarbon date of 840±80 CE for this phase (I-8649, Veloz Maggiolo et al. 1976b). The necropolis has two occupation phases called the Atajadizo Phase (840-1000 CE) and Guayabal Phase (1000-1400 CE) on the basis of a series of radiocarbon dates made at the time of the excavations in 1975 (Veloz Maggiolo et al 1976b; Wilson, 2007). European-made objects were also found during site excavation, but not in the burials (Veloz Maggiolo et al. 1976b; Keehnen 2019).

Mounds (Monticulos) 1 and 2 are placed in the Atajadizo Phase. A skeleton in Monticulo 1 was recovered associated with Chicoid pottery fragments. In deeper levels, fragments of Ostionoid ceramics were found. Mounds 4 and 5 are placed in the Guayabal Phase. Mound 3 contained no artifacts, and Mound 6 was disturbed by looters who left the skeletons *in situ*. These mounds cannot be attributed to either phase due to the lack of archaeological material.

In addition, there were burials in Trench 1 from the Plaza Sud Ouest (Luna Calderón 1976a, 1976b; Veloz Maggiolo et al. 1976a, 1976b) and a bioanthropological study of 47 burials was conducted. Several of the burials in Mound 4 (Guayabal Phase) show signs of violence, which was interpreted as evidence of inter-tribal struggles (Luna Calderón 1976a, 1976b). Finally, burial 6 of an adult in Mound 5 (Guayabal Phase) is missing its skull (as also seen in the La Union site), which could be a consequence of the Taíno practice of curating human skulls in their houses (Veloz Maggiolo 1993).

Subsistence during the older Atajadizo Phase was based on the gathering of terrestrial foods (land snails and hutia) and, to a lesser degree, fishing and gathering of seafood from mangroves. For the more recent Guayabal Phase, there is an increase in the gathering of mollusks from the mangroves as well as fishing. Gathering was also reported for both phases to be of greater importance than agricultural activities. The faunal data show that the marine resources comprise on average about 50% of the sample, including littoral mollusks and crustaceans (Rímoli 1976). The presence of both Chicoid and Ostionoid ceramics indicates that this site was occupied during a transition in pottery traditions.

**Cueva Roja (Pedernales)**

The excavation of the site of Cueva Roja took place in 1978, financed by the National Geographic Society and the Universidad Central del Este. The deposits are in a small cave near the sea and comprise skeletal remains with some associated cultural materials. The skeletal remains of 98 individuals in secondary deposition were recovered, of which 54 were adults over the age of 25 years,

10 were between 10 and 24 years of age, and 34 were under 10 years old. Some remains have been cremated.

The preliminary analyses conducted on the morphology and morphometry of the teeth from individuals from this necropolis identified marked differences in comparison with those of later ceramic users from El Soco and Juan Dolio. These differences generated the hypothesis that different mobility patterns are reflected between the two cultural and chronological phases (Coppa et al. 1995). The high fracture index at Cueva Roja is compatible with a mobile, transhumant way of life. This hypothesis subsequently was confirmed on a larger number of ancient individuals using more sophisticated multivariate statistics. The analysis also showed that the population of Cueva Roja exhibited a high biological affinity with the pre-Ceramic Guanahatabey of Cuba (Cucina et al. 2003; Coppa et al. 2004, 2008). Our genetic analysis of the Cueva Roja individuals which suggests entirely Archaic-related ancestry in all individuals is strongly consistent with this inference. An examination for evidence of syphilis showed that this population, as opposed to those of the Ceramic Age, was not affected by this pathology (Rothschild et al. 2000).

**PUERTO RICO:**

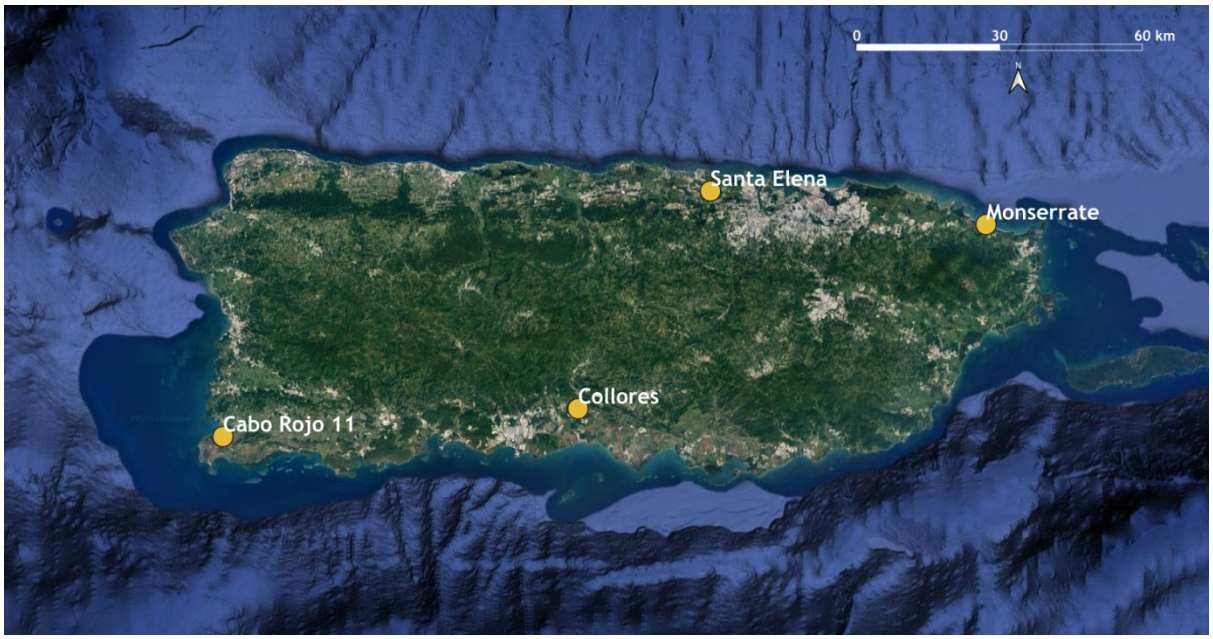



**Figure S10: Location of four archaeological sites with known locations in Puerto Rico included in this study.** Map created using QGIS Geographic Information System v3.6 (http://qgis.org); basemaps from Google Earth.

The remains from Puerto Rico included in this work were acquired by Rainey in 1934 and in 1935, (ANTPA.000161, ANTPA.000163, and ANTPA.000165) and in 1938 by Irving Rouse (ANTPA.000150, ANTPA.000151, ANTPA.000152, ANTPA.000153). The human remains from Puerto Rico were described by Drew (2009). Rouse (1952a, 1952b) refers to the Puerto Rican sites by more than one name,

including several sites that have the same name and a numerical suffix. For example, Cabo Rojo has four such variations in publications (Rouse 1952a: 311) and seventeen in unpublished notes.

**Collores**

Collores is located on Puerto Rico's mid-Southern coast, in the foothills approximately 2 kilometers northwest of the town of Juana Díaz (Rouse 1952b: 532). Shell debris covered approximately half an acre of the site. Modern agriculture had impacted the site, but two large middens, each "50 centimeters high", were still present when Rainey and Rouse examined the site (Rouse 1952b: 532). A modern road cut through one of the middens (Midden A), but the other midden (Midden B) was intact when Rainey encountered it. Rainey excavated two sections of Midden A. Excavation area 1 contained pottery of the Cuevas style (late Saladoid) and Ostiones and Santa Elena styles (Ostionoid) distributed throughout, with Ostiones pottery in the highest density. Rouse found the mixture of styles to be "unusual," and likely a disturbed deposit (Rouse 1952b: 533). According to Rouse (1952b: 532-533), "Charcoal, shells, animal bones, and potsherds, probably of the Ostiones style, were observed in the black loam of both middens."

ANTPA.000161 was recovered from the Collores site (Juana Díaz 1). This site was excavated by Rainey in 1934, and Rouse (1952b: 532) says that it is located in "almost the exact center of the south-coast area" of Puerto Rico. Rouse visited the site on September 15, 1936. Describing the earlier excavations, Rouse (1952b: 535) says: "Two burials were encountered in the lowest, "red culture" layer ... The first, which lay at a depth of 150 centimeters in section Dl, consisted of an adult female skeleton lying flexed on its left side. Little more than a meter away, and at a slightly lower depth, were the remains of a baby, too badly disintegrated to determine the position of the body."

Rouse sorted excavation area 2 into two divisions based on ceramic styles: a Cuevas division (late Saladoid) and an Ostiones (Ostionoid) division. Both of the burials were found in the Cuevas division. After evaluating Rainey's excavation notes and artifacts from area 2, Rouse felt it possible that the lowest layer, which contained the burials, were deposited "*in situ*", and that the rest of the material "eroded down the hillside at a later date." (Rouse 1952b: 537).

**Monserrate**

The Monserrate site (Luquillo 1) was excavated by Rainey in 1934 and 1935. The site is located near the mouth of a small lagoon on Punta Embarcadero in Barrio Mameyes of the municipality of Luquillo (Rouse 1952a: 419), east of San Juan near the northeast corner of the island. Rainey noted five distinct mounds, rising 1-1.5 meters above ground, and abundant surface ceramics at the site. ANTPA.000163 was one individual in a group of three burials in section B-5, level 1.25 meters, Mound A, Barrio Monserrate, according to museum catalogue records. Rainey (1940: 78) states that this section of the mound contained many flexed burials, and in the level where this individual was found "...the deposit was so filled with skeletons that some sections appeared to contain a massed burial." Shell refuse was abundant in the mound, along with the remains of other animals and artifacts. The most abundant pottery in Mound A was Ostionian Ostionoid (Rouse 1952: 421).

**Cañas/Unknown**

Cañas is located three kilometers north of the Caribbean Sea on the east bank of the Río Cañas (Rouse 1952b: 552), in a plain between the ocean and sea on the southwest coast of Puerto Rico near Ponce (Rainey 1940: 7). Cañas was Rainey's "principal site" where he first defined his Crab (red-on-white Saladoid) and shell (incised) pottery (Ostionoid) cultures (Rouse 1952b: 522). According to Rainey (1940: 7), the archaeological material at Cañas was well known to locals and prior to his excavations antiquities collectors would gather artifacts after field plowing exposed them. Rainey excavated in an overgrown land plot that contained "several mounds of considerable elevation" (Rainey 1940: 7). Cultural refuse was abundant on the surface of the site. Rainey excavated the largest mound and put test pits in two additional mounds. Two sections of the large mound were excavated; we analyzed an individual from section # 2 (ANTPA.000164; I13542), the southwestern area of the mound. Nine burials were encountered in this area. According to Rainey (1940: 12), seven were from a substratum of land-crab shells but were so poorly preserved that they could not be adequately studied. We analyzed one of the two individuals preserved well enough to be characterized osteologically (a morphologically female adult, Drew 2005); however, the individual failed to produce working ancient DNA data.

In addition to the failed Cañas individual, we analyzed two individuals of uncertain provenience in the Yale Peabody collections that could potentially be from Cañas. Rouse inherited a collection of materials from Rainey. The collection contained human remains, a large assortment of high-quality pottery, and other artifacts (Drew 2005). According to Drew (2005: 528), a piece of highly-polished redware labelled "Cañas #2 G-4 1.00" was in the materials (probably Ostionoid), which may suggest a provenience for the materials. Drew (2005: 528) also surmises that "the state of preservation of these remains is consistent with coastal locations where the abundance of shell fragments inhibited decomposition." The individuals we analyzed were part of a set of multiple co-mingled individuals comprising at least three adults and two infants. They are cataloged as being from Cañas, Collores or Monserrate, three different sites in Puerto Rico.

**Cabo Rojo 11**

From 1937-1938 Rouse surveyed and excavated a number of sites around the Cabo Rojo municipality. ANTPA.000150 was recovered from the Cabo Rojo 11/Llanos Tuna site (Rouse 1952a: 391), the only definite ball court site Rouse excavated during his 1930s fieldwork. This site is located in Barrio Llanos Tuna of the municipality of Cabo Rojo, about four kilometers southeast of the town of Cabo Rojo in the western part of the island. This site is near an unnamed stream, believed to flow northward and westward into the Rio Guanajibo and the bay of Mayagüez. Most of the pottery from this site was of the Ostiones type (Ostionoid). According to Rouse (1952a: 391), Ostiones sherds were common on the surface along with other artifacts, but not charcoal, ash or animal bones. Rouse excavated a 4 x 4-meter test at Cabo Rojo. One burial was encountered in this test unit. Rouse (Rouse 1952a: 393) describes the burial as:

> "A skeleton of an adult was found in section Bl of level 2, apparently flexed and lying on its left side. This skeleton was much disintegrated partially because the shells

> were closely packed and partially because it had been penetrated by the roots of a tree. Only fragments of the skull, parts of the pelvis, and the long bones remained. The latter were inclined, some one way and some the other, as if they had been moved out of position. The burial was directly in the refuse, without associated artifacts."

Drew (2005: 525) determined the individual to be morphologically an adult male.

**Toa Baja 2**

The site of Toa Baja 2 (sometimes referred to as Santa Elena) is located on Puerto Rico's north coast. The large village site is situated where the tracks of a cane railroad crossed the valley of the La Plata river, about 1.5 kilometers above Toa Baja and 5 kilometers from the sea near the north, central shore of the island. ANTPA.000151, ANTPA.000152, and ANTPA.000153 were excavated from this site (Rouse 1952a: 426). Rouse (1952a) noted that the site was well known to locals prior to archaeological investigation. According to Rouse (1952a), the site was located on a bench above a river floodplain and about five acres in size. Rouse excavated four 2x2 meter units at the site during the 1937-1938 field seasons. The ceramics at the site suggested it had both a pre-contact occupation as well as a contact and post-contact occupation (European ceramics were found on the uppermost layers). Sherds in the Precontact layers were primarily Santa Elena style (Elenan Ostionoid).

Rouse (1952a: 428-429) encountered three burials in the pre-contact strata. The first burial was found at the lowest shell stratum in the northwestern corner of section A2, at an average depth of 118 centimeters. The individual was in a flexed position lying on its right side. The bones were in good condition and there were no associated artifacts. The second burial was found in section Al, at a depth of from 136 to 160 centimeters. The burial included the remains of an infant that were badly decomposed and included the skull, vertebrae, ribs, and a few long bones. This infant was between the legs and arms of an adult. Most of the adult skeleton was outside of the excavation unit, but it lay on its left side in a flexed position with no associated artifacts. The third burial was found about 20 centimeters under the second burial, and also included an infant associated with an adult. The infant remains were in poor condition whereas the adult was better preserved. The adult was buried in a flexed position on its right side with no associated artifacts.

**VENEZUELA:**

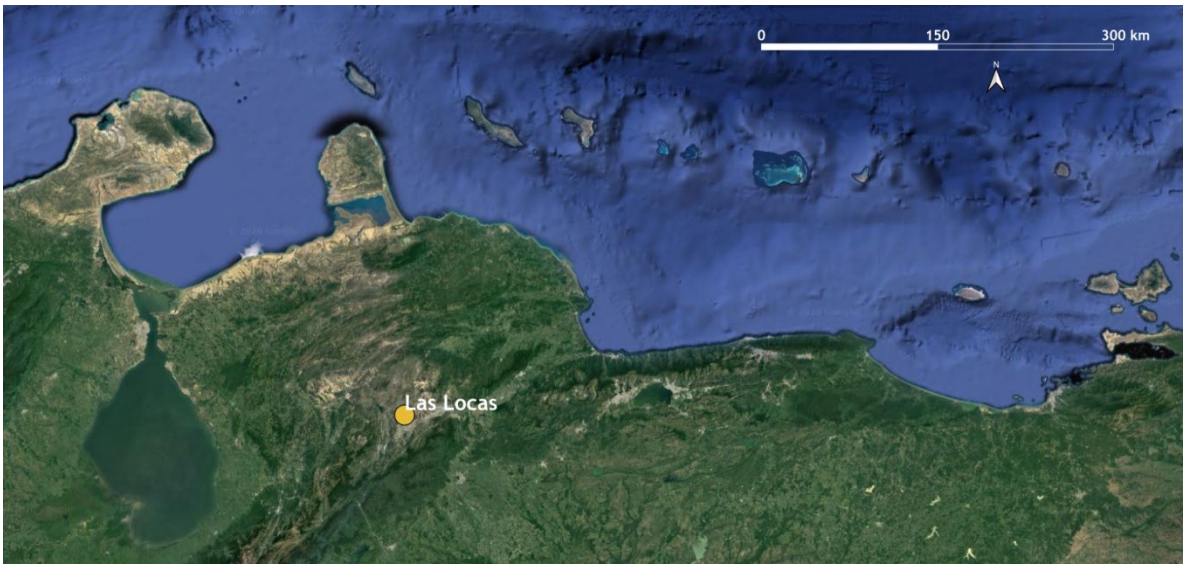



**Figure S11: Location of the archaeological site in Venezuela included in this study.** Map created using QGIS Geographic Information System v3.6 (http://qgis.org); basemaps from Google Earth.

**Las Locas (Quíbor Valley, Jiménez Municipality, Venezuela)**

Las Locas is the oldest necropolis described in the Quíbor Valley, located in the west-central region of Venezuela. It is located 11 kilometers northwest of the city of Quíbor in the Jiménez Municipality in Lara. The cemetery was first excavated by Mario Sanoja and Iraida Vargas as part of the Archaeological Project of the West of Venezuela (Sanoja and Vargas 1967). The site was estimated to date to around 500 BCE, based on the time range established for the Tradition Santa Ana style of the ceramics collected in the cemetery (Sanoja and Vargas 2007). We obtained slightly later dates on bone from two genetically homogenous individuals directly analyzed in this study: 2360-2315 calBP (PSUAMS-7365) for I17892 and 2360-2330 calBP (PSUMAS-7364) for I17889 (see Supplementary Information section 3 and Supplementary Data 3 for additional information on newly generated radiocarbon dates).

The skeletal remains studied as part of this work are deposited in the "Gonzalo Rincón Gutiérrez" Archaeological Museum of the University of Los Andes, Mérida-Venezuela. The archaeological remains found in the necropolis (mortuary treatment associated with exuberant funerary ceramics) provide evidence concerning the emergence of hierarchical societies in northwestern Venezuela.

Our genetic data from Las Locas are notable in two ways. First, while the Las Locas individuals were members of a community that used ceramics, this does not exclude the possibility that the region where they lived was a possible point of origin for the populations who moved into the Caribbean at a much earlier time during the Archaic Age and established an ancestry profile at least in the Greater Antilles that we examine in this study. Population structure analysis suggests similar ancestry patterns between the Las Locas individuals and our Archaic-related individuals from the Greater Antilles, while in maximum likelihood trees the latter are positioned in a node leading to Las Locas and present-day

Chibchan-speaking groups. This position, however, is not clearly confirmed with $f_4$-statistics as we could not find a closer affinity of the Archaic-related individuals to Las Locas or any Chibchan-speaking group when compared to other present-day or ancient populations. While additional ancient DNA from the region from earlier in time would allow further testing of a scenario where the Archaic Age movement into the Caribbean involved people living in this part of South America in groups ancestral to the people of Las Locas, it is possible that the Las Locas individuals we analyzed would be best seen as an Archaic-related population impacted by later admixture, despite their intensive ceramic use. Second, a striking genetic inference from Las Locas is that the ancestry of the individuals we analyzed is a good proxy source for about ~25% of the ancestry of the individuals we analyzed from Curaçao (see also a discussion of this in the section on Curaçao). Combined with the archaeological evidence of the spread of Dabajuroid pottery around ~500 CE (which represents a material connection between Curaçao and the region of west Venezuela where the site of Las Locas is found), our results suggest that Las Locas-related ancestry spread to Curaçao from the mainland in association with the spread of Dabajuroid material culture, largely displacing whatever ancestry was previously present in Curaçao (as the other component of ancestry in the Curaçao individuals we analyzed was primarily associated with that seen in people living in the Lesser Antilles during the Ceramic Age, see Supplementary Information section 9).

## SI3- Newly-reported direct $^{14}C$ Dates and isotope data

Direct radiocarbon ($^{14}C$) dates obtained from skeletal material from the Caribbean have the potential to improve our understanding of a range of topics of interest, including the timing of population movements into and around the Caribbean as well as the onset of new cultural traditions and transitions between traditions. Radiocarbon dates require calibration to account for changes in the global radiocarbon concentration over time (Bronk Ramsey 2008; Taylor 2009), and dates obtained using skeletal material from the Northern Hemisphere are most often calibrated using the IntCal13/IntCal20 curve (Reimer et al. 2013, 2020), which represents the mid-latitude Northern Hemisphere atmospheric reservoir.

It has been argued that radiocarbon dates from insular biomaterials should undergo corrections for the marine radiocarbon reservoir effect, as $^{14}C$ is not equally distributed across the biosphere and marine ecological zones which can lead to an offset in $^{14}C$ age between contemporaneous organisms from the terrestrial environment and organisms deriving carbon from marine environments (Aitken 2013). Here, the use of isotopic information from ancient individuals (specifically $\delta^{13}C_{collagen}$, $\delta^{13}C_{apatite}$, and $\delta^{15}N_{collagen}$ values) can provide insight into the proportion of an individual's diet composed of marine sources and assist in performing accurate calibrations.

In the Caribbean, the contribution of plant foods to the diet increased substantially with the introduction of agriculture at the onset of the Ceramic Age. While manioc was the staple crop in South American tropical forests (and probably the Caribbean, along with sweet potato), maize also arrived early to the islands (probably with the earliest inhabitants; Pagán-Jiménez et al. 2015) and seems to be especially important because of its high protein content. However, maize quickly depletes tropical soils due to a high nutrient requirement and therefore is a secondary crop outside river drainages where soils are replenished by annual floods. Maize was certainly a part of Caribbean diets, though this percentage varied by location and environmental conditions (Figueredo 2015). Important within the context of the Caribbean, maize (a $C_4$ plant) has a $\delta^{13}C$ signature of around 10 per mil, similar to the $C_4$-like signature observed in fish and mollusks from Caribbean coral reef environments (Keegan and DeNiro 1988). It is therefore difficult to determine the proportion of $\delta^{13}C$ contributed by carbohydrates (maize) versus protein (marine organisms) using bone collagen alone. Though fish could potentially be a major component of diet for people on an island, many of the Greater Antillean sites included in this study are not immediately on the coast, and so lacked direct access to marine foods. Furthermore, the success of fishing is limited by weather, seasonal and other conditions that limit catches, and there is also evidence for fairly rapid local resource depletion, including the size of fish caught, even at low population densities (Carlson and Keegan 2004; Fitzpatrick and Keegan 2007).

Here, we generate $\delta^{13}C_{collagen}$, $\delta^{13}C_{apatite}$, and $\delta^{15}N_{collagen}$ values and use them to assist in the calibration of direct radiocarbon dates from the pre-contact Caribbean. A discussion of method and interpretation of results is provided below. We report all direct $^{14}C$ dates in the main manuscript as

calibrated years before present (calBP), using the IntCal20 curve data for calibration (dates in Supplementary Data 3).

*Stable carbon and nitrogen isotopes*

Carbon and nitrogen isotope ratios were measured on extracted and purified bone collagen gelatin (>30 kDa gelatin) after ultrafiltration, or on purified amino acid hydrolysate of extracted bone collagen gelatin (N=41; see Supplementary Data 3 for preparation method by individual). Bone surfaces were physically removed and approximately 200-400 mg of sample was demineralized for 24 to 36 hours in 0.5 N HCl at 5°C. The pseudomorph was gelatinized in 0.01N HCl for 12 hours. The resulting gelatin solution was lyophilized and weighed to determine percent yield as a first evaluation of the degree of bone collagen preservation. Rehydrated gelatin solution was pipetted into precleaned Centriprep ultrafilters (retaining >30-kDa molecular weight gelatin) and centrifuged three times for 20 min, diluted with $H_2O$ (18.2 megohm per cm), and centrifuged three more times for 20 min to desalt the solution.

Bone collagen samples with low gelatin yields were processed using amino acid hydrolysis and XAD purification (Lohse et al. 2014). Gelatin was hydrolyzed in 2 ml of 6 N HCl for 24 hours at 110°C. Supelco ENVI-Chrom SPE (solid-phase extraction; Sigma-Aldrich) columns with 0.45-µm polyvinylidene difluoride filters were equilibrated with 50 ml of 6 N HCl, and the washings were discarded. Two milliliters of hydrolyzate were pipetted onto the SPE column and driven with an additional 10 ml of 6 N HCl dropwise with a syringe into a 20-mm culture tube. The hydrolyzate was dried into a viscous syrup by passing UHP (ultra-high purity) $N_2$ gas over the sample heated at 50°C for ~12 hours. Though we note that the isotope ratios of XAD-purified amino acids may not be directly comparable to collagen ratios, the differences are minor (Lohse et al. 2014). Carbon and nitrogen concentrations and stable isotope ratios of the collagen or amino acid samples were measured at the Yale Analytical and Stable Isotope Center with a Costech elemental analyzer (ECS 4010) and Thermo DELTAPlus analyzer. Sample quality was evaluated by % crude gelatin yield, %C, %N, and C/N ratios (atomic). C:N ratios ranging between 3.15 and 3.44 indicated good collagen or amino acid preservation (Kennett et al. 2017).

The isotopic information was used to identify ancient Caribbean individuals with diets exclusively derived from breastfeeding rather than higher trophic level foods (Kennett et al. 2016). Potential nursing $\delta^{15}N$ signatures were assessed using a general linear model comparing the relationship between $\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$ for the Caribbean data. We evaluated if the $\delta^{15}N$ values were enriched relative to the total sample by taking the absolute average of the ancient Caribbean dataset (10.9‰) plus 1/2 trophic level (2.5‰). Furthermore, assuming that nursing infants are enriched approximately 2-3‰ compared to mothers (Fogel et al. 1989; Fuller et al. 2006), we estimated that the lower bound threshold for a nursing signal is the female $\delta^{15}N$ average (11.2‰) plus 2‰. We estimated that $\delta^{15}N_{collagen}$ values over 13.4‰ are likely breast-feeding trophic effects. Potential infants were removed from the statistical analysis.

We compared 41 ancient Caribbean individuals in this study to a reference database that included 90 previously published pre-contact Caribbean individuals (Keegan and DeNiro 1988; Krigbaum et al. 2013; Laffoon et al. 2016; Chinique de Armas et al. 2017; Pestle and Laffoon 2018), 47 Coastal Maya individuals (Williams et al. 2009), 101 Classic Maya individuals (Sommerville et al. 2013), and 38 Pre- and Post-maize individuals from the Maya Mountains of Belize (Mayahak Cab Pek (MHCP) and Saki Tzul (ST)) (Kennett et al. 2020) (Figure S12).

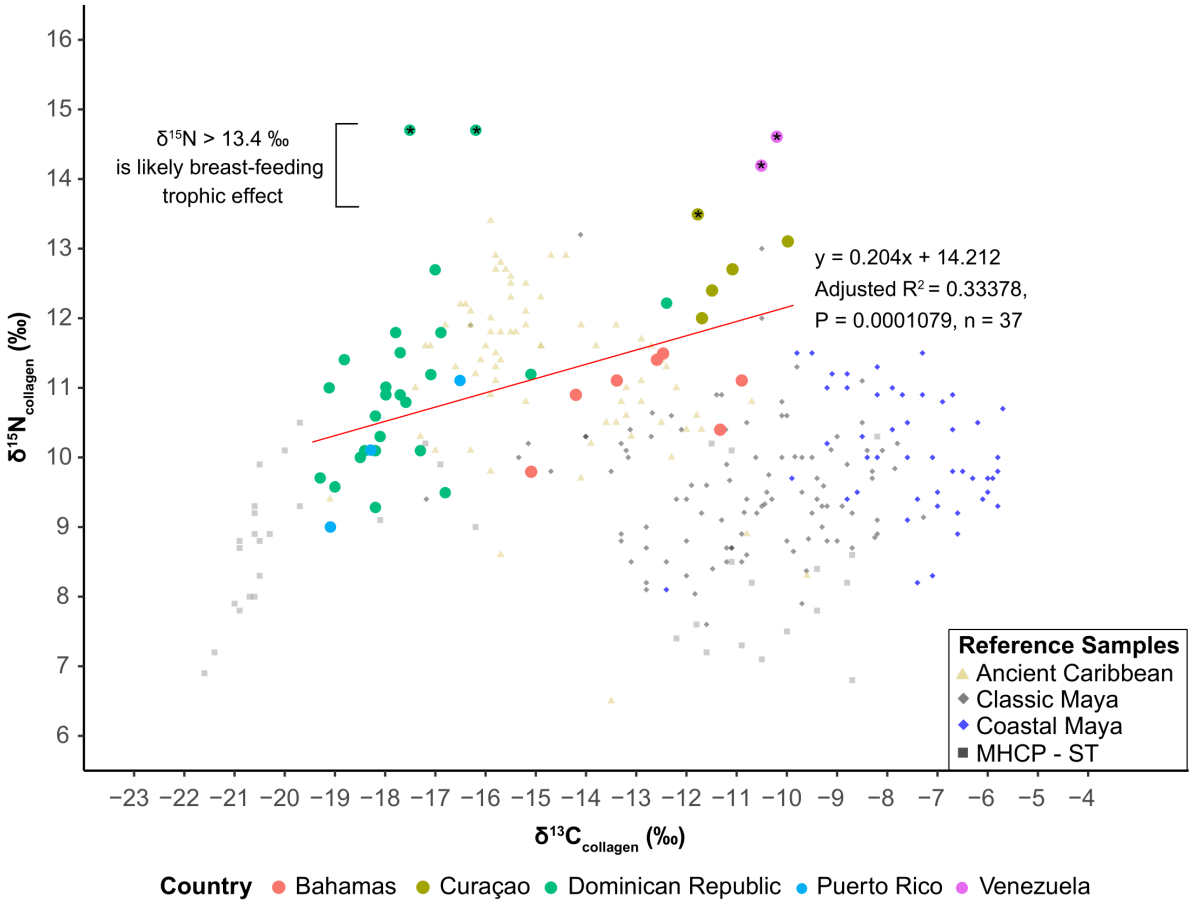



**Figure S12: $\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$ values from this study (N=41) plotted relative to the Caribbean and Maya reference data (N=276).** Included are the results of a general linear analysis comparing the relationship between $\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$ for the Caribbean data in this study. The red line shows the slope of the linear regression model and the adjusted R2 indicates the proportion of variance explained by the linear association between $\delta^{13}C_{collagen}$ and $\delta^{15}N_{collagen}$. The statistical test is two-sided. All individuals that are ~2.5‰ enriched in $\delta^{15}N_{collagen}$ relative to the sample mean (10.9‰) are considered to be elevated due to the breast-feeding trophic effect; individuals with breast-feeding trophic levels are noted by an asterisk (*) and were removed from the statistical analysis. Collagen isotope data show stark differences among island protein sources: Dominican Republic and Puerto Rico (high $C_3$ contribution), The Bahamas (mixed $C_3$-$C_4$ contribution), and Curaçao (high $C_4$ contribution).

*Stable carbon isotopes (apatite)*

Sample preparation for bone carbonate analysis was conducted in the Human Paleoecology and Isotope Geochemistry Laboratory at University of California, Santa Barbara using a modified version of procedures outlined in Koch et al. (1997) and Kennett et al. (2020) (N=24). The outer surface of a 2.5 × 2.5 cm fragment of dense bone was mechanically removed and bone samples were ground into a fine powder using an agate mortar and pestle. For each sample, approximately 100 mg of bone powder was treated in 2% bleach (NaOCl) for 24 hours at room temperature with vented capped vials and then rinsed three times in $H_2O$ (18.2 megohm per cm) to remove residual bleach. This step was repeated to ensure the removal of organic materials. Samples were treated in 0.1 M acetic acid ($CH_3COOH$) for 24 hours at room temperature with vented capped vials (Garvie-Lok et al. 2004) and then rinsed again to a neutral pH with water (18.2 megohm per cm). The samples were then dried overnight at 60°C and analyzed at the Center for Stable Isotopes, University of New Mexico, by continuous-flow isotope ratio mass spectrometry using a GasBench device coupled to a Thermo Fisher Scientific Delta V Plus isotope ratio mass spectrometer. The results are reported using the delta notation measured against VPDB (Vienna Pee Dee Belemnite). Reproducibility was better than 0.1‰ for both $\delta^{13}C$ based on repeats of a laboratory standard (Carrara Marble). The laboratory standard is calibrated versus National Institute of Standards and Technology (NBS)-19, for which the $\delta^{13}C$ is 1.95‰.

*Simple Carbon Isotope Model*

A simple carbon isotope model was used to assess the relative proportion of $C_3$ and $C_4$ foods in the diet of 24 ancient Caribbean individuals compared with data from 242 ancient Caribbean and Maya reference individuals (Figure S13; data for newly-reported ancient Caribbean individuals in Supplementary Data 3). Researchers note that $\delta^{13}C_{collagen}$ and $\delta^{13}C_{apatite}$ alone fail to capture the full range of dietary energy or protein sources (Kellner and Schoeninger 2007; Froehle et al. 2012) and plotting $\delta^{13}C_{collagen}$ against $\delta^{13}C_{apatite}$ allows for a more complete picture of whole diet and the dietary plant protein component. The model was developed using experimental feeding data and dietary data from human populations with reasonably well-known diets to estimate $C_3$ vs. $C_4$/marine protein and non-protein sources in the total diet (Kellner and Schoeninger 2007; Froehle et al. 2012). When applied to human dietary reconstructions, the position of an individual's stable carbon value relative to the $\delta^{13}C_{collagen}$ regression line estimates information on protein sources while their position relative to the $\delta^{13}C_{apatite}$ line shows the ratio of $C_3$ to $C_4$ plants in the total diet. The result is a more complete understanding of dietary constituents than was afforded by previous methods.

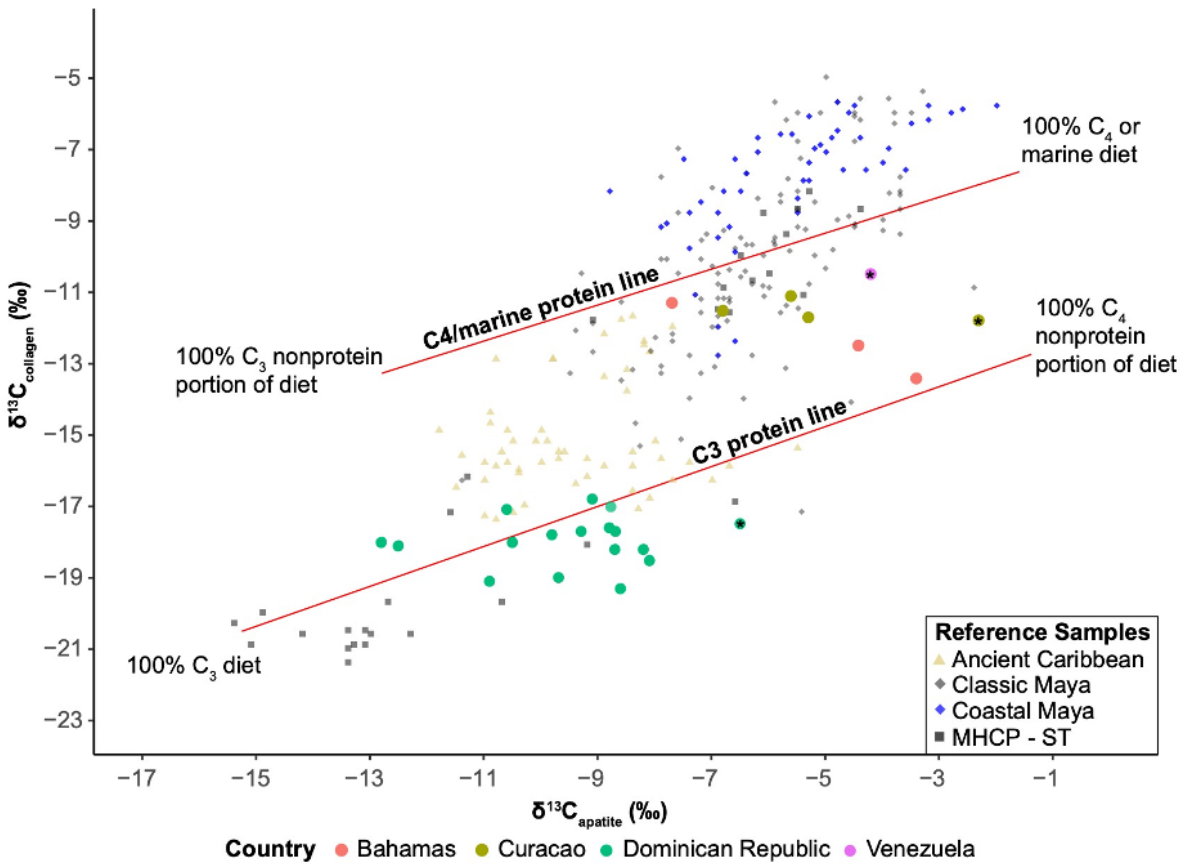



**Figure S13: $\delta^{13}C_{collagen}$ and $\delta^{13}C_{apatite}$ values from this study (N=24) plotted against the simple carbon model regression lines[16] relative to the ancient Caribbean and Maya reference data (N=242).** Ancient Caribbean individuals are plotted along $C_3$ and $C_4$ protein lines. All individuals from the Dominican Republic fall between ~50-80% $C_3$ diet along the $C_3$ protein line. Individuals from The Bahamas and Curaçao are either off-set along the $C_4$ protein line (between ~40-60%) suggesting either higher proportions of marine proteins or maize in the whole diet, or along the lower portion of the $C_3$ protein line, suggesting more $C_4$ or CAMs plants in the whole diet. Individuals with breast-feeding trophic levels are noted by an asterisk (*).

*Multi-Isotope Model*

Ancient Caribbean individuals with $\delta^{13}C_{collagen}$, $\delta^{15}N_{collagen}$, and $\delta^{13}C_{apatite}$ data were assigned to specific dietary clusters using a multivariate model developed by Froehle et al. (2012) and compared to reference data from 242 previously published ancient Caribbean and Maya individuals (Figure S14; data for newly-reported ancient Caribbean individuals in Supplementary Data 3). The multi-isotope approach uses a k-means cluster analysis on archaeological populations with well-defined diets to generate dietary groupings based on ratios in stable carbon and nitrogen isotopes, resulting in five distinct dietary clusters and a discriminant function model to "flatten" the three isotope variables ($\delta^{13}C_{collagen}$, $\delta^{13}C_{apatite}$, and $\delta^{15}N_{collagen}$) into two-dimensional plotting space. The two discriminant function equations account for 98.8% of the variance in the five dietary clusters. The resulting plot

space contains the previously generated dietary clusters where each cluster is characterized by a box that represents the range of isotope values for each function within the clusters. The location in plotting space where an individual's isotopic signature falls reveals information about protein sources and total diet, including whether or not an individual's diet contained marine protein sources.

Function 1 (Carbon) = (0.322 * $\delta^{13}C$ carbonate) + (0.727 * $\delta^{13}C$ collagen+ (0.219 * $\delta^{15}N$) + 9.354

Function 2 (Nitrogen) = (-0.393 * $\delta^{13}C$ carbonate) + (0.133 * $\delta^{13}C$ collagen + (0.622 * $\delta^{15}N$) – 8.703

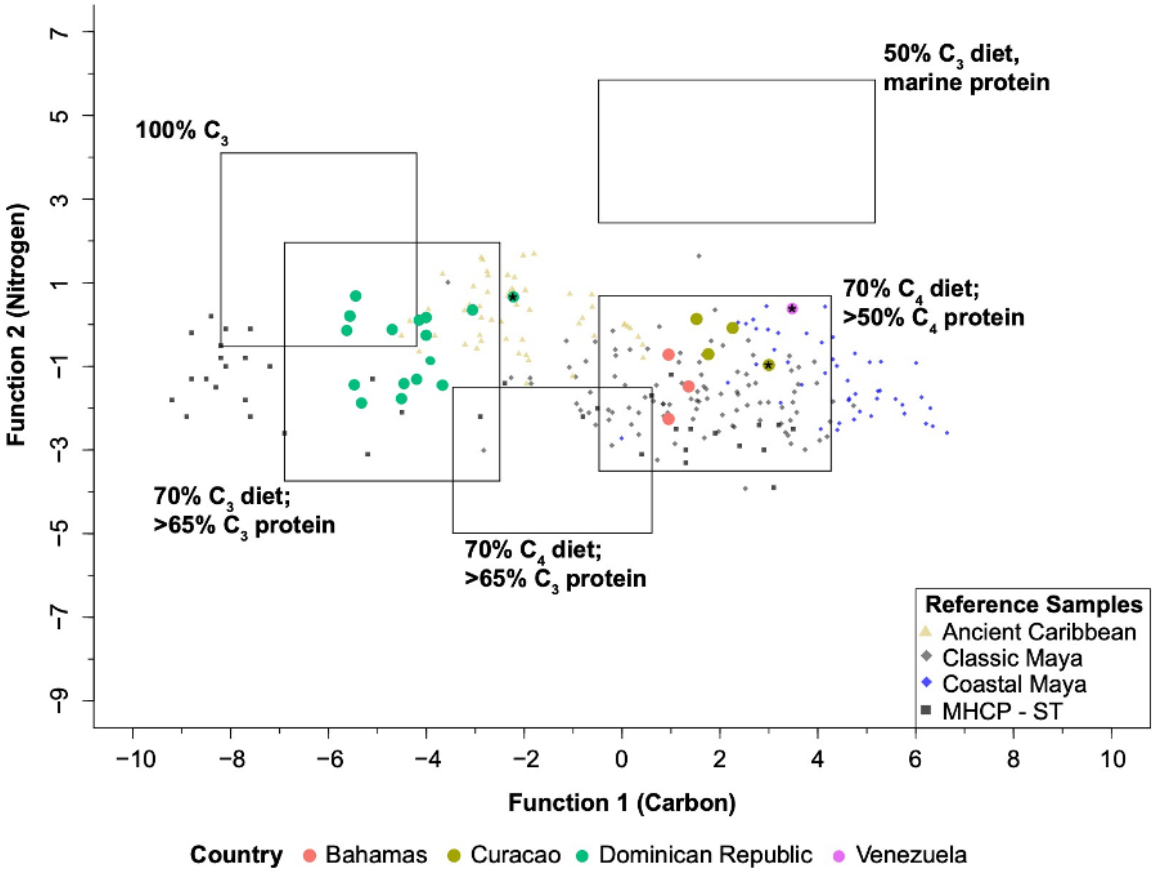



**Figure S14: Ancient Caribbean function 1 (carbon) and function 2 (nitrogen) discriminant function values (N=24) plotted against dietary clusters (Froehle et al. 2012) relative to the Caribbean and Maya reference dataset (N=242).** The results support a stark difference between the island ecological patterns. The Dominican Republic dietary cluster (70% $C_3$ diet and >65% $C_3$ protein) indicates a diet higher in $C_3$ terrestrial resources while The Bahamas and Curaçao individuals fall in the dietary cluster high in $C_4$ resources (70% $C_4$ diet and >50% $C_4$ protein). Individuals with breast-feeding trophic levels are noted by an asterisk (*).

*FTIR Quality Control analysis*

Bone apatite for stable carbon analysis was prepared in the Paleoecology and Isotope Geochemistry Laboratory at University of California, Santa Barbara (UCSB) using a modified version of Koch et al. (1997) following procedures detailed by Kennett et al. (2020). Spectral band ratios in biogenic hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] were evaluated to test diagenetic alterations in the carbonate ($CO_3$)

analysis (Wright and Schwarcz 1999; Bayarı et al. 2020; Leskovar et al. 2020). Diagenetic alterations in carbonate (V2 and V3) and phosphate (V2 and V3) spectral domains are indicative of postmortem changes in the apatite crystal structure of the sample (Weiner and Bar-Yosef 1990; Hedges et al. 1995). Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectra were generated for all pretreated bone carbonate samples at the UCSB Materials Reach Laboratory using the Thermo Scientific Nicolet is10 FTIR spectrometer with a Smart Diamond ATR accessory. Each spectrum was compiled from 32 scans of ~3-5 mg of pretreated powdered sample for the mid-range IR domain (4000 to 525 $cm^{-1}$). ATR-FTIR spectra of all samples are displayed in supplemental Figure S15 and quality control parameters are reported in Table S1. The degree of apatite recrystallization was quantified using the crystallinity index ($CI_{IRSF}$; Turner-Walker et al. 2002) by calculating the phosphate V2 domain peaks at 603 and 565 $cm^{-1}$ divided by the low point at ~595 $cm^{-1}$. All CI values were consistent with well-preserved biogenic signals (2.5 – 3.4). The $V_3$ carbonate band peak at 1415 $cm^{-1}$ (type B carbonate peak) was used to obtain the relative amount of A (API) and B-type (BPI) carbonates, the relative proportion of type B carbonates to type A carbonates and to evaluate the carbonate to phosphate (C/P) ratio (Thompson et al. 2009; Dal Sasso et al. 2018). The amide I to $V_3$ phosphate peak ratio (AmI/P) was used to quantify bone collagen content in the bioapatites (Lebon et al. 2016). All ratios were consistent with compositional and structural changes in previous studies (Snoeck and Pellegrini 2015; Kennett et al. 2020; Leskovar et al. 2020).

**Table S1: Descriptive quality control statistics obtained from ATR-FTIR spectra.**

| Master No. | $CI_{IRSF}$ | C/P | C/C | API | BPI | AmI/P |
|---|---|---|---|---|---|---|
| I7969 | 3.0 | 0.33 | 1.96 | 0.44 | 0.58 | 0.26 |
| I7973 | 3.4 | 0.22 | 2.02 | 0.39 | 0.50 | 0.18 |
| I8118 | 3.1 | 0.30 | 1.95 | 0.41 | 0.62 | 0.19 |
| I8547 | 2.9 | 0.32 | 1.94 | 0.44 | 0.63 | 0.22 |
| I8548 | 3.0 | 0.34 | 1.95 | 0.45 | 0.61 | 0.25 |
| I10126 | 3.0 | 0.32 | 1.94 | 0.42 | 0.62 | 0.22 |
| I10758 | 3.0 | 0.32 | 1.93 | 0.40 | 0.61 | 0.21 |
| I11892 | 2.6 | 0.55 | 1.98 | 0.64 | 0.74 | 0.50 |
| I13189 | 3.1 | 0.29 | 1.96 | 0.42 | 0.58 | 0.21 |
| I13195 | 3.4 | 0.27 | 1.94 | 0.39 | 0.62 | 0.16 |
| I13198 | 2.5 | 0.60 | 1.97 | 0.71 | 0.82 | 0.54 |
| I13208 | 3.1 | 0.30 | 1.97 | 0.43 | 0.58 | 0.22 |
| I13472 | 2.9 | 0.35 | 1.94 | 0.44 | 0.64 | 0.25 |
| I15109 | 3.1 | 0.30 | 1.96 | 0.41 | 0.58 | 0.21 |
| I15595 | 3.1 | 0.31 | 1.96 | 0.43 | 0.58 | 0.23 |
| I15601 | 3.0 | 0.37 | 1.96 | 0.46 | 0.61 | 0.27 |
| I15676 | 3.3 | 0.23 | 2.00 | 0.38 | 0.50 | 0.18 |
| I15968 | 3.1 | 0.30 | 1.93 | 0.45 | 0.66 | 0.20 |
| I15978 | 3.1 | 0.29 | 1.95 | 0.43 | 0.63 | 0.20 |
| I17889 | 2.6 | 0.55 | 1.94 | 0.61 | 0.79 | 0.45 |
| I17901 | 2.7 | 0.45 | 1.96 | 0.53 | 0.69 | 0.35 |
| I14876 | 3.4 | 0.24 | 1.92 | 0.34 | 0.51 | 0.17 |
| I14920 | 3.0 | 0.27 | 1.90 | 0.38 | 0.58 | 0.19 |
| I14922 | 2.7 | 0.51 | 1.89 | 0.50 | 0.80 | 0.33 |

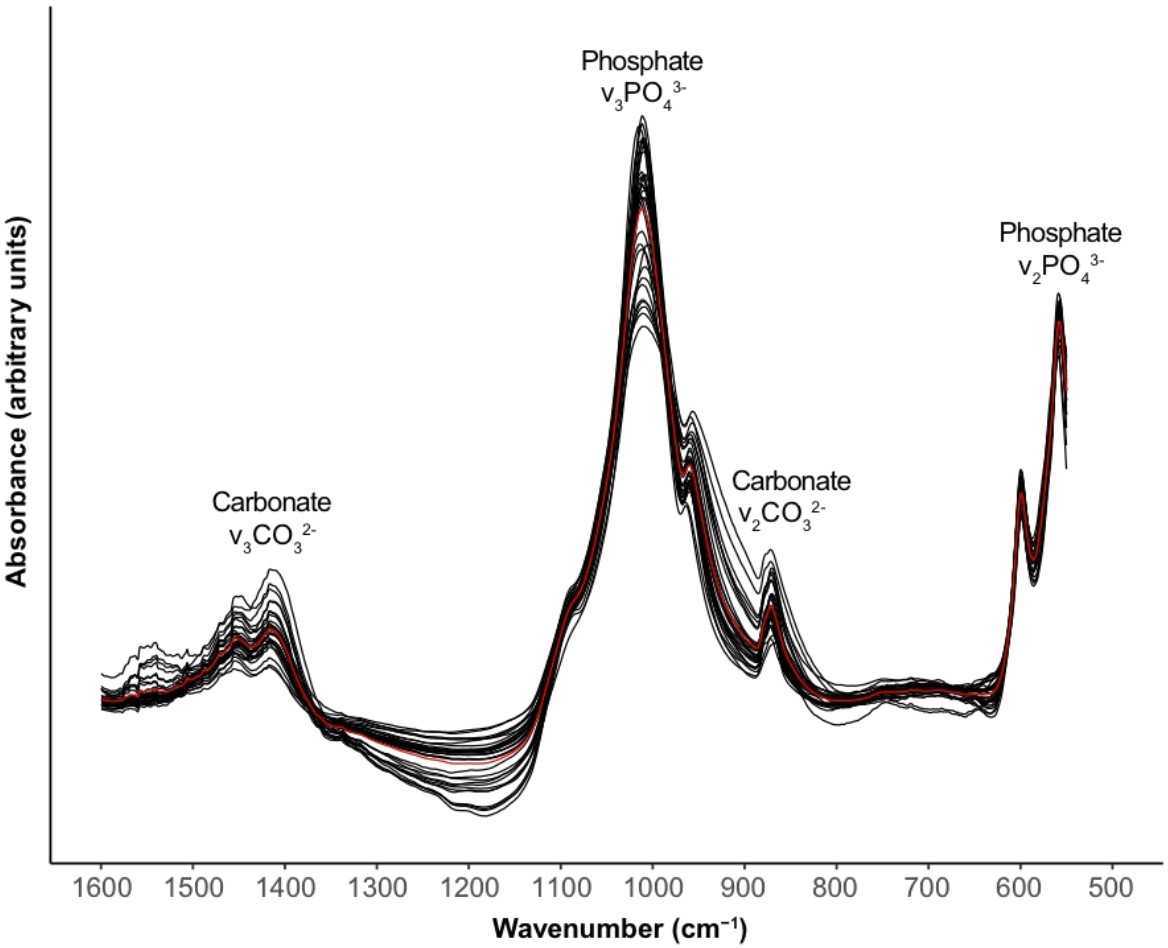



**Figure S15: Normalized average ATR-FTIR spectra of ancient Caribbean pretreated bone apatite samples, expanded on the 550-1600 $cm^{-1}$.** Carbonate and phosphate band positions used to calculate the crystallinity index, C/P ratio, and Amide I/P ratio are displayed above the main peaks. The red line corresponds to the average wavenumber of all samples.

*Radiocarbon dating and calibration*

We directly radiocarbon dated 41 individuals with ancient DNA data at the Pennsylvania State University. Four additional individuals (DSH) were dated at the Center for Isotopic Research on Cultural and Environmental Heritage (CIRCE). Bone collagen or XAD-purified amino acids from bone tissues were directly dated at the Pennsylvania State University (PSU) AMS radiocarbon dating facility. Samples (Ultrafiltration, ~2.1 mg; XAD, ~3.5 mg) were combusted for 3 hours at 900°C in vacuum-sealed quartz tubes with CuO and Ag wires. Sample $CO_2$ was reduced to graphite at 550°C using $H_2$ and an Fe catalyst, with reaction water drawn off with $Mg(ClO_4)_2$ (Kennett et al. 2017). Graphite samples were pressed into targets in Al targets and loaded on a cathode wheel, and $^{14}C$ measurements were made on a modified National Electronics Corporation (NEC) compact spectrometer with a 0.5-MV accelerator (NEC 1.5SDH-1). The $^{14}C$ ages were corrected for mass-dependent fractionation, with $\delta^{13}C$ values measured on the AMS (Stuvier and Polach 1977) and compared with samples of Pleistocene whale bone (backgrounds, >48,000 $^{14}C$ B.P.), late Holocene bison bone (~1850 $^{14}C$ B.P.), late 1800s

C.E. cow bone, and OX-2 oxalic acid standards for normalization. CIRCE radiocarbon dates were processed following a modified Longin method (Longin 1971) and are detailed in (Passariello et al. 2012). After purification, combustion and zinc reduction for graphite target production was applied (Marzaioli et al. 2008) and measurements by 3MV NEC AMS system were taken according to Terrasi et al. (2008).

All calibrated $^{14}C$ ages were computed using OxCal v4.4 (Ramsey 2009) using the IntCal20 (Reimer et al. 2020). Overall our stable isotope analysis detected minimal consumption of marine resources and calibration with the marine calibration curve (Marine20; Heaton et al. 2020), or a mixture of the two, is not warranted. We cannot rule out the consumption of reef fish in a small number of individuals from Curaçao (I13472 (PSUAMS-7355), I11892 (PSUAMS-7387)) and The Bahamas (I14922 (PSUAMS-7371)) due to the overlapping carbon isotopic values with $C_4$ and CAM plants (Keegan and DeNiro 1988; Krigbaum et al. 2013; Laffoon et al. 2016; Chinique de Armas et al. 2017; Pestle and Laffoon 2018; see Figures S13 and S14).

## SI4- Co-analysis of newly-reported data with the data reported in Nägele et al. 2020

We merged the genome-wide data for 93 ancient Caribbean individuals first reported in Nägele et al. 2020 with our 1240K capture dataset for co-analysis. Consistent with their processing of the data, we used a hybrid strategy of trimming two base pairs from each end of the read for UDG-half libraries and 10 base pairs from each end of the read for UDG-minus libraries (n=4 from Anse à la Gourde). We excluded four individuals from their dataset in our co-analysis:

- PCV002: this individual is a lower coverage duplicate of another individual (I14878) in our newly-reported dataset;
- CAO004: this individual is a possible duplicate of CAO032 and shows evidence of contamination;
- CAO018: this individual is a lower coverage duplicate or first-degree relative of CAO015;
- PCA010: this individual is low coverage (~13K SNPs covered) and shows evidence of contamination.

All duplicates were determined following the method in Olalde et al. (2019).

We determined that two individuals reported in Nägele et al. (PDI008 and PDI010) were duplicates of PI-420a and PI-51 (respectively), initially reported in Nieves-Colón et al. (2020). We include PID008 and PDI010 in our co-analysis, and also include the Nieves-Colón et al. whole-genome sequencing data as well as the Nägele et al. capture data for these individuals on our PCA and ADMIXTURE plots because of the different data types.

**No evidence of admixture in PDI009**

Nägele et al. report that the individual PDI009 from Paso del Indio in Puerto Rico was admixed, harboring both Ceramic- and Archaic-related ancestry. They base this conclusion on Fig. 2C of their study, where Paso del Indio is a visual outlier among Ceramic Age sites. They further support this conclusion by fitting a 2-way *qpAdm* model that estimates this individual to have 13.8 ± 7.7% Archaic-related ancestry at p=0.33 (Table S7 of their study). No other individual in their dataset is assessed as having evidence of a significant mixture of Ceramic- and Archaic-related ancestry.

Using our own curated dataset and basic data analysis pipeline, we did not initially identify this individual as admixed, so we carried out a focused re-examination of the evidence for admixture in PDI009. First, while Fig. 2C from Nägele et al. visually suggests that Paso del Indio is an outlier among Ceramic-associated sites, the error bars associated with Paso del Indio in this plot are large and the outlier status is not a specific observation about PDI009 but rather about the pool of all seven individuals from the site. Second, there is no formal *qpWave* test or $f_4$-statistic reported in their work showing that this individual is inhomogeneous with other Ceramic Age individuals - e.g., a statistic such as $f_4$(Mbuti, ArchaicAssociatedIndividualOrGroup; PDI009, OtherCeramicAssociatedIndividualOrGroup). Without a statistic like this and a correction for multiple-hypothesis testing, there is no statistical support for the claim of admixture. Third, we re-examined Table S7 of Nägele et al., which reports *qpAdm* modeling of all individuals as a mixture of

Ceramic- and Archaic-related ancestry, forcing two-way admixture without testing whether a one-way clade model works. Here, the deviation report for PDI009 is only 1.8-standard errors from zero, which does not reach significance. We took Table S7 of Nägele et al. and computed a Z-score for the difference of the Ceramic estimate from the group mean (0 for Archaic-associated, and 1 for Ceramic-associated), and focused on the Z-score of lower absolute magnitude. There are 10 outliers at |Z|>2, none of which are significant outliers after Bonferroni correction for 94 hypotheses; the list does not include PID009 because the signal is only Z=-1.79, which is p=0.037 without Bonferroni correction and p=0.97 with this correction, placing this individual 14th on the list of 94 individuals in terms of significance of deviation from 0 or 1 (Table S2).

**Table S2: Revisiting the statistics reported in Table S7 of Nägele et al. shows that the analyses reported in that study provide no significant evidence of Archaic-/Ceramic-related admixture in PDI009 or indeed any other individual in that study.** This table uses the values obtained through *qpAdm* analysis reported in Table S7 of Nägele et al. and computes Z-scores and p-values for deviation from either 100% Archaic or 100% Ceramic-associated ancestry based on the numbers from that table. The table is ordered by significance of deviation from the expectation of 100% ancestry from one of these groups. P-values reported in Nägele et al. below p=0.05 are in red, and PDI009 is highlighted in gray.

| Test individual | Primary genetic association | p-value for qpAdm fit (quoting from Table S7 of Nägele et al. 2020) | Estimate of Ceramic-associated ancestry proportion | Standard error of Ceramic-associated ancestry proportion | Z-score for deviation from the closest genetic grouping (Archaic or Ceramic associated) | p-value for deviation from expectation without any multiple hypothesis testing correction | p-value for deviation from expectation, Bonferroni corrected for 94 hypotheses tested |
|---|---|---|---|---|---|---|---|
| CAO017 | Archaic | 0.346 | -0.089 | 0.03 | -2.97 | 0.0015 | 0.13 |
| CIP011 | Archaic | 0.596 | -0.142 | 0.054 | -2.63 | 0.0043 | 0.33 |
| CIP | Archaic | 0.107 | -0.059 | 0.024 | -2.46 | 0.0070 | 0.48 |
| PDM010 | Archaic | 0.857 | 0.086 | 0.036 | 2.39 | 0.0084 | 0.55 |
| PDI012013 | Ceramic | 0.467 | 1.083 | 0.036 | 2.31 | 0.011 | 0.63 |
| LAV007 | Ceramic | 0.531 | 0.912 | 0.04 | -2.20 | 0.014 | 0.73 |
| LOI001 | Ceramic | 0.000000013 | 0.828 | 0.081 | -2.12 | 0.017 | 0.80 |
| PDM009 | Archaic | 0.041 | -0.095 | 0.045 | -2.11 | 0.017 | 0.81 |
| PCA | Ceramic | 0.128 | 0.931 | 0.033 | -2.09 | 0.018 | 0.82 |
| PDM004 | Archaic | 0.839 | 0.063 | 0.031 | 2.03 | 0.021 | 0.86 |
| CIP001 | Archaic | 0.082 | -0.143 | 0.073 | -1.96 | 0.025 | 0.91 |
| CIP007012 | Archaic | 0.265 | 0.11 | 0.06 | 1.83 | 0.033 | 0.96 |
| CAO006 | Archaic | 0.782 | -0.124 | 0.069 | -1.80 | 0.036 | 0.97 |
| PDI009 | Ceramic | 0.335 | 0.862 | 0.077 | -1.79 | 0.037 | 0.97 |
| PDM | Archaic | 0.749 | 0.034 | 0.019 | 1.79 | 0.037 | 0.97 |
| CAO014 | Archaic | 0.708 | -0.051 | 0.029 | -1.76 | 0.039 | 0.98 |
| CUC002 | Archaic | 0.019 | -0.056 | 0.032 | -1.75 | 0.040 | 0.98 |
| CUC004 | Archaic | 0.496 | 0.117 | 0.067 | 1.75 | 0.040 | 0.98 |
| PDM003 | Archaic | 0.554 | 0.123 | 0.073 | 1.68 | 0.046 | 0.99 |
| CAO024 | Archaic | 0.150 | -0.047 | 0.028 | -1.68 | 0.047 | 0.99 |
| PDI | Ceramic | 0.391 | 1.033 | 0.02 | 1.65 | 0.049 | 0.99 |
| CAO019 | Archaic | 0.196 | -0.064 | 0.04 | -1.60 | 0.055 | 0.99 |
| CIP008 | Archaic | 0.765 | -0.099 | 0.062 | -1.60 | 0.055 | 1 |
| CAO | Archaic | 0.617 | -0.017 | 0.011 | -1.55 | 0.061 | 1 |
| PDM001 | Archaic | 0.275 | -0.067 | 0.044 | -1.52 | 0.064 | 1 |
| PDM006 | Archaic | 0.684 | 0.056 | 0.04 | 1.40 | 0.081 | 1 |
| CAO029 | Archaic | 0.835 | -0.039 | 0.029 | -1.34 | 0.089 | 1 |
| PCA006 | Ceramic | 0.578 | 0.916 | 0.064 | -1.31 | 0.095 | 1 |
| PCA002 | Ceramic | 0.245 | 0.95 | 0.039 | -1.28 | 0.10 | 1 |
| CAO002008 | Archaic | 0.097 | 0.04 | 0.032 | 1.25 | 0.11 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CAO030 | Archaic | 0.052 | -0.036 | 0.03 | -1.20 | 0.12 | 1 |
| CUC | Archaic | 0.027 | -0.026 | 0.022 | -1.18 | 0.12 | 1 |
| CAO007 | Archaic | 0.785 | 0.037 | 0.032 | 1.16 | 0.12 | 1 |
| CAO011 | Archaic | 0.473 | 0.031 | 0.027 | 1.15 | 0.13 | 1 |
| ALG002 | Ceramic | 0.201 | 1.062 | 0.056 | 1.11 | 0.13 | 1 |
| PCA001 | Ceramic | 0.119 | 0.916 | 0.084 | -1.00 | 0.16 | 1 |
| PCV004 | Ceramic | 0.889 | 1.069 | 0.071 | 0.97 | 0.17 | 1 |
| LAV006 | Ceramic | 0.496 | 0.968 | 0.034 | -0.94 | 0.17 | 1 |
| CAO027 | Archaic | 0.238 | -0.028 | 0.03 | -0.93 | 0.18 | 1 |
| PDI008 | Ceramic | 0.861 | 1.034 | 0.037 | 0.92 | 0.18 | 1 |
| ALG003 | Ceramic | 0.114 | 1.036 | 0.04 | 0.90 | 0.18 | 1 |
| CAO022026 | Archaic | 0.144 | -0.026 | 0.029 | -0.90 | 0.18 | 1 |
| CIP003 | Archaic | 0.053 | -0.037 | 0.043 | -0.86 | 0.19 | 1 |
| CDE005 | Ceramic | 0.933 | 0.972 | 0.034 | -0.82 | 0.21 | 1 |
| PCV003 | Ceramic | 0.176 | 1.039 | 0.048 | 0.81 | 0.21 | 1 |
| ELM001 | Ceramic | 0.917 | 0.972 | 0.035 | -0.80 | 0.21 | 1 |
| CDE002 | Ceramic | 0.171 | 0.973 | 0.034 | -0.79 | 0.21 | 1 |
| TIB002 | Ceramic | 0.265 | 1.037 | 0.049 | 0.76 | 0.23 | 1 |
| GUY001 | Archaic | 0.257 | 0.023 | 0.031 | 0.74 | 0.23 | 1 |
| LAV | Ceramic | 0.898 | 0.989 | 0.015 | -0.73 | 0.23 | 1 |
| CIP010 | Archaic | 0.556 | 0.047 | 0.067 | 0.70 | 0.24 | 1 |
| LAV008 | Ceramic | 0.287 | 1.041 | 0.061 | 0.67 | 0.25 | 1 |
| LAV012 | Ceramic | 0.788 | 0.918 | 0.123 | -0.67 | 0.25 | 1 |
| ALG001 | Ceramic | 0.580 | 0.967 | 0.05 | -0.66 | 0.25 | 1 |
| LAV010 | Ceramic | 0.922 | 1.027 | 0.041 | 0.66 | 0.26 | 1 |
| ALG004 | Ceramic | 0.257 | 1.037 | 0.059 | 0.63 | 0.27 | 1 |
| CAO028 | Archaic | 0.210 | 0.017 | 0.028 | 0.61 | 0.27 | 1 |
| CAO020 | Archaic | 0.098 | 0.017 | 0.029 | 0.59 | 0.28 | 1 |
| CAO015 | Archaic | 0.137 | 0.017 | 0.03 | 0.57 | 0.29 | 1 |
| TIB | Ceramic | 0.531 | 1.023 | 0.041 | 0.56 | 0.29 | 1 |
| PDM008 | Archaic | 0.212 | 0.022 | 0.041 | 0.54 | 0.30 | 1 |
| PCV | Ceramic | 0.242 | 1.015 | 0.028 | 0.54 | 0.30 | 1 |
| LAV002 | Ceramic | 0.939 | 0.984 | 0.032 | -0.50 | 0.31 | 1 |
| LAV001 | Ceramic | 0.183 | 1.017 | 0.035 | 0.49 | 0.31 | 1 |
| CAO021 | Archaic | 0.484 | 0.014 | 0.03 | 0.47 | 0.32 | 1 |
| ALG | Ceramic | 0.025 | 1.013 | 0.028 | 0.46 | 0.32 | 1 |
| CUC003 | Archaic | 0.730 | -0.013 | 0.03 | -0.43 | 0.33 | 1 |
| CAO009013 | Archaic | 0.406 | -0.012 | 0.028 | -0.43 | 0.33 | 1 |
| CAO023025 | Archaic | 0.438 | -0.012 | 0.028 | -0.43 | 0.33 | 1 |
| CAO031 | Archaic | 0.698 | -0.009 | 0.031 | -0.29 | 0.39 | 1 |
| CIP002 | Archaic | 0.149 | -0.004 | 0.092 | -0.04 | 0.48 | 1 |
| CAO010 | Archaic | 0.128 | 0 | 0.038 | 0.00 | 0.50 | 1 |
| PDM002 | Archaic | 0.428 | 0.001 | 0.045 | 0.02 | 0.49 | 1 |
| CAO032 | Archaic | 0.062 | 0.002 | 0.029 | 0.07 | 0.47 | 1 |
| GUY003 | Archaic | 0.719 | 0.004 | 0.058 | 0.07 | 0.47 | 1 |
| CAO012 | Archaic | 0.185 | 0.006 | 0.03 | 0.20 | 0.42 | 1 |
| GUY002 | Archaic | 0.680 | 0.009 | 0.034 | 0.26 | 0.40 | 1 |
| GUY | Archaic | 0.594 | 0.008 | 0.023 | 0.35 | 0.36 | 1 |
| CAO016 | Archaic | 0.356 | 0.014 | 0.033 | 0.42 | 0.34 | 1 |
| CDE | Ceramic | 0.804 | 0.993 | 0.018 | -0.39 | 0.35 | 1 |
| LAV004 | Ceramic | 0.407 | 0.986 | 0.038 | -0.37 | 0.36 | 1 |
| CDE001 | Ceramic | 0.272 | 0.988 | 0.036 | -0.33 | 0.37 | 1 |
| LAV011 | Ceramic | 0.173 | 0.993 | 0.078 | -0.09 | 0.46 | 1 |
| LAV005 | Ceramic | 0.250 | 0.997 | 0.037 | -0.08 | 0.47 | 1 |
| TIB001 | Ceramic | 0.370 | 0.997 | 0.078 | -0.04 | 0.48 | 1 |
| PDI011 | Ceramic | 0.251 | 1.004 | 0.038 | 0.11 | 0.46 | 1 |
| CDE004 | Ceramic | 0.020 | 1.006 | 0.037 | 0.16 | 0.44 | 1 |
| LAV003 | Ceramic | 0.658 | 1.007 | 0.034 | 0.21 | 0.42 | 1 |
| PCV001 | Ceramic | 0.819 | 1.014 | 0.049 | 0.29 | 0.39 | 1 |
| PDI006 | Ceramic | 0.305 | 1.02 | 0.062 | 0.32 | 0.37 | 1 |
| PDI010 | Ceramic | 0.162 | 1.012 | 0.037 | 0.32 | 0.37 | 1 |
| PCV002 | Ceramic | 0.109 | 1.015 | 0.04 | 0.37 | 0.35 | 1 |
| CDE003 | Ceramic | 0.421 | 1.014 | 0.035 | 0.40 | 0.34 | 1 |
| LAV009 | Ceramic | 0.851 | 1.064 | 0.146 | 0.44 | 0.33 | 1 |

We also attempted to replicate the test for admixture in PDI009 following the *qpAdm* methodology reported in Nägele et al., specifically using the Archaic- and Ceramic-associated individuals covered

by over 50,000 SNPs as sources; however, we used our processing of the data from that study. As such, we excluded Archaic-associated individuals CUC001 and CIP002 and Ceramic-associated individuals LAV009 and LAV012 (in addition to the individuals listed above that we exclude from all analyses). We include data from PCV002 for this analysis only, instead of using the data from the same individual that we newly report here as I14878 (in analyses using the combined dataset, we use the higher coverage data for I14878 over data for PCV002 generated by Nägele et al.). In addition, we do not exclude CAO019 which is reported in Nägele et al. as having ~40K SNPs (their Table S1), but which we determined to have ~360K SNPs. We used the same outgroup set of Mbuti.DG, Onge.DG, Papuan.DG, Han.DG, Russia_MA1_HG.SG, USA_Ancient_Beringian.SG, USA_Anzick.SG, Mixe.DG, Mexico_Zapotec.DG, Belize_MayahakCabPek_9300, Karitiana.DG, Piapoco.DG, CIP009. We find that both a clade test using Ceramic-associated individuals only, as well as a 2-way model considering both Ceramic-related and Archaic-related ancestry contributions, fit the data (p=0.86 and 0.84, respectively), with our 2-way model providing an ancestry estimate of 10.1±9.7% Archaic-related ancestry. This large standard error suggests that while this individual could harbor Archaic-related ancestry as suggested in Nägele et al., it is also entirely consistent with having none at all (95% confidence interval computed as [max(0,10.1-1.96*9.7),10.1+1.96*9.7] truncated at zero of 0-29% in our analysis, and 0-28% in the analysis reported by Nägele et al.).

We leveraged additional statistical power from the newly-reported data in this work to test if PDI009 was inhomogeneous with the other individuals from the Paso del Indio site. Using the statistic $f_4$(Mbuti, *GreaterAntilles_Archaic; PDI009, *PasoDelIndio_Ceramic_notPDI009), where *GreaterAntilles_Archaic is all Archaic-associated individuals from Cuba plus I10126 (all sub-clades used for $f_4$-statistics are described in detail in Supplementary Information section 8) and *PasodelIndio_Ceramic is a pool of the six remaining individuals from Paso del Indio who were not assessed by Nägele et al. as having Archaic-related ancestry, we find a non-significant result (Z=-0.44), indicating no excess allele-sharing between PDI009 and Archaic-associated individuals relative to the other individuals from Paso del Indio (Table S3).

**Table S3: $f_4$-statistic results for PDI009.**

| Pop X | Pop Y | Pop W | Pop Z | f4 | SE | Z | SNPs |
|---|---|---|---|---|---|---|---|
| Mbuti | *GreaterAntilles_Archaic | PDI009 | *PasoDelIndio_Ceramic | -0.000258 | 0.000584 | -0.442 | 94957 |

Finally, after determining that all individuals from the site of Paso del Indio were part of the *Caribbean_Ceramic clade and specifically part of the *EasternGreaterAntilles_Ceramic sub-clade (described in Supplementary Information section 8), we assessed if PDI009 had more Archaic-related ancestry than the pool of all other individuals in *EasternGreaterAntilles_Ceramic, and obtained no results supporting this (Z=0.571; see Supplementary Data 8).

Overall, without the ability to exclude a working 1-way *qpAdm* model or statistical evidence from a significant $f_4$-statistic, there is poor support for the finding reported in Nägele et al. that PDI009 has

significantly more Archaic-related ancestry than the other individuals from Paso del Indio or other Ceramic-associated individuals either in the analyses we report or those reported in the Nägele et al. study. We therefore PDI009 included in our *EasternGreaterAntilles_Ceramic sub-clade for all analyses. An alternative possible explanation for a signal that can be assessed as differing slightly from other individuals from Paso del Indio is contamination. We note that this sample is reported as having a mean mtDNA contamination estimate of 0.06 (range 0.02-0.1), substantially higher than most individuals reported in Nägele et al. No ANGSD contamination estimate was provided for this individual.

## SI5 - Principal Component Analysis (PCA)

In Figure S16, we show the same PCA as in Extended Data Fig. 1b, but here we separate the projected ancient individuals by site. For The Bahamas, we cluster all sites from each island together.

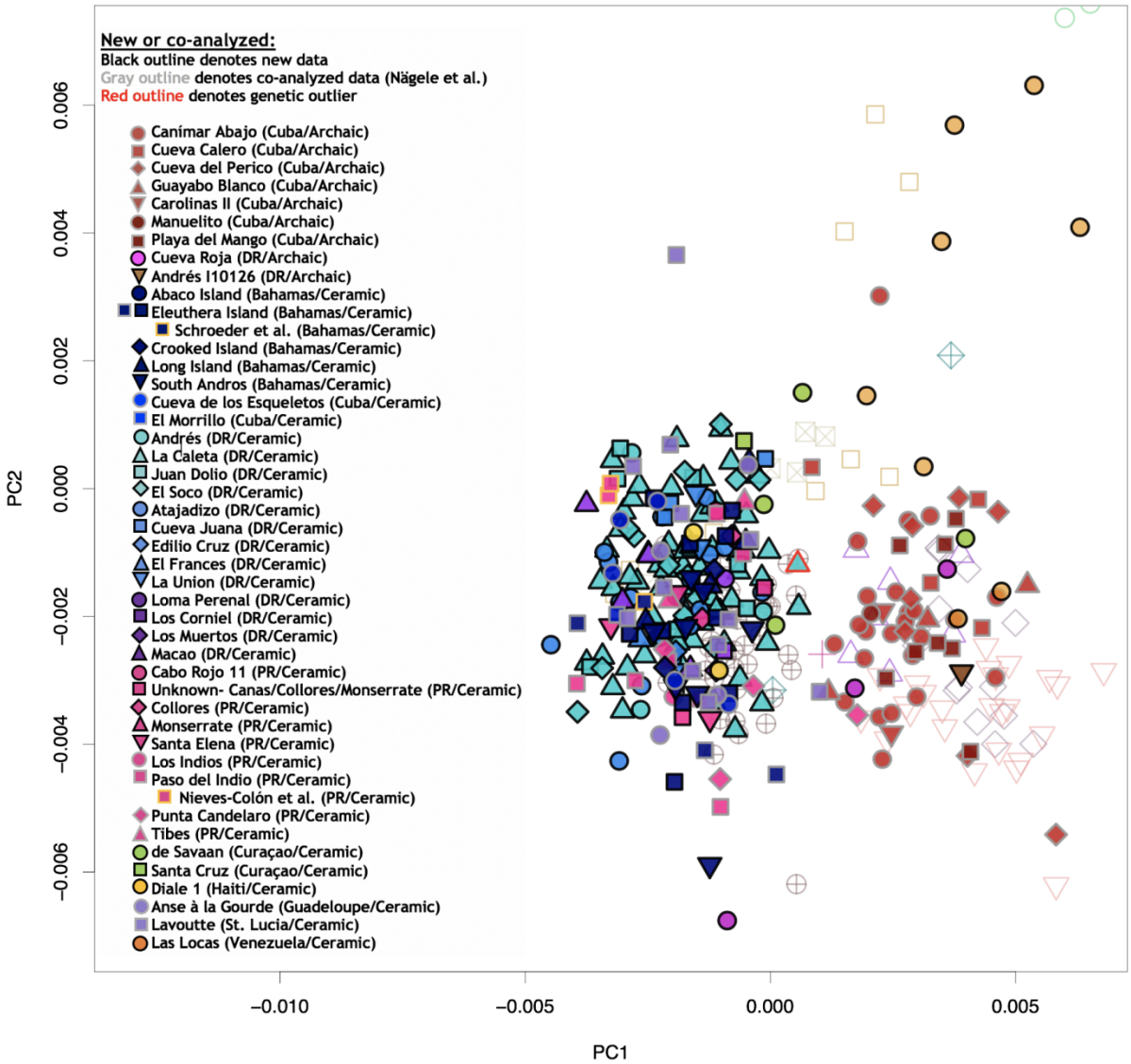



**Figure S16: PCA with 174 new ancient individuals and 92 previously-published individuals (89 from Nägele et al. (2020) co-analyzed in this work) separated by site**. Information in parentheses includes the present-day country where the site is located (DR, Dominican Republic; PR, Puerto Rico) and predominant associated technology. Modern populations shown are detailed in the legend of Extended Data Fig. 1b.

In order to visualize the Ceramic and Archaic-associated clusters in greater detail, the PCA presented as Extended Data Fig. 1b is zoomed; as such, some of the present-day South American individuals are not included in this view. In Figure S17, we provide a non-zoomed version of the same PCA to show the extent of genetic diversity of the modern American populations and the clustering of the ancient Caribbean and Venezuelan individuals amidst this diversity.

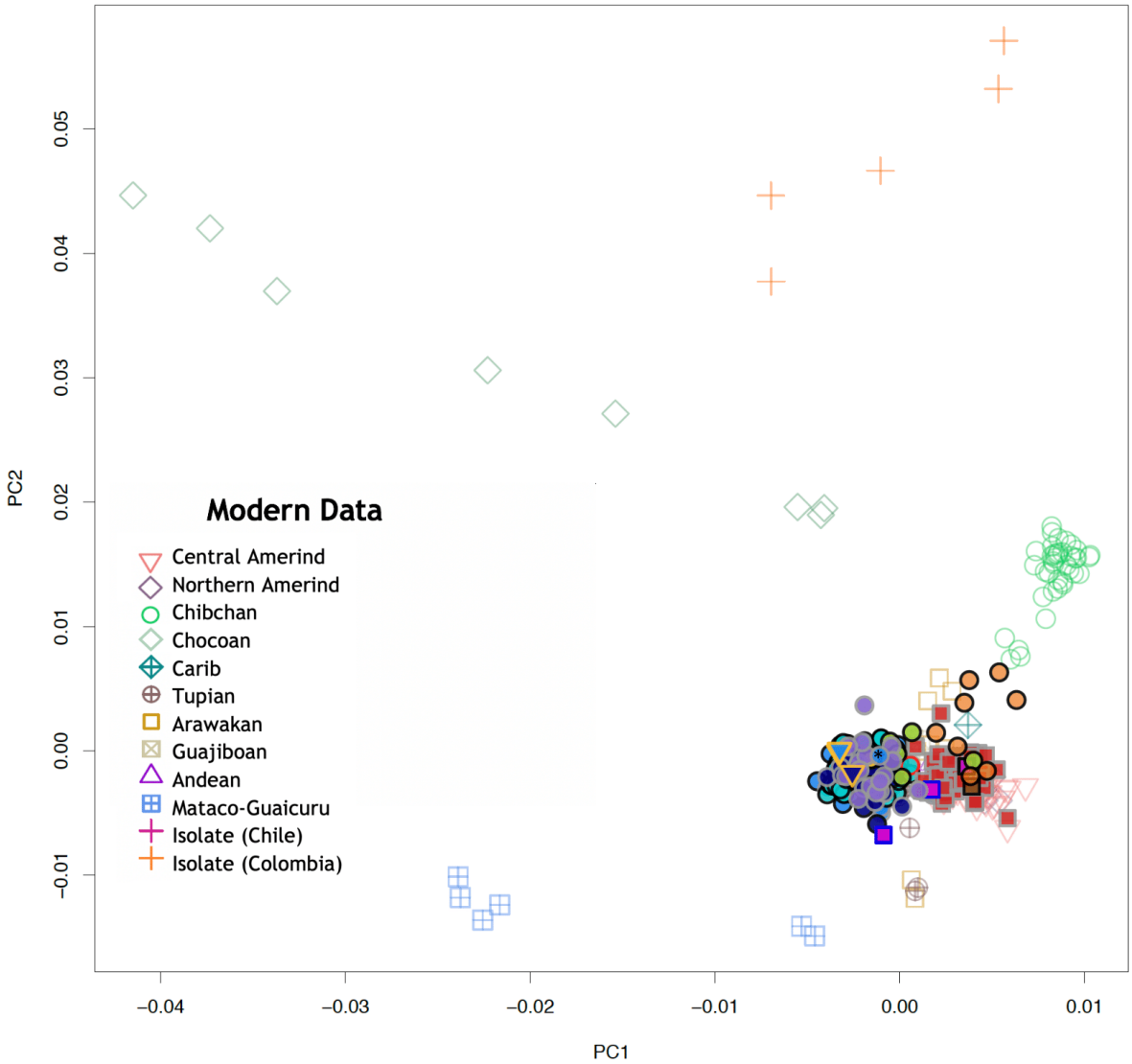



**Figure S17: Non-zoomed version of PCA in Fig. 2a.** Ancient populations shown are detailed in the legend of Extended Data Fig. 1b.

To confirm that our results were not substantially affected by errors induced by ancient DNA damage, we performed PCA using a dataset that excluded all CpG sites (Figure S18). We evaluate this result as similar to the zoomed PCA created when CpG sites are included (Extended Data Fig. 1b in the main manuscript), suggesting that the projected location of our ancient individuals is not affected by damage-induced artifacts.


**Figure S18: PCA (zoomed as in Extended Data Fig. 1b of main manuscript) excluding CpG sites.**

Finally, to better represent the signals of genetic differentiation among the ancient individuals, we computed axes using only high coverage (>500K SNPs), non-related, non-outlier ancient individuals from *Cuba_Archaic, *Venezuela_Ceramic, *EasternGreaterAntilles_Ceramic, *BahamasCuba_Ceramic, and *SECoastDR_Ceramic and projected all other ancient and modern individuals onto these axes (Figure S19). Individuals used for computation of axes are listed in Supplementary Data 4. When these ancient individuals are used instead to calculate PCs, ancient Venezuelans and Archaic-associated individuals form distinct clusters and ancient individuals from Curaçao and Haiti shift in the direction of Venezuelans and Archaic-associated people respectively, consistent with ADMIXTURE analysis suggesting they harbor a minor proportion of those ancestries and *qpAdm* analysis confirming their admixture.

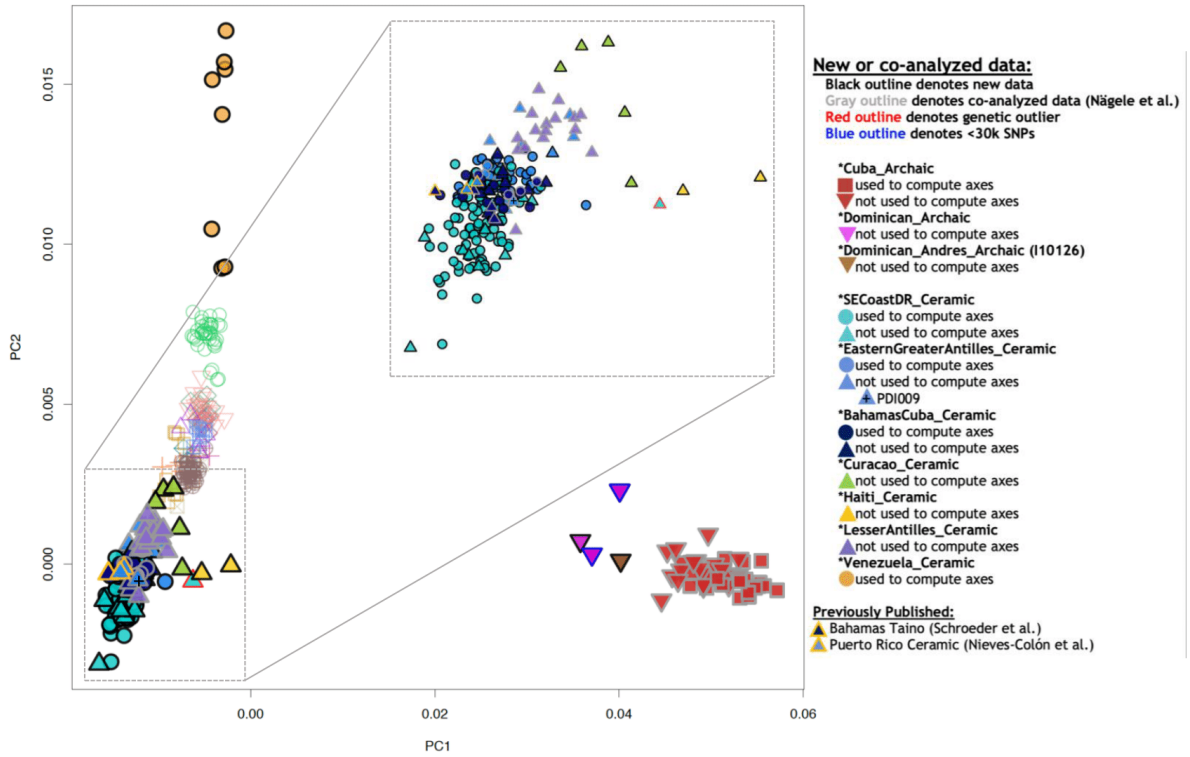



**Figure S19: PCA with axes computed using ancient non-related, non-outlier samples from *Cuba_Archaic, *Venezuela_Ceramic, *EasternGreaterAntilles_Ceramic, *BahamasCuba_Ceramic, and *SECoastDR_Ceramic with >500K SNPs.** Modern populations shown are detailed in the legend of Extended Data Fig. 1b.

## SI6 - Unsupervised population structure analysis

We performed unsupervised population structure analysis using ADMIXTURE (Alexander et al. 2009; Alexander and Lange 2011) with a reference panel of 286 modern individuals from populations across the world, three published ancient individuals from the Caribbean with genome-wide data (Schroeder et al. 2018; Nieves-Colón et al. 2020), and the 89 individuals from Nägele et al. (2020) that we co-analyze along with our 174 newly-reported individuals. We display K=6 in the main manuscript (Extended Data Fig. 1c), selected as best representing the observed ancestry components, and provide the following table of cross-validation errors for five replicates of K=2-10 (Table S4).

**Table S4: Cross-validation errors for five replicates of K=2-10.**

| | | Run 1 | Run 2 | Run 3 | Run 4 | Run 5 |
|---|---|---|---|---|---|---|
| | 2 | 0.67334 | 0.67331 | 0.67343 | 0.67331 | 0.67336 |
| | 3 | 0.67119 | 0.66733 | 0.66890 | 0.67104 | 0.67084 |
| | 4 | 0.66489 | 0.66506 | 0.66460 | 0.66480 | 0.66466 |
| | 5 | 0 0.65890 | 0.65884 | 0.65807 | 0.65877 | 0.65831 |
| K value | 6 | 0.66177 | 0.66154 | 0.65422 | 0.65427 | 0.65428 |
| | 7 | 0.65667 | 0.65688 | 0.65689 | 0.065668 | 0.65707 |
| | 8 | 0.65510 | 0.66261 | 0.65464 | 0.66249 | 0.65511 |
| | 9 | 0.65369 | 0.65401 | 0.66089 | 0.65437 | 0.65424 |
| | 10 | 0.66065 | 0.65983 | 0.65925 | 0.65795 | 0.66006 |

In Figure S20, we show results for each value of K between 2 and 10, providing a visualization of the full reference panel. The colors of modern American populations correspond to language groups as in Extended Data Fig. 2 of the main manuscript.

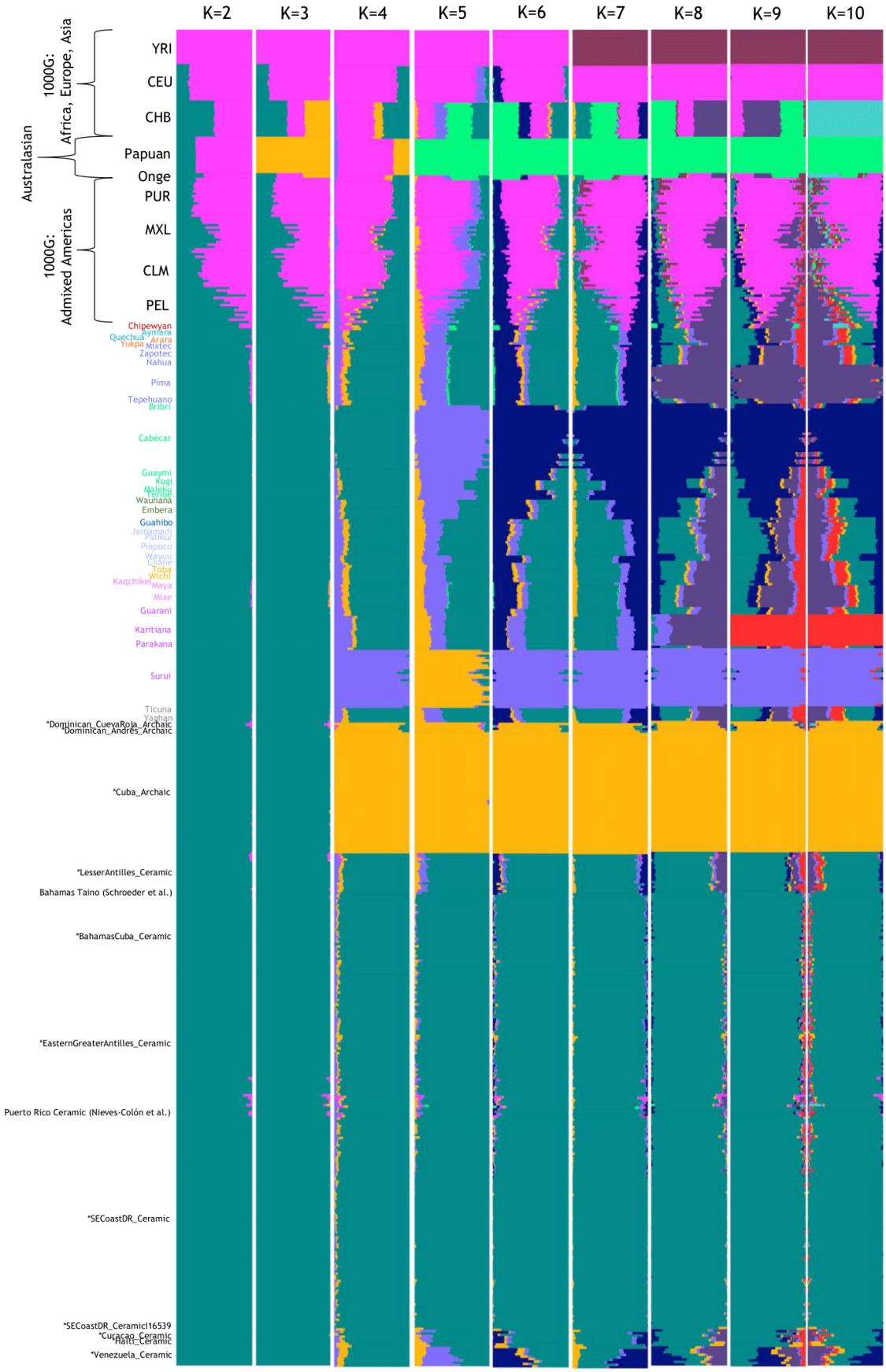



**Figure S20: Model-based ancestry analysis using ADMIXTURE for values of K between 2 and 10.**

## SI7 - Kinship and consanguinity analysis

### Kinship analysis

We looked for close kin relationships between all individuals in our study (including the co-analyzed data from Nägele et al. 2020) following the method of Olalde et al. (2019). In brief, this method compares the mean mismatch rate of all autosomal SNPs with at least one sequencing read between individuals (randomly selecting one read if coverage is greater than one at a particular SNP for a given individual). The mismatch rate is then used to estimate a relatedness coefficient *r*. This method is specifically applicable for estimating relatedness using haploid SNP data (often the data type used in ancient DNA analyses), providing accurate estimates of kinship up to third-degree relatives. We also used ngsrelate v2 (Hanghøj et al. 2019) to differentiate between relationships assessed by the Olalde et al. method as "second to third-degree," allowing us to differentiate these kin pairs as "second-degree" and "third-degree or greater." We find that the majority of individuals who had a kin relationship with another individual in our dataset (n=37) were from the site of La Caleta (Table S5). While this site has the highest number of individuals (n=63) of any site analyzed, a high proportion of individuals (~59%) share a kin relationship with another individual in this study. Other sites also exhibited high frequencies of related individuals (albeit with lower overall numbers of individuals): we find that the two studied individuals from Diale 1 in Haiti were related (second-degree relatives), as were all four individuals from the site of Macao in the Dominican Republic (one pair of second-degree relatives and two pairs of third-degree or higher relatives). It is possible that this could be explained by non-random sampling (for example, selecting two individuals buried near to each other), as well as relatively small population sizes at these locales.

We identify close relatives (second- and third-degree relatives) buried at different sites in the southern Dominican Republic. First-degree relatives I17906 and I17903 are buried at Atajadizo and are related to individual I15601 from La Caleta (I17906 is a second-degree relative of this individual while I17903 is a third-degree relative of this individual; Extended Data Fig. 6, Table S5). All three individuals were determined to be genetically male (Supplementary Data 2). While the numbers are too few to test for a statistically significant difference between behaviors of males and females, these results suggest that males engaged in intra-island mobility and connectivity.

We also identify kin pairs that were unreported in Nägele et al. (Table S5). Analyzing their data, we identify four relationships between five individuals out of 12 total individuals studied from the site of Lavoutte in St. Lucia. We find a single kin pair at the Archaic-associated sites of Canímar Abajo, Cueva Calero, and Playa del Mango (all in Cuba) as well as a single pair of relatives at the Ceramic-associated site of Cueva de los Esqueletos (also in Cuba).

**Table S5: Identified pairs of close relatives per site.** Number in parentheses is the number of individuals studied from the site. Gray cells identify inter-site relatives from La Caleta (I15601) and Atajadizo (I17903 and I17906).

| La Caleta (N=63) | | |
|---|---|---|
| I16687 | I15590 | 1st degree |
| I15050 | I16540 | 2nd degree |
| I16175 | I16181 | 2nd degree |
| I15082 | I16181 | 2nd degree |
| I15599 | I15964 | 2nd degree |
| I15598 | I15590 | 2nd degree |
| I16172 | I16174 | 2nd degree |
| I15675 | I15678 | 2nd degree |
| I15598 | I16687 | 2nd degree |
| I15682 | I16556 | 2nd degree |
| I15051 | I15604 | 2nd degree |
| I15964 | I15977 | 2nd degree |
| I16520 | I16539 | 2nd degree |
| I15597 | I15602 | 2nd degree |
| I15594 | I16180 | 2nd degree |
| I15596 | I15595 | 2nd degree |
| I15596 | I15678 | 2nd degree |
| I15596 | I15671 | 2nd degree |
| I15965 | I15977 | 3rd degree or higher |
| I15604 | I15970 | 3rd degree or higher |
| I15674 | I15973 | 3rd degree or higher |
| I16520 | I16687 | 3rd degree or higher |
| I16520 | I15590 | 3rd degree or higher |
| I15598 | I16520 | 3rd degree or higher |
| I15597 | I15601 | 3rd degree or higher |
| I15971 | I16173 | 3rd degree or higher |
| I15977 | I16556 | 3rd degree or higher |
| I15601 | I15602 | 3rd degree or higher |
| I15595 | I15678 | 3rd degree or higher |
| I15671 | I15678 | 3rd degree or higher |
| I15671 | I15965 | 3rd degree or higher |
| I15671 | I15977 | 3rd degree or higher |
| I15671 | I15964 | 3rd degree or higher |
| I15051 | I16540 | 3rd degree or higher |
| I16180 | I16540 | 3rd degree or higher |
| I15596 | I16171 | 3rd degree or higher |
| I15587 | I16171 | 3rd degree or higher |
| I16174 | I16687 | 3rd degree or higher |
| I15596 | I15965 | 3rd degree or higher |
| I15964 | I15965 | 3rd degree or higher |

| Macao (N=4) | | |
|---|---|---|
| I7973 | I7976 | 2nd degree |
| I7972 | I7974 | 3rd degree or higher |
| I7972 | I7976 | 3rd degree or higher |

| Atajadizo (N=18) | | |
|---|---|---|
| I17903 | I17906 | 1st degree |

| Diale 1 (N=2) | | |
|---|---|---|
| I12575 | I12576 | 2nd degree |

| El Soco (N=13) | | |
|---|---|---|
| I13189 | I13203 | 1st degree |

| Sanctuary Blue Hole (N=7) | | |
|---|---|---|
| I13558 | I14883 | 2nd degree |

| Inter-Site: La Caleta - Atajadizo | | |
|---|---|---|
| I15601 | I17906 | 2nd degree |
| I15601 | I17903 | 3rd degree or higher |

## From Nägele et al. (2020)

| Lavoutte (N=12) | | |
|---|---|---|
| LAV008 | LAV010 | 2nd degree |
| LAV007 | LAV010 | 2nd degree |
| LAV001 | LAV012 | 2nd degree |
| LAV007 | LAV008 | 3rd degree or higher |

| Playa del Mango (N=8) | | |
|---|---|---|
| PDM001 | PDM002 | 3rd degree or higher |

| Cueva de los Esqueletos (N=5) | | |
|---|---|---|
| CDE002 | CDE004 | 2nd degree |

| Cueva Calero (N=4) | | |
|---|---|---|
| CUC002 | CUC004 | 3rd degree or higher |

| Canímar Abajo (N=24) | | |
|---|---|---|
| CAO016 | CAO019 | 3rd degree or higher |

## Consanguinity analysis

We estimated the size and amount of runs of homozygosity (ROH) >4cM for 202 individuals from our dataset (including 151 newly-reported individuals and 51 first reported in Nägele et al. 2020) with sufficient coverage. ROH blocks longer than 20cM provide a clear indication of recent parental relatedness (up to five generations ago), as recombination quickly breaks up blocks back in time, making this signal independent of demographic processes occurring in the deeper past. In Figure S21 we visualize our results, grouped per archaeological site (data in Supplementary Data 12).

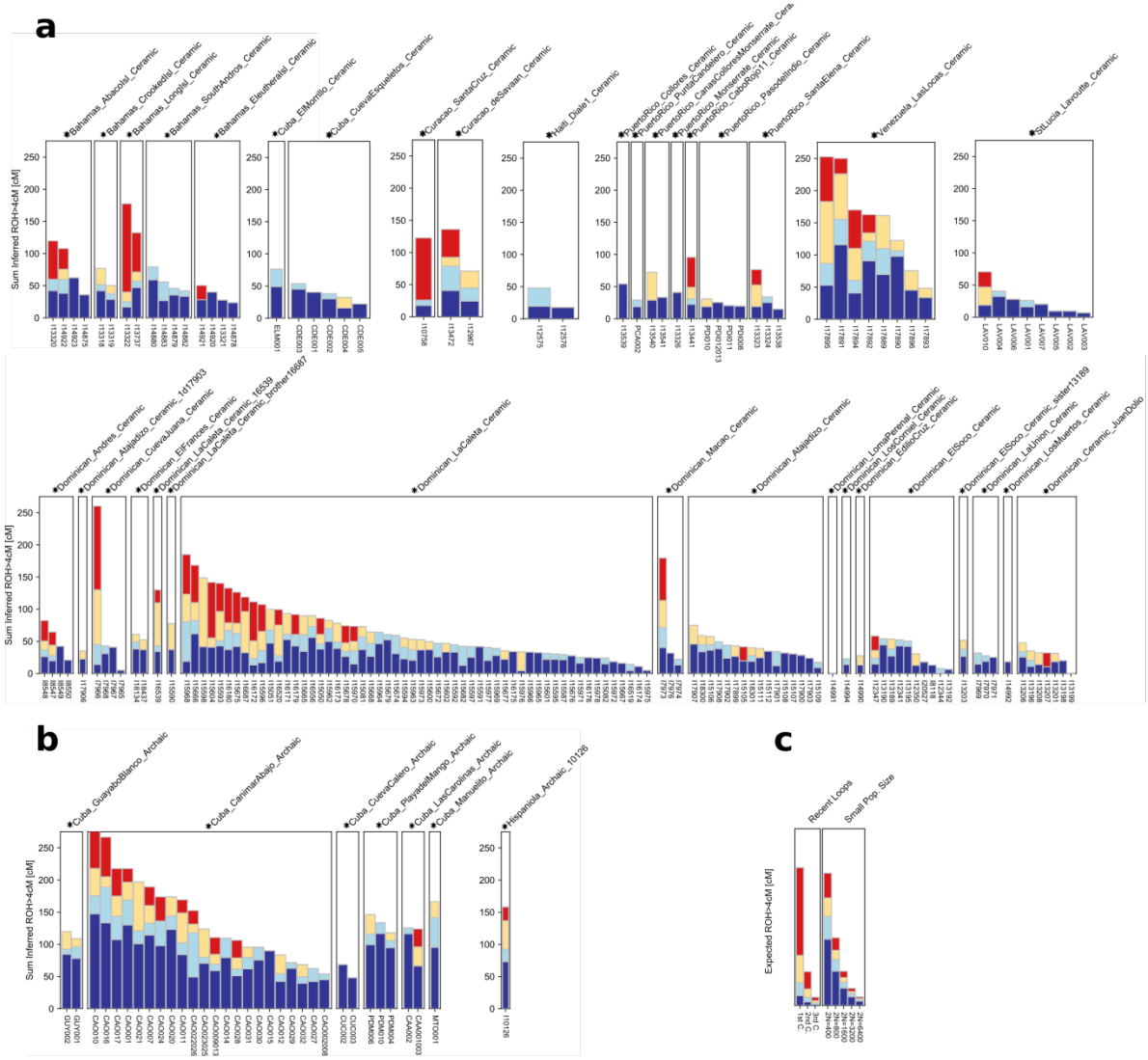



**Figure S21: ROH calls in ancient individuals.** We depict inferred ROH for 202 ancient individuals from the (**a**) Ceramic-associated (**b**) Archaic-associated clades, grouped here by site. Each bar represents one ancient individual, and we depict the total sums of ROH that fall into four length categories: 4-8cM (dark blue), 8-12cM (light blue), 12-20cM (yellow), and >20cM (red) for each individual. (**c**) The legend shows analytical expectations, calculated using the formulas reported in Ringbauer et al. (2020). Letters "b", "s", and "1d" at the end of labels represent "brother of", "sister of", and "1st-degree relative of," respectively.

We also depict the expected ROH for close cousin relationships and panmictic populations of small effective population size ($N_e$), calculated using the formulas reported in Ringbauer et al. (2020). We stress that for most populations, ancestry spreads geographically when tracing it backward in time (Ralph and Coop 2013), so effective population size values should be seen as comparison in an idealized case. Moreover, these are expectations, as there is considerable variation due to randomness of recombination per individual (Ringbauer et al. 2020); for instance, 50-500cM ROH in total are plausible values for offspring of first cousins.

We evaluated heterozygosity levels and found lower genetic diversity in the individuals from *GreaterAntilles_Archaic than in any of the tested Ceramic-related groups from the Greater Antilles, Lesser Antilles, The Bahamas, Curaçao, and Venezuela (Extended Data Fig. 5). These results agree with the median sums of ROH suggesting lower genetic diversity reflective of a smaller population pool at Archaic-related sites than at Ceramic-related sites. Ceramic-associated Caribbean groups were found to have an overall similar genetic diversity to that of some contemporary groups from continental South America, such as from the Peruvian Middle and Late Horizon periods (Nakatsuka et al. 2020).

**Maximum Likelihood Estimation of effective population size using Runs of Homozygosity**

We developed a method to fit the effective population size $N_e$ from observed ROH lengths, using the observed lengths of ROH blocks $\mathbf{l} = l_1, \ldots l_n$ for a given set of individuals $i_1 .. i_k$. The method is based on a maximum likelihood inference scheme, calculates the likelihood $\Pr(\mathbf{l} \mid N_e)$ for the length distribution of a single individual and then estimates the population size that maximizes the product of the likelihoods. The likelihood is based on a well-known formula for the probability density of observing ROH (or IBD blocks) of length $x$, $b(x|t)$, between a pair of haplotypes originating from time t in the past; see Browning and Browning (2015) and Ringbauer, Coop, and Barton (2017) for details:

$$b(x|t)\,dx = \left( \underbrace{(G-x)(2t)^2 \exp(-2tx)\,dx}_{(i)} + \underbrace{2(2t)\exp(-2tx)\,dx}_{(ii)} \right) \psi(t) \tag{1}$$

Here $\psi(t)$ denotes the probability of coalescence $t$ generations ago, $G$ the length of the haplotype, and (i) describes blocks in the interior, and (ii) blocks at one of the two ends of the haplotype. For a continuous panmictic population consisting of N haplotypes the coalescence probability time t ago, we plug in

$$\psi_N(t) = \exp\left(-\frac{t}{N}\right)\frac{1}{N}. \tag{2}$$

Integrating over all $t$ yields a straightforward analytical solution:

$$f_N(x)dx = \left( \frac{8(G-x)}{N} \frac{1}{(2x+\frac{1}{N})^3} + \frac{4}{N} \frac{1}{(2x+\frac{1}{N})^2} \right) dx \tag{3}$$

For multiple chromosomes, this formula needs to be summed over all chromosomes. For each small length interval $\Delta x$, the product $f_N(x)\Delta x$ gives the expected number of ROH within this length interval. Following the approach of Ralph and Coop (2013), applied and explained in detail in Ringbauer, Coop, and Barton (2017), we arrive at an approximate likelihood by binning shared blocks into small length bins $x_1, \ldots, x_l$ of width $\Delta x$ by modelling ROH counts in each of these bins as independent Poisson counts with expected rates $f_N(x_i)\Delta x$. We then calculate a composite likelihood of the observed data given $N_e$ using this Poisson model, and computationally maximize this overall likelihood. Estimates for the uncertainty of the estimator can be obtained via the curvature of the likelihood function (Fisher information matrix), or also via bootstrap over individuals or chromosomes. An implementation of this method is available via the Python package *hapROH* (https://pypi.org/project/hapROH/), which accelerates the calculations explained in Ringbauer, Coop, and Barton (2017) that finish optimization and calculation for ROH of 10 individuals within much less than a second on a standard CPU.

**Testing the $N_e$ Inference Method**

To test the inference method, we generated simulated ROH for a panmictic population. We used the software *msprime* (Kelleher, Etheridge, and McVean 2016) and simulated all autosomes, with lengths determined between the genetic map difference between the first and last 1240k SNP on each autosome (the set of SNPs we used in our inference scheme). We simulated four population sizes (250, 500, 1000, 2000) and validated the simulations by comparing the averages to our analytical expectations (obtained from integrating Formula (3); Figure S22). We then ran our inference method on genome-wide ROH of groups of 10 individuals, testing two scenarios: data where ROH blocks are i) determined by each true recombination event (recorded using the full ARG), and ii) by continuous tracts of co-ancestry within the last 100 generations - that merge "ineffective recombination events" which are detected in empirical data only as single continuous stretches (Chiang, Ralph, and Novembre 2016). Our results demonstrate that our method can robustly recover the population size used in these simulations. Moreover, the confidence intervals (obtained from the likelihood profile) accurately reflect the estimator uncertainty (Figure S23). Merging non-detectable recombination events starts having an observable effect for $2N_e$ below 1000, causing a downward bias of estimates (as more ROH are expected than expected from true recombination). Above $N_e$=500 this effect is negligible, and down to ~$N_e$=250 the observed bias remains small (<20%). For the empirical analysis, we filtered individuals who are possibly offspring of closely related parents, with more than 50cM of their genome in ROH>20cM blocks.

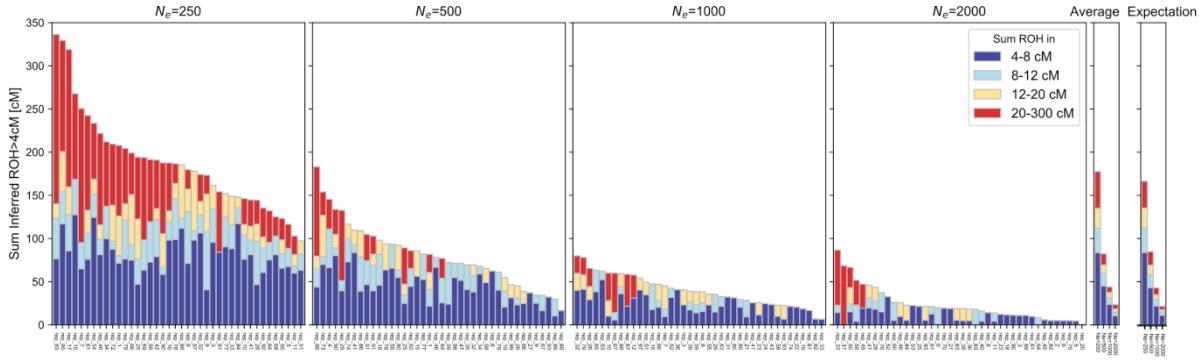



**Figure S22: Simulated ROH for four population sizes.** We visualize simulated ROH distribution on all autosomes ($N_e$=250,500,1000,2000). Each bar visualizes ROH of one individual (40 individuals per population size). The "average" panel gives the empirical average for each of these groups, and the "Small Pop. Size" panel gives the analytical average calculated from formula (3).

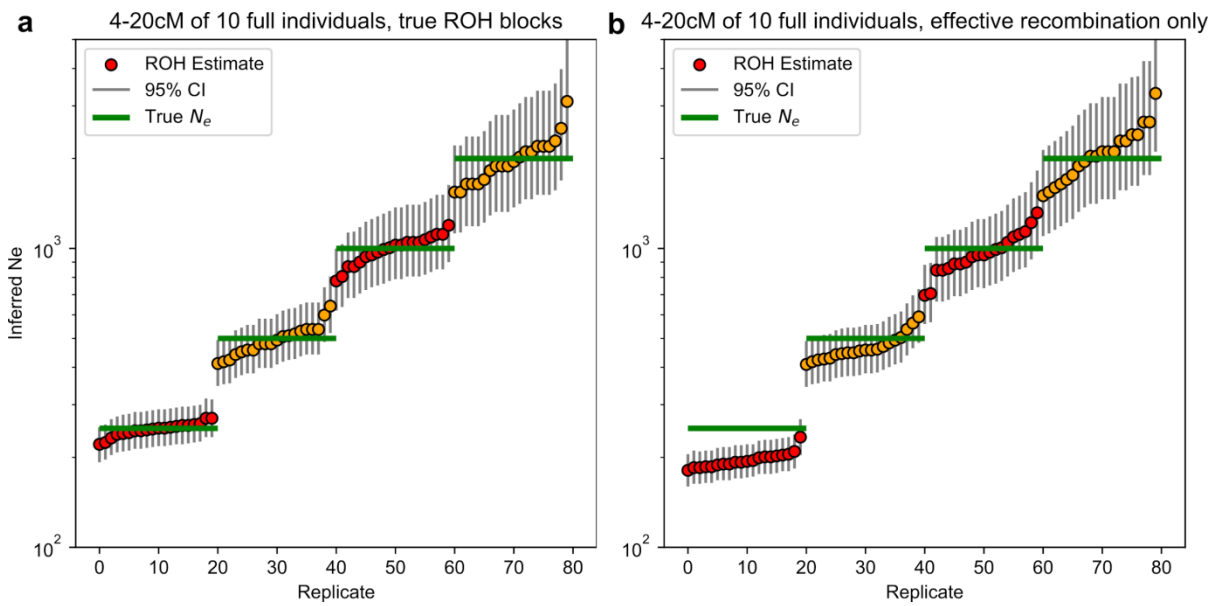



**Figure S23: Inferred Ne for various population sizes.** We inferred $N_e$ using 10 individuals for $N_e$= 250, 500, 1000 and 2000 (green lines). Each point represents one replicate experiment of simulation and inferred data, vertical bars represent 95% CI. **(a)** Using all ROH defined by all recombination breakpoints (using the ARG). **(b)** ROH is defined as stretches with coalescent times less than 100 generations ago.

### IBD sharing on the X chromosome

Shared segments between pairs of individuals, so-called Identity by Descent segments ("IBD blocks") are signatures of recent co-ancestry. We identified shared genomic segments on the X chromosome between pairs of male individuals ("IBD_X"). We used a simple extension of the *hapROH* approach because male X chromosomes consist of only a single haplotype. We paired pseudo-haploid data from two male X chromosomes and ran *hapROH* on each of these artificial individuals, treating the paired data as read counts. We manually confirmed a large number of these calls (see Figure S24 for an

example of comparison). For downstream analysis, we measured the X chromosome length and IBD map lengths as ⅔ of the map length of female X (as X chromosome lineages spend on average ⅔ of their time in females and do not recombine in males). We identified 19 pairs of individuals who were distant cousins and shared X chromosome IBD segments 8.7-22.7cM that lived on different islands and 34 pairs of individuals from different sites on the same island who shared X chromosome IBD segments 8.0-17.3cM (Supplementary Data Table 13).

To infer population sizes from IBD, we applied the same likelihood approach as described for ROH. We note that inferred population sizes from the X chromosome depend on male and female effective size in a way different from the autosomes, as X lineages spend on average ⅔ of their time in female ancestors, and males have only one copy of it. Assuming female and male effective sizes are equal, this effective population size is ¾ of the effective size of males. However, in certain scenarios, higher variation in male offspring success can increase the effective size of the X chromosome even slightly beyond the effective size of the autosomes (Vicoso and Charlesworth 2009). We therefore did not correct our effective population size estimates from the X chromosome.

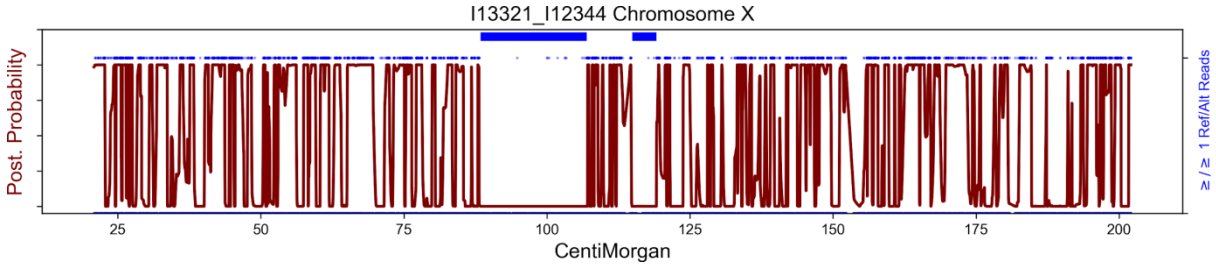



**Figure S24: IBD segment on the X chromosomes between a pair of males from different islands.** We inferred IBD segments in pairs of male X chromosomes by using *hapROH* on paired data. Such IBD segments are characterized by an absence of heterozygous reads in the paired data (apart from rare genotyping or contamination errors). We depict inferred IBD calls >2cM (blue bars) as well as the *hapROH* posterior for the non-ROH state. Here we show a long shared IBD segment on the X between individual I3321 from Eleuthera Island (The Bahamas) and individual I12344 from El Soco (Dominican Republic). The overall rate of such genetic cousins is informative about recent population sizes and provides information about effective population size on a pan-Caribbean scale when detected across islands (Fig. 3c; Extended Data Fig. 4b).

Our results show that patterns of short IBD_X (2-8cM) are shared between different comparisons within Ceramic sites, whereas for longer IBD_X (10-20cM) we observe a clear geographic pattern (Fig. 3c). IBD sharing across pairs within the La Caleta site is an order of magnitude higher than for comparisons across islands. We note that there are some individuals from La Caleta with long IBD_X shared with a relatively large number of other La Caleta individuals, for instance individual I16180 shares IBD_X>10cM with 14 other individuals. Such clusters of distant cousins could explain why IBD_X >10cM is elevated compared to ROH >10cM (Fig. 3c) in La Caleta. However, even across sites in Hispaniola and across islands, there is an abundance of long IBD_X indicative of effective population sizes below 10,000. This is an overestimate of the recent population size (which is measured by long

ROH, see Figs. 3b, Extended Data Fig. 4a), as limited migration reduces cross-coalescent rates beyond the ones expected within a panmictic population of the same size. It is also an overestimate because variation in the dates of the individuals we analyzed causes the average length of shared haplotypes to be smaller than would be expected if the individuals lived at the same time.

## SI8 - Clade grouping and substructure analysis with *qpWave*, *TreeMix*, and $f_4$-statistics

We used a five-step framework involving *qpWave*, *TreeMix*, and $f_4$-statistics—which we considered together with admixture profiles and proportions from *qpAdm*—to create genetically-driven groupings of sites and individuals blinded to archaeologically-based material culture assignments (based on chronology or material culture). We recognize that genetic groupings may or may not reflect the true nature of social organization in the past. However, the ability to use ancient DNA data to document ancestry change provides an important new tool for understanding the past because it makes it possible to document intensive interactions between groups of people that occurred. In particular, the observation of substantial ancestry change occurring in concert with a cultural transition provides evidence that movement of people contributed to that change, while conversely the absence of ancestry change associated with cultural transition increases the weight of evidence that the cultural change occurred through local developments or transmission of ideas. Thus, by grouping individuals entirely based on genetic data and geography, and then asking the extent to which these groupings correlated to cultural evidence, we can ask questions that meaningfully allow us to improve our understanding of the past.

As many genetic analyses require genetic information from more than one individual, we systematically grouped people together using the method described below to facilitate analyses that require us to distinguish people who were relatively genetically closer to each other from people who were more genetically distinct. We emphasize that all groups or sub-clades presented in this study are composed of individuals from a broadly homogeneous genetic pool, also demonstrated by low pairwise $F_{ST}$ distances (Extended Data Fig. 2), and they represent a gradient of genetic relationships that may change with additional data and increased analytical power.

### Step 1: Initial assignment of major clades with *qpWave*

For the first step, we ran pairwise *qpWave* tests between all sites included in this study and in Nägele et al. (2020), treating all individuals within sites as part of the same analysis unit if they were plausibly part of the same genetic cluster in PCA and ADMIXTURE analyses (Extended Data Fig. 1a, 1b). Only one individual newly-reported in this work was a clear outlier from other individuals from the same site in PCA and ADMIXTURE, and we confirmed that this individual was a chronological outlier compared to the other individuals from the site as well through direct radiocarbon dating. This individual, I10126 from Andrés (Dominican Republic) was directly dated to the Archaic Age and had a genomic profile clearly different from all other individuals from the predominantly Ceramic-associated Andrés site; for these reasons, we analysed I10126 independently. In Step 1 (Figure S25), we identified three major clades, where all sites or individuals (in cases where a site was represented by only a single individual) within each clade formed a clade with the majority of other sites or individuals within that same clade, and therefore are consistent with being descended from an ancestral population that was genetically homogeneous since their separation from every other clade:

A) *GreaterAntilles_Archaic - composed of 7 sites from Cuba, all matching an Archaic archaeological association, along with individual I10126 from the site of Andrés (Dominican Republic);

B) *Caribbean_Ceramic - composed of Ceramic-associated sites from Cuba, The Bahamas, Haiti, Dominican Republic, Puerto Rico, St. Lucia, Guadeloupe, and Curaçao (although the high number of failed comparisons might suggest that the latter might not be a clade with a pool of the others, discussed in greater detail below and in Supplementary Information section 9);

C) *Venezuela_Ceramic - composed of the site of Las Locas (Venezuela).

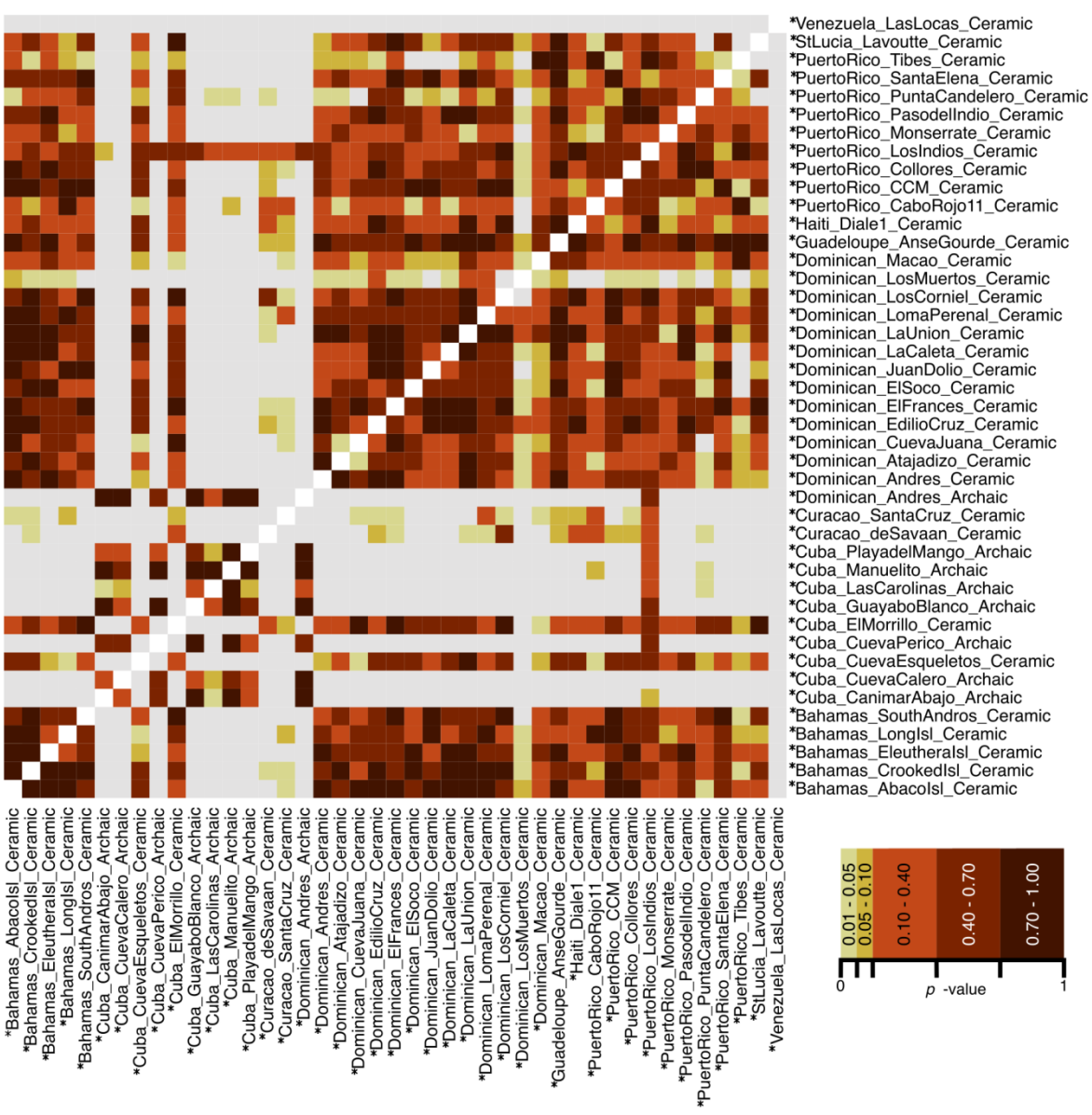



**Figure S25: Pairwise *qpWave* Step 1 results for all sites in this study, using a threshold of *p*>0.01.** Colors represent *p*-values of *qpWave* tests.

**Step 2: Exploring substructure within Step 1 clades using a model competition approach**

Step two consisted of repetition of pairwise models between members of the three major clades with *qpWave*, but this time using a "model competition" approach, as previously used in the related software *qpAdm* (Figure S26). With this approach, a group on the "Left" ("sources") is moved to the "Right" ("references") if it is not currently being used on the "Left" (Lazaridis et al. 2016, Narasimhan et al. 2019, Fernandes et al. 2020). Sites were merged if they formed an exclusive clade with each other.

Following this rule, we merged the individuals from the two Ceramic-associated sites from Curaçao (de Savaan and Santa Cruz), which did not form a clade with any of the other individuals from other sites in the *Caribbean_Ceramic clade, but were marginally consistent with forming a clade with each other (p=0.011). They were merged as *Curacao_Ceramic and removed from *Caribbean_Ceramic. In Supplementary Information section 9 we show how this behavior is explained by the presence of admixture in Curaçao.

Individuals from the site of Diale 1 in Haiti also did not form a clade with any other site (again due to the presence of admixture) and were therefore labelled as *Haiti_Ceramic and removed from the *Caribbean_Ceramic clade (see Supplementary Information section 9 for more information about admixture at Diale 1). Lastly, we merged the only two sites from the Lesser Antilles, Lavoutte (St. Lucia) and Anse à la Gourde (Guadeloupe), as *LesserAntilles_Ceramic. Individuals from the latter site did have a p=0.026 with *PuertoRico_LosIndios_Ceramic, which is above the threshold of p>0.01 we use for our splitting procedure, but we ignore this signal for our grouping analysis because (a) *PuertoRico_LosIndios_Ceramic comprises a single low-coverage individual and similar patterns are not observed for higher coverage individuals, and (b) the low coverage *PuertoRico_LosIndios_Ceramic also groups well with almost all other Greater Antilles Ceramic-associated individuals.

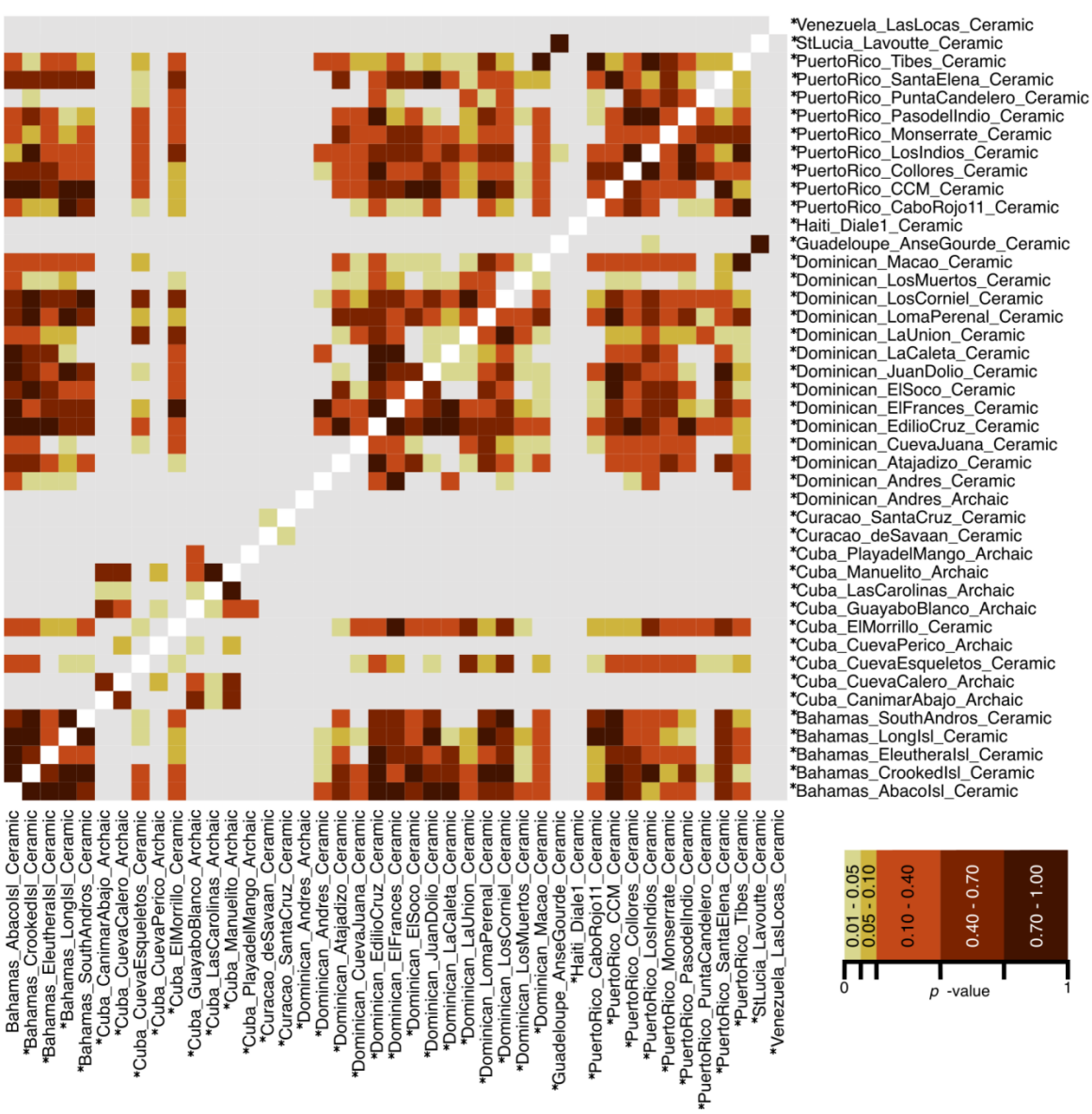



**Figure S26: Pairwise *qpWave* Step 2 results, as a repetition of the tests from Step 1, but now using a model competition approach.** Threshold of $p>0.01$ used. Colors represent $p$-values of *qpWave* tests.

**Step 3: Exploring structure using TreeMix in *Caribbean_Ceramic**

For step three, we investigated structure within *Caribbean_Ceramic and *GreaterAntilles_Ceramic. We first ran *TreeMix* on the populations from the Illumina dataset allowing no migration events (Figure S27a). The residuals indicated a non-optimal fit for *Haiti_Ceramic (Figure S27b) so we re-ran TreeMix allowing up to as many admixture events as necessary until admixture was modelled into *Haiti_Ceramic (Figures S27, S28). At "-m 1" *TreeMix* identified a migration event between *Haiti_Ceramic and *GreaterAntilles_Archaic and the maximum residuals were reduced from 10.5 to 5.1 standard errors (Figure S28).

Next, we identified persistent branches or nodes that maintained the same populations in the two tree fits (representing 0 or 1 migration events) relative to the other ancient sites, and explored if they formed statistically significant sub-groups (or sub-clades) on the tree with 1 migration event allowed using $f_4$-statistics. Those sites were:

- All sites/islands from The Bahamas, and the two Cuban Ceramic sites from Nägele et al. (2020) - Cueva de los Esqueletos *(CDE)* and El Morrillo (*ELM*);

- The southeastern coastal Dominican Republic Ceramic-associated sites of La Caleta, Andrés, Juan Dolio, and El Soco, with Macao and El Frances on a sister branch;

- Two Ceramic sites from Puerto Rico: Santa Elena and Cañas/Collores/Monserrate, with a sister branch that included CaboRojo11 (another Puerto Rican site) and the Dominican site of Atajadizo;

- Two Ceramic sites from the Dominican Republic: Los Corniel and Edilio Cruz;

- The Nägele et al. (2020) Archaic-associated sites of Canímar Abajo *(CAO)* and Manuelito *(MTO)*;

- The Nägele et al. (2020) Archaic-associated site of Playa del Mango (*PDE*);

- The Archaic-related individual I10126 from the Ceramic-associated site of Andrés.

The majority of the sites that moved between different locations in the trees when we altered the number of migration events comprised single individuals where we have a lower resolution due to lower sample size.

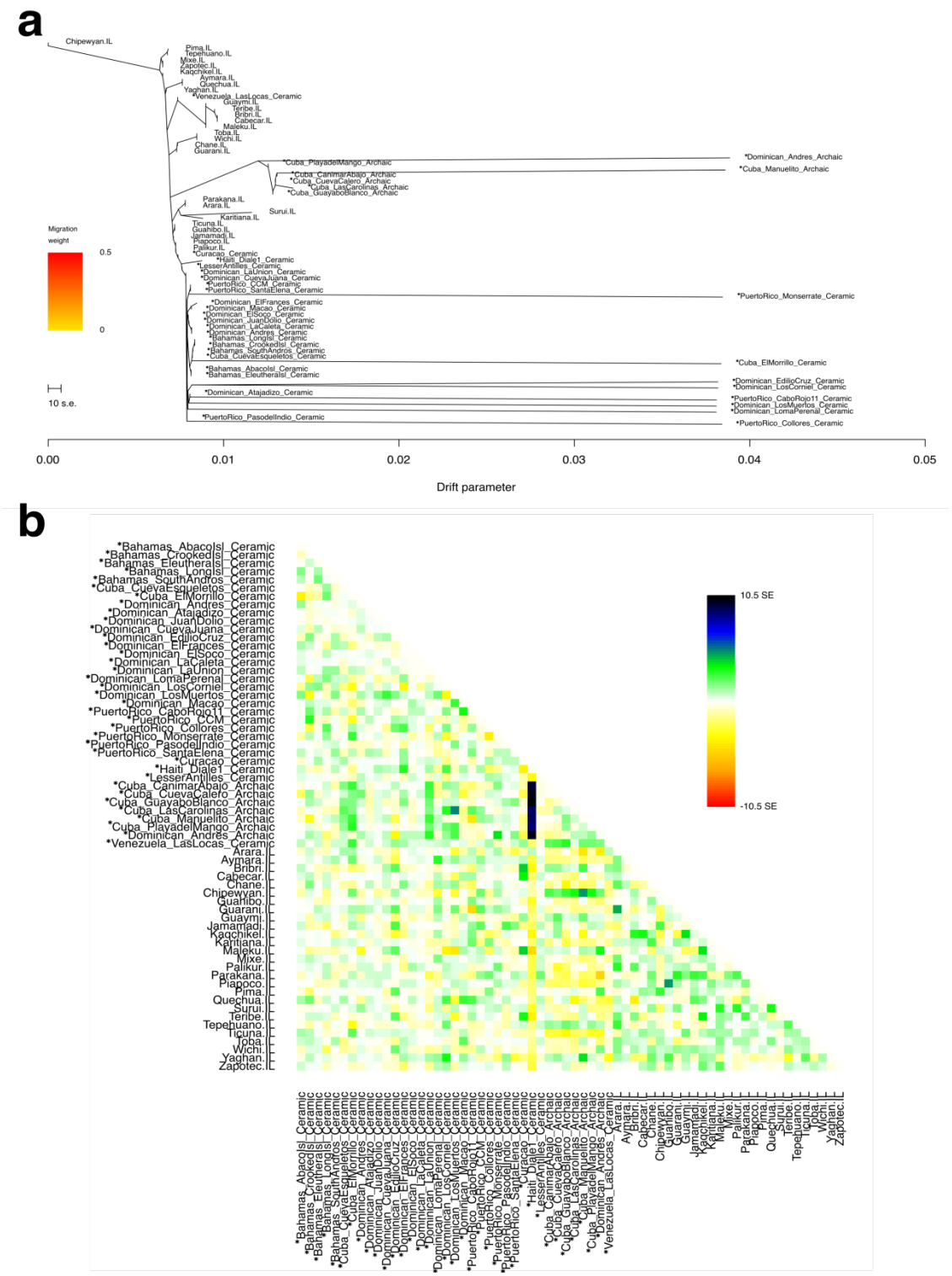



**Figure S27: *TreeMix* results with no migration/admixture events allowed (a) and residuals of the corresponding fits (b).**

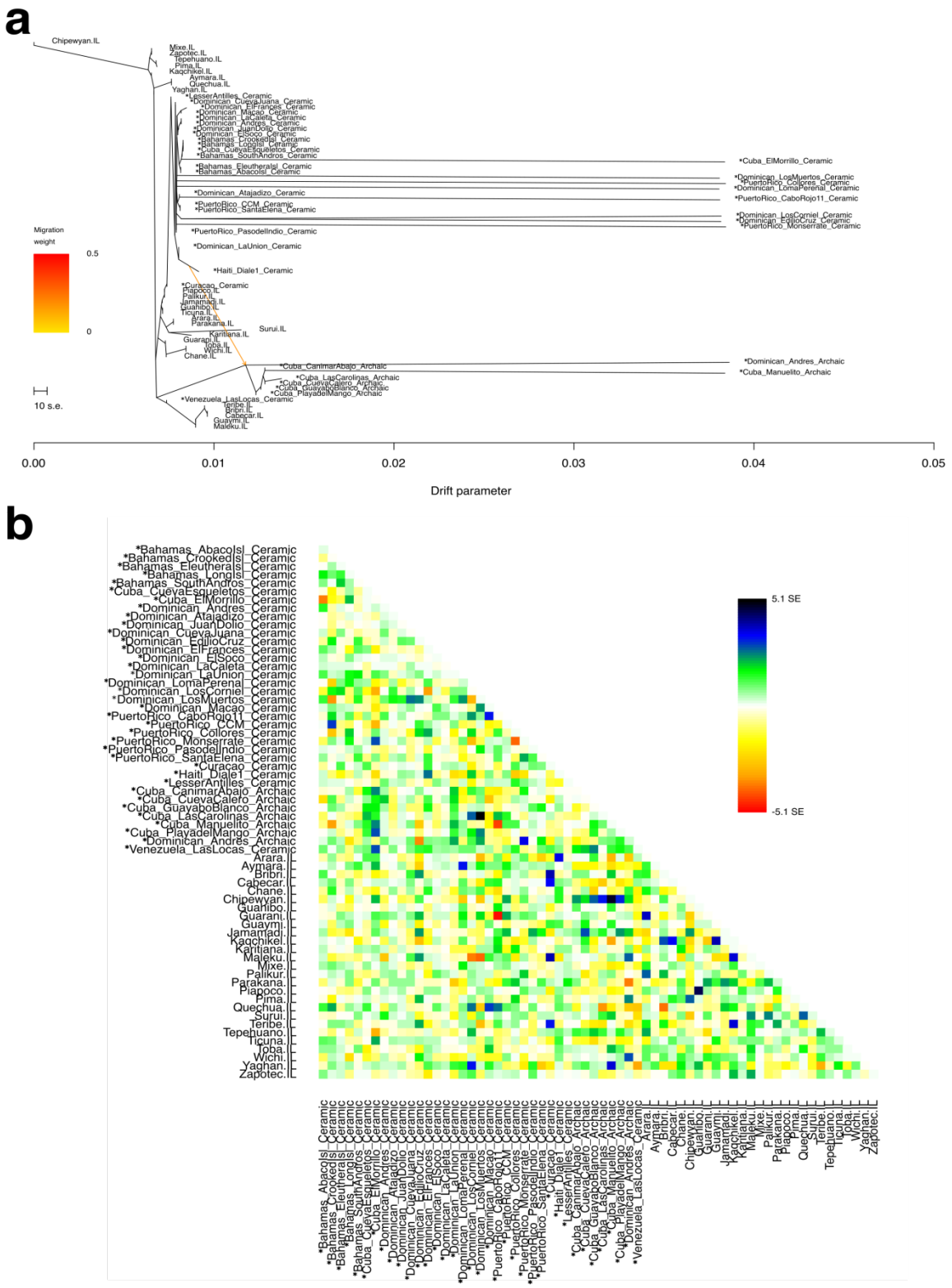



**Figure S28: *TreeMix* results with 1 migration/admixture events allowed (a) and residuals of the corresponding fits (b).**

**Step 4: Using $f_4$-statistics to confirm *TreeMix's* structure**

For the branches identified in step 3 we computed $f$-statistics to evaluate if they formed clades to the exclusion of pools of other sites, hence suggesting structure within the *Caribbean_Ceramic clade.

The large *TreeMix* branch that included all sites from the Bahamas as well as the two Ceramic-associated sites from Cuba (Cueva de los Esqueletos and El Morrillo) is supported by $f_4$-statistics. All tests involving these sites produced a |Z| < 2.8 for the first test $f_4$(Mbuti, Pool; Test1; Test2) and |Z| > 2.8 for at least one of the other two $f_4$-statistics (as described above and in Table S6) when compared against a pool of Ceramic-associated sites excluding the Bahamas and Cuba. An exception was found for *Cuba_CuevaEsqueletos_Ceramic and *Bahamas_LongIsl_Ceramic as the Test populations, where the pool of Dominican and Puerto Rican Ceramic-associated sites was closer to the latter (|Z| = 3.174). However, statistics of the form $f_4$(Mbuti, *Cuba_CuevaEsqueletos_Ceramic/*Cuba_ElMorrillo_Ceramic; PoolOfBahamas, PoolOfNonBahamas), with PoolofBahamas as all Ceramic-associated Bahamian sites from our study and PoolOfNonBahamas as all Dominican and Puerto Rican sites, clearly support the inclusion of Cueva de los Esqueletos and El Morrillo within the Bahamian group (Z = -7.729 and -5.214, respectively), and thus the formation of a sub-clade within *Caribbean_Ceramic. We therefore merged all sites from the Bahamas as well as the two Ceramic-associated sites from Cuba into the sub-clade *BahamasCuba_Ceramic.

We were not able to confirm a sub-clade with El Frances and Macao, or a closer relationship to the sister branch containing the Dominican sites of La Caleta, Andrés, Juan Dolio, and El Soco, but found clear evidence of a sub-clade with La Caleta and Andrés to the exclusion of the other tested nodes/pools (Table S6). For El Soco and Juan Dolio we found contradictory results, with El Soco sharing significantly more alleles with Juan Dolio than with the node containing La Caleta and Andrés (|Z| = 3.244, Table S6). La Caleta, Andrés, El Soco, and Juan Dolio are located within a 50 kilometer stretch of coast in the southeast part of the Dominican Republic (Figure S9), so we further explored the relationship between these sites with $f_4$-statistics of the form $f_4$(Mbuti, Site1; Site2, *PoolOfAllOtherCaribbean_Ceramic) on all possible permutations between the sites. The results show a west-to-east cline of relatedness for the coastal sites (the sites from west to east are La Caleta, Andrés, Juan Dolio, and El Soco) as statistically significant tests (|Z| > 2.8) were found for the geographically proximate pairs La Caleta-Andrés, La Caleta-Juan Dolio, Andrés-Juan Dolio, and Juan Dolio-El Soco (Table S7). The three westernmost sites, La Caleta, Andrés, and Juan Dolio, always shared more alleles with each other than with the remaining *Caribbean_Ceramic sites, while the easternmost site, El Soco, only produced statistically significant results with La Caleta and with the site geographically closest to it (Juan Dolio, located 22 kilometers to the west) (Table S7). Considering this evidence together, we merged the four coastal sites of La Caleta, Andrés, El Soco, and Juan Dolio as the sub-clade *SECoastDR_Ceramic due to their geographical proximity and the identification of a genetic cline connecting them.

All Archaic-associated sites from Cuba were merged into *Cuba_Archaic, to the exclusion of the only individual from this major clade from the Dominican Republic (I10126), as even Playa del Mango, (the Archaic site from Cuba which appeared closest to I10126 using *TreeMix*) was found to be significantly

closer to the pool of the remaining Cuban sites than to I10126 (|Z| = 4.125), while the $f_4$-statistic testing whether Playa del Mango was a clade with the other Cuban Archaic sites relative to the Dominican Archaic site passed (|Z|=0.454) (Table S6).

**Table S6: $f_4$-statistic Z-scores of the triple tests for the branches with unchanged relative location as identified in step 3.** Tests of the form $f_4$(Mbuti, Pool; Test1, Test2), $f_4$(Mbuti, Test1; Pool, Test2), $f_4$(Mbuti, Test2; Test1, Pool), with respective Z-scores shown as Z1, Z2, and Z3. *PuertoRico_CCM_Ceramic is the abbreviation used for the individuals from an uncertain context (either Cañas, Collores, or Monserrate).

| | Test1<br>*Bahamas_EleutheraIsl_Ceramic | Test2<br>*Bahamas_AbacoIsl_Ceramic | | |
|---|---|---|---|---|
| Pool1 | *Bahamas_CrookedIsl_Ceramic, *Bahamas_EleutheraIsl_Ceramic, *Bahamas_LongIsl_Ceramic, *Bahamas_SouthAndros_Ceramic, *Cuba_CuevaEsqueletos_Ceramic, *Cuba_ElMorrillo_Ceramic | | 0.453 | Z1 |
| | | | 0.442 | Z2 |
| | | | 0.032 | Z3 |
| Pool2 | *Dominican_Andres_Ceramic, *Dominican_Atajadizo_Ceramic, *Dominican_JuanDolio_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_ElFrances_Ceramic, *Dominican_ElSoco_Ceramic, *Dominican_LaCaleta_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosCorniel_Ceramic, *Dominican_LosMuertos_Ceramic, *Dominican_Macao_Ceramic, *PuertoRico_CaboRojo11_Ceramic, *PuertoRico_CCM_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic, *PuertoRico_SantaElena_Ceramic | | 1.498 | Z1 |
| | | | 4.083 | Z2 |
| | | | -2.895 | Z3 |
| | **Test1**<br>***Cuba_CuevaEsqueletos_Ceramic** | **Test2**<br>***Bahamas_LongIsl_Ceramic** | | |
| Pool1 | *Bahamas_CrookedIsl_Ceramic | | 4.297 | Z1 |
| | | | 2.730 | Z2 |
| | | | 1.578 | Z3 |
| Pool2 | *Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic | | 3.610 | Z1 |
| | | | 3.555 | Z2 |
| | | | 0.223 | Z3 |
| Pool3 | *Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic | | 3.714 | Z1 |
| | | | 5.138 | Z2 |
| | | | -1.275 | Z3 |
| Pool4 | *Dominican_Andres_Ceramic, *Dominican_Atajadizo_Ceramic, *Dominican_JuanDolio_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_ElFrances_Ceramic, *Dominican_ElSoco_Ceramic, *Dominican_LaCaleta_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosCorniel_Ceramic, *Dominican_LosMuertos_Ceramic, *Dominican_Macao_Ceramic, *PuertoRico_CaboRojo11_Ceramic, *PuertoRico_CCM_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic, *PuertoRico_SantaElena_Ceramic | | 3.174 | Z1 |
| | | | 7.726 | Z2 |
| | | | -5.084 | Z3 |
| | **Test1**<br>***Cuba_CuevaEsqueletos_Ceramic, *Bahamas_LongIsl_Ceramic** | **Test2**<br>***Bahamas_CrookedIsl_Ceramic** | | |
| Pool1 | *Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic | | -0.741 | Z1 |
| | | | 0.066 | Z2 |
| | | | -0.949 | Z3 |
| Pool2 | *Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic | | 0.036 | Z1 |
| | | | 1.558 | Z2 |
| | | | -1.787 | Z3 |
| Pool3 | *Dominican_Andres_Ceramic, *Dominican_Atajadizo_Ceramic, *Dominican_JuanDolio_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_ElFrances_Ceramic, *Dominican_ElSoco_Ceramic, *Dominican_LaCaleta_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosCorniel_Ceramic, *Dominican_LosMuertos_Ceramic, *Dominican_Macao_Ceramic, *PuertoRico_CaboRojo11_Ceramic, *PuertoRico_CCM_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic, *PuertoRico_SantaElena_Ceramic | | 0.149 | Z1 |
| | | | 4.062 | Z2 |
| | | | -5.308 | Z3 |
| | **Test1**<br>***Cuba_CuevaEsqueletos_Ceramic, *Bahamas_LongIsl_Ceramic, *Bahamas_CrookedIsl_Ceramic** | **Test2**<br>***Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic** | | |
| Pool1 | *Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic | | 0.290 | Z1 |
| | | | 1.881 | Z2 |
| | | | -1.711 | Z3 |
| Pool2 | *Dominican_Andres_Ceramic, *Dominican_Atajadizo_Ceramic, *Dominican_JuanDolio_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_ElFrances_Ceramic, *Dominican_ElSoco_Ceramic, *Dominican_LaCaleta_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosCorniel_Ceramic, *Dominican_LosMuertos_Ceramic, *Dominican_Macao_Ceramic, *PuertoRico_CaboRojo11_Ceramic, *PuertoRico_CCM_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic, *PuertoRico_SantaElena_Ceramic | | -0.463 | Z1 |
| | | | 6.201 | Z2 |
| | | | -7.457 | Z3 |
| | **Test1**<br>***Cuba_CuevaEsqueletos_Ceramic, *Bahamas_LongIsl_Ceramic, *Bahamas_CrookedIsl_Ceramic, *Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic** | **Test2**<br>***Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic** | | |
| Pool1 | *Dominican_Andres_Ceramic, *Dominican_Atajadizo_Ceramic, *Dominican_JuanDolio_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_ElFrances_Ceramic, *Dominican_ElSoco_Ceramic, *Dominican_LaCaleta_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosCorniel_Ceramic, *Dominican_LosMuertos_Ceramic, *Dominican_Macao_Ceramic, *PuertoRico_CaboRojo11_Ceramic, *PuertoRico_CCM_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic, *PuertoRico_SantaElena_Ceramic | | -0.124 | Z1 |
| | | | 5.475 | Z2 |
| | | | -6.228 | Z3 |
| | **Test1**<br>***PuertoRico_CCM_Ceramic** | **Test2**<br>***PuertoRico_SantaElena_Ceramic** | | |
| Pool1 | *Dominican_Atajadizo_Ceramic, *PuertoRico_CaboRojo11_Ceramic | | -0.292 | Z1 |
| | | | -0.177 | Z2 |
| | | | -0.123 | Z3 |

| | | | |
|---|---|---|---|
| **Pool2** | *Cuba_CuevaEsqueletos_Ceramic, *Bahamas_LongIsl_Ceramic, *Bahamas_CrookedIsl_Ceramic, *Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic, *Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic, *Dominican_Andres_Ceramic, *Dominican_JuanDolio_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_ElFrances_Ceramic, *Dominican_ElSoco_Ceramic, *Dominican_LaCaleta_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosCorniel_Ceramic, *Dominican_LosMuertos_Ceramic, *Dominican_Macao_Ceramic, *PuertoRico_Collores_Ceramic, | -0.493 | Z1 |
| | | 0.345 | Z2 |
| | | -0.789 | Z3 |
| | **Test1** ***PuertoRico_CCM_Ceramic, *PuertoRico_SantaElena_Ceramic** | **Test2** ***Dominican_Atajadizo_Ceramic, *PuertoRico_CaboRojo11_Ceramic** | |
| **Pool1** | *Cuba_CuevaEsqueletos_Ceramic, *Bahamas_LongIsl_Ceramic, *Bahamas_CrookedIsl_Ceramic, *Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic, *Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic, *Dominican_Andres_Ceramic, *Dominican_JuanDolio_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_ElFrances_Ceramic, *Dominican_ElSoco_Ceramic, *Dominican_LaCaleta_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosCorniel_Ceramic, *Dominican_LosMuertos_Ceramic, *Dominican_Macao_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic | 0.262 | Z1 |
| | | 0.965 | Z2 |
| | | -0.425 | Z3 |
| | **Test1** ***Dominican_Atajadizo_Ceramic** | **Test2** ***PuertoRico_CaboRojo11_Ceramic** | |
| **Pool1** | *PuertoRico_CCM_Ceramic, *PuertoRico_SantaElena_Ceramic | 2.016 | Z1 |
| | | 3.048 | Z2 |
| | | -1.270 | Z3 |
| **Pool2** | *Cuba_CuevaEsqueletos_Ceramic, *Bahamas_LongIsl_Ceramic, *Bahamas_CrookedIsl_Ceramic, *Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic, *Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic, *Dominican_Andres_Ceramic, *Dominican_JuanDolio_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_ElFrances_Ceramic, *Dominican_ElSoco_Ceramic, *Dominican_LaCaleta_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosCorniel_Ceramic, *Dominican_LosMuertos_Ceramic, *Dominican_Macao_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic | 2.611 | Z1 |
| | | 3.484 | Z2 |
| | | -1.922 | Z3 |
| | **Test1** ***Dominican_LosCorniel_Ceramic** | **Test2** ***Dominican_EdilioCruz_Ceramic** | |
| **Pool1** | *Cuba_CuevaEsqueletos_Ceramic, *Bahamas_LongIsl_Ceramic, *Bahamas_CrookedIsl_Ceramic, *Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic, *Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic, *Dominican_Andres_Ceramic, *Dominican_JuanDolio_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_ElFrances_Ceramic, *Dominican_ElSoco_Ceramic, *Dominican_LaCaleta_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosMuertos_Ceramic, *Dominican_Macao_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic, *Dominican_Atajadizo_Ceramic, *PuertoRico_CaboRojo11_Ceramic, *PuertoRico_CCM_Ceramic, *PuertoRico_SantaElena_Ceramic | 0.339 | Z1 |
| | | 2.754 | Z2 |
| | | -2.468 | Z3 |
| | **Test1** ***Dominican_ElFrances_Ceramic** | **Test2** ***Dominican_Macao_Ceramic** | |
| **Pool1** | *Dominican_LaCaleta_Ceramic, *Dominican_Andres_Ceramic, *Dominican_JuanDolio_Ceramic, *Dominican_ElSoco_Ceramic | -0.640 | Z1 |
| | | 2.077 | Z2 |
| | | -2.769 | Z3 |
| **Pool2** | *Cuba_CuevaEsqueletos_Ceramic, *Bahamas_LongIsl_Ceramic, *Bahamas_CrookedIsl_Ceramic, *Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic, *Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosMuertos_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic, *Dominican_Atajadizo_Ceramic, *PuertoRico_CaboRojo11_Ceramic, *PuertoRico_CCM_Ceramic, *PuertoRico_SantaElena_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_LosCorniel_Ceramic | -1.001 | Z1 |
| | | 2.052 | Z2 |
| | | -3.118 | Z3 |
| | **Test1** ***Dominican_ElFrances_Ceramic, *Dominican_Macao_Ceramic** | **Test2** ***Dominican_LaCaleta_Ceramic, *Dominican_Andres_Ceramic, *Dominican_JuanDolio_Ceramic, *Dominican_ElSoco_Ceramic** | |
| **Pool1** | *Cuba_CuevaEsqueletos_Ceramic, *Bahamas_LongIsl_Ceramic, *Bahamas_CrookedIsl_Ceramic, *Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic, *Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosMuertos_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic, *Dominican_Atajadizo_Ceramic, *PuertoRico_CaboRojo11_Ceramic, *PuertoRico_CCM_Ceramic, *PuertoRico_SantaElena_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_LosCorniel_Ceramic | 0.499 | Z1 |
| | | 1.693 | Z2 |
| | | -0.329 | Z3 |
| | **Test1** ***Dominican_LaCaleta_Ceramic** | **Test2** ***Dominican_Andres_Ceramic** | |
| **Pool1** | *Dominican_JuanDolio_Ceramic | 2.268 | Z1 |
| | | 3.076 | Z2 |
| | | -1.042 | Z3 |
| **Pool2** | *Dominican_ElSoco_Ceramic | 1.143 | Z1 |
| | | 4.467 | Z2 |
| | | -4.340 | Z3 |
| **Pool3** | *Dominican_ElFrances_Ceramic, *Dominican_Macao_Ceramic | 1.133 | Z1 |
| | | 4.809 | Z2 |
| | | -3.843 | Z3 |
| **Pool4** | *Cuba_CuevaEsqueletos_Ceramic, *Bahamas_LongIsl_Ceramic, *Bahamas_CrookedIsl_Ceramic, *Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic, *Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosMuertos_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic, *Dominican_Atajadizo_Ceramic, *PuertoRico_CaboRojo11_Ceramic, *PuertoRico_CCM_Ceramic, *PuertoRico_SantaElena_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_LosCorniel_Ceramic | 2.055 | Z1 |
| | | 7.147 | Z2 |
| | | -8.742 | Z3 |
| | **Test1** ***Dominican_LaCaleta_Ceramic** | **Test2** ***Dominican_Andres_Ceramic** | |
| **Pool1** | *Dominican_JuanDolio_Ceramic, *Dominican_ElSoco_Ceramic | 1.827 | Z1 |
| | | 4.146 | Z2 |
| | | -3.267 | Z3 |
| | | 1.133 | Z1 |

| | | | |
|---|---|---|---|
| **Pool2** | *Dominican_ElFrances_Ceramic, *Dominican_Macao_Ceramic | 4.809 | Z2 |
| | | -3.843 | Z3 |
| **Pool3** | *Cuba_CuevaEsqueletos_Ceramic, *Bahamas_LongIsl_Ceramic, *Bahamas_CrookedIsl_Ceramic, *Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic, *Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosMuertos_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic, *Dominican_Atajadizo_Ceramic, *PuertoRico_CaboRojo11_Ceramic, *PuertoRico_CCM_Ceramic, *PuertoRico_SantaElena_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_LosCorniel_Ceramic | 2.055 | Z1 |
| | | 7.147 | Z2 |
| | | -8.742 | Z3 |
| | **Test1** | **Test2** | |
| | ***Dominican_LaCaleta_Ceramic, *Dominican_Andres_Ceramic, *Dominican_JuanDolio_Ceramic, *Dominican_ElSoco_Ceramic** | ***Dominican_ElFrances_Ceramic, *Dominican_Macao_Ceramic** | |
| **Pool1** | *Cuba_CuevaEsqueletos_Ceramic, *Bahamas_LongIsl_Ceramic, *Bahamas_CrookedIsl_Ceramic, *Bahamas_SouthAndros_Ceramic, *Cuba_ElMorrillo_Ceramic, *Bahamas_EleutheraIsl_Ceramic, *Bahamas_AbacoIsl_Ceramic, *Dominican_CuevaJuana_Ceramic, *Dominican_LaUnion_Ceramic, *Dominican_LomaPerenal_Ceramic, *Dominican_LosMuertos_Ceramic, *PuertoRico_Collores_Ceramic, *PuertoRico_Monserrate_Ceramic, *PuertoRico_PasodelIndio_Ceramic, *Dominican_Atajadizo_Ceramic, *PuertoRico_CaboRojo11_Ceramic, *PuertoRico_CCM_Ceramic, *PuertoRico_SantaElena_Ceramic, *Dominican_EdilioCruz_Ceramic, *Dominican_LosCorniel_Ceramic | -0.499 | Z1 |
| | | 0.329 | Z2 |
| | | -1.693 | Z3 |
| | **Test1** | **Test2** | |
| | ***Dominican_ElSoco_Ceramic** | ***Dominican_JuanDolio_Ceramic** | |
| **Pool1** | *Dominican_JuanDolio_Ceramic, *Dominican_ElSoco_Ceramic | 1.775 | Z1 |
| | | 3.244 | Z2 |
| | | -1.561 | Z3 |
| | **Test1** | **Test2** | |
| | ***Cuba_CanimarAbajo_Archaic** | ***Cuba_Manuelito_Archaic** | |
| **Pool1** | *Cuba_PlayaDelMango_Archaic, *Cuba_GuayaboBlanco_Archaic, *Cuba_LasCarolinas_Archaic, *Cuba_CuevaCalero_Archaic | 1.476 | Z1 |
| | | 4.297 | Z2 |
| | | -4.556 | Z3 |
| **Pool2** | *Dominican_Andres_Archaic | 1.834 | Z1 |
| | | 9.018 | Z2 |
| | | -5.506 | Z3 |
| | **Test1** | **Test2** | |
| | ***Cuba_CanimarAbajo_Archaic, *Cuba_Manuelito_Archaic** | ***Cuba_PlayaDelMango_Archaic, *Cuba_GuayaboBlanco_Archaic, *Cuba_LasCarolinas_Archaic, *Cuba_CuevaCalero_Archaic** | |
| **Pool1** | *Dominican_Andres_Archaic | 0.971 | Z1 |
| | | 7.626 | Z2 |
| | | -6.966 | Z3 |
| | **Test1** | **Test2** | |
| | ***Cuba_GuayaboBlanco_Archaic** | ***Cuba_PlayaDelMango_Archaic, *Cuba_LasCarolinas_Archaic, *Cuba_CuevaCalero_Archaic, *Cuba_CanimarAbajo_Archaic, *Cuba_Manuelito_Archaic** | |
| **Pool1** | *Dominican_Andres_Archaic | 0.486 | Z1 |
| | | 5.869 | Z2 |
| | | -6.440 | Z3 |
| | **Test1** | **Test2** | |
| | ***Cuba_PlayaDelMango_Archaic** | ***Cuba_GuayaboBlanco_Archaic, *Cuba_LasCarolinas_Archaic, *Cuba_CuevaCalero_Archaic, *Cuba_CanimarAbajo_Archaic, *Cuba_Manuelito_Archaic** | |
| **Pool1** | *Dominican_Andres_Archaic | -0.454 | Z1 |
| | | 4.125 | Z2 |
| | | -4.684 | Z3 |

**Table S7: Testing the relationship between the 4 coastal sites from southeastern Dominican Republic against a pool of other *Caribbean_Ceramic sites.**

| Pop X | Pop Y | Pop W | Pop Z | f4 | SE | Z | SNPs |
|---|---|---|---|---|---|---|---|
| Mbuti | *Dominican_Ceramic_Andres | *Dominican_Ceramic_LaCaleta | Ceramic_Pool | -0.000737 | 0.000085 | -8.720 | 983005 |
| Mbuti | *Dominican_Ceramic_Andres | *Dominican_Ceramic_ElSoco | Ceramic_Pool | -0.000186 | 0.000118 | -1.582 | 973498 |
| Mbuti | *Dominican_Ceramic_Andres | *Dominican_Ceramic_JuanDolio | Ceramic_Pool | -0.000578 | 0.000140 | -4.118 | 955569 |
| Mbuti | *Dominican_Ceramic_LaCaleta | *Dominican_Ceramic_ElSoco | Ceramic_Pool | -0.000280 | 0.000095 | -2.955 | 1064638 |
| Mbuti | *Dominican_Ceramic_LaCaleta | *Dominican_Ceramic_JuanDolio | Ceramic_Pool | -0.000486 | 0.000112 | -4.333 | 1023924 |
| Mbuti | *Dominican_Ceramic_ElSoco | *Dominican_Ceramic_JuanDolio | Ceramic_Pool | -0.000505 | 0.000129 | -4.042 | 1012067 |

The site composition of the sub-clades created after these 4 steps are as follows:

*Cuba_Archaic - Canímar Abajo, Carolinas II, Cueva Calero, Guayabo Blanco, Manuelito, Playa del Mango;

*Dominican_Andres_Archaic - Andrés (I10126) (here we keep the site's name in the sub-clade label to indicate that three low-coverage Dominican Archaic-associated individuals from Cueva Roja are not included in this sub-clade);

*SECoastDR_Ceramic - La Caleta, Andrés, Juan Dolio, El Soco;

*BahamasCuba_Ceramic - Abaco Island (Bill Johnson's Cave, Hopetown, Randy's Cave, Imperial Lighthouse Cave), Crooked Island (Burial Cave #1, Gordon Hill Cave, unknown site), Eleuthera Island (Garden Cave, Preacher's Cave, Valentine's Blue Hole, Wemyss Bight Cave), Long Island (Rolling Heads Site, Cave near Clarence Town), South Andros Island (Sanctuary Blue Hole, Stargate Blue Hole), El Morrillo, Cueva de los Esqueletos;

*EasternGreaterAntilles_Ceramic - El Francés, Edilio Cruz, La Unión, Loma Perenal, Macao, Collores, Monserrate, Cabo Rojo 11, Santa Elena, Cañas/Collores/Monserrate, Atajadizo, Cueva Juana, Los Corniel, Los Muertos, Paso del Indio;

*LesserAntilles_Ceramic - Anse à la Gourde, Lavoutte;

*Venezuela_Ceramic - Las Locas;

*Haiti_Ceramic - Diale 1;

*Curacao_Ceramic - de Savaan, Santa Cruz;

After identifying all sub-clades, we evaluated whether the four sites not used in the *TreeMix* and $f_4$ workflow due to reduced coverage (Cueva del Perico I (CIP), Los Indios (LOI), Punta Candelero (PCA), Tibes (TIB)) shared closer affinities to any of sub-clades relative to the others (Supplementary Data 7). The Ceramic-associated sites of Los Indios, Punta Candelero, and Tibes showed no specific affinities to any sub-clade, so they were merged into the *Caribbean_Ceramic sub-clade comprising sites without specific affinities: *EasternGreaterAntilles_Ceramic. In contrast, the Archaic-associated site of Cueva del Perico (Cuba) was found to be significantly closer to *Cuba_Archaic than to *Dominican_Andres_Archaic, and so was merged into the former ($|Z| = 6.410$, Supplementary Data 7). The composition of all sub-clades is presented in the main manuscript as Fig. 1b.

Next, we used the intra-sub-clade statistic $f_4$(Mbuti, *GreaterAntilles_Archaic; CeramicIndividual, *CeramicSubClade-Without-Individual), with CeramicIndividual as every individual from within a Ceramic-associated sub-clade, and *CeramicSubClade-Without-Individual as their sub-clade minus the individual being tested, to assess if any Ceramic-associated individuals had more Archaic-related ancestry than the remainder of their sub-clade (significant results in Table S8, all results in Supplementary Data 8). For any statistically significant case ($Z < -2.8$) we removed the individual from the sub-clade and labeled them as their sub-clade plus their Master ID (lab) as a suffix (e.g.

*CladeOrSub-Clade_Technology8888). Any individuals determined to have excess Archaic-related ancestry were not included in further analyses of their sub-clade.

**Table S8: Testing each individual's Archaic-related affinities within each sub-clade.** Results for |Z|>2.8 are presented; complete table found in Supplementary Data 6.

| Pop X | Pop Y | Pop W | Pop Z | f4 | SE | Z | SNPs |
|---|---|---|---|---|---|---|---|
| Mbuti | *GreaterAntilles_Archaic | *SECoastDR_Ceramic 16539 | *SECoastDR_Ceramic _ excl_16539 | -0.002111 | 0.000384 | -5.502 | 699782 |
| Mbuti | *GreaterAntilles_Archaic | *EasternGreaterAntilles_ Ceramic7969 | *EasternGreaterAntilles_ Ceramic_excl_7969 | -0.001219 | 0.000350 | -3.482 | 825578 |
| Mbuti | *GreaterAntilles_Archaic | *SECoastDR_Ceramic 16520 | *SECoastDR_Ceramic_ excl_16520 | -0.000995 | 0.000334 | -2.981 | 759450 |

As shown above, three individuals produced statistically significant statistics that suggested having higher levels of Archaic-related ancestry when compared to their sub-clade: I16539 from the site of La Caleta in the *SECoastDR_Ceramic sub-clade (Z = -5.502), I7969 from the site of La Union in the *EasternGreaterAntilles_Ceramic sub-clade (Z = -3.482), and I16520 also from La Caleta in the *SECoastDR_Ceramic sub-clade (Z = -2.981). In Supplementary Information section 9 (and before performing any other analyses) we estimated the amount of this Archaic-related ancestry in these individuals using *qpAdm*. Because we were not able to reject the formation of a clade between I7969 and I16520 and their respective sub-clades (even with *GreaterAntilles_Archaic on the "Right") we did not remove these individuals from their sub-clades; however, I16539 was renamed as *SECoastDR_Ceramic16539 after showing definitive outlier status.

**Step 5: Exploring substructure with $f_4$-statistics**

We finally investigated substructure within the *Caribbean_Ceramic sub-clades using $f_4$-statistics. Substructure was interpreted as present if a site produced statistically significant results (average pairwise Z-score > 2.8) with at least 50% of the remaining sites from that sub-clade.

For the *BahamasCuba_Ceramic group, all islands had an average Z-score > 2.8 with at least 50% of the remaining sites, suggesting the presence of substructure in this clade (Supplementary Data 6).

For *SECoastDR_Ceramic, the individuals from La Caleta shared more alleles with each other than with individuals from El Soco and Juan Dolio, but not with individuals from the geographically more proximate site of Andrés. This further supports the genetic cline in this sub-clade (discussed above, with data in Table S7).

Within the large *EasternGreaterAntilles_Ceramic sub-clade, 22 pairwise tests had an average Z > 2.8, but only the Dominican site of Macao showed significant evidence of structuring relative to the other sites in the broader clade, according to the rules defined above. We note that it is possible that this result is driven at least in part by the close relatedness of the studied individuals from Macao, as we observe one pair of second-degree relatives and two pairs of third-degree or higher relatives among the four individuals studied (Supplementary Information section 7).

Lastly, within *Cuba_Archaic the sites of Canímar Abajo, Cueva Calero, Cueva Perico, and Playa del Mango also showed signs of substructure (Supplementary Data 6). Of note is Playa del Mango, for which all 60 tests produced an average Z=9.194, agreeing with the site's outlier position in *TreeMix* (Figure S27 and S28).

For Extended Data Fig. 3 we allowed as many migration/admixture events ('- m') as necessary until all the admixture events identified for our (sub-)clades in other analysis such as *f*-statistics and *qpAdm* (see sections below) were introduced by *TreeMix*. This happened at '-m 4' where individual I16539 received admixture from *GreaterAntilles_Archaic, also reducing the standard error of the residuals from |12.5| to |6.2|.

## SI9 - Admixture modeling and estimates of ancestry proportions

We first used *qpAdm* to estimate the amount of Archaic-associated ancestry in individuals I16520, I16539 and I7969, who showed higher affinities to *GreaterAntilles_Archaic than the rest of their Ceramic-associated sub-clades (although marginally for I16520). We then attempted to understand if the exclusion of *LesserAntilles_Ceramic, *Haiti_Ceramic and *Curacao_Ceramic from the major *Caribbean_Ceramic clade could possibly be explained by admixture, after finding that these sub-clades initially formed a clade with other Ceramic-associated sites in *qpWave*, but no longer grouped with them upon the application of the model competition approach. In the tables in this section "Anc_X" represents the proportion of ancestry from a source population, and "SE_X" the associated standard error.

***SECoastDR_Ceramic16520, *SECoastDR_Ceramic16539, *EasternGreaterAntilles_Ceramic7969**

Three individuals from two sub-clades within the *Caribbean_Ceramic major clade (I16520, I16539 and I7969) had some evidence of Archaic-related ancestry. Two individuals, *SECoastDR_Ceramic16520 and *SECoastDR_Ceramic16539, fall on the PCA at the border of the *Caribbean_Ceramic* cluster in the direction of the *GreaterAntilles_Archaic cluster. We first tested if these individuals might be outliers using *qpAdm*, beginning by confirming that these individuals formed a clade with the sub-clade to which they were initially assigned by the method explained above, and then adding *GreaterAntilles_Archaic to the "Right." If the latter contributed ancestry to these individuals, it is expected that the previously-working model would now fail (i.e., $p<0.05$, although we allow a buffer zone between 0.01 and 0.05 in cases where standard errors are substantially reduced during model competition). We also tested both *Cuba_Archaic and *Dominican_Andres_Archaic separately as sources of Archaic-related ancestry, with the source on the "Left" added to the "Right."

Individual I16539 was modeled as having between 11.8±1.9% and 14.7±3.0% Archaic-related ancestry, depending on the Archaic source used (*Dominican_Andres_Archaic and *Cuba_CanimarAbajo_Archaic, respectively); the remaining ancestry was *SECoastDR_Ceramic-related (Table S9**)**. We discuss the model with the lower standard error in the main manuscript.

In contrast, we could not reject the 1-way models for the other two individuals (I7969 and I16520) using their own sub-clades as sources. Although the tests with *GreaterAntilles_Archaic on the "Right" did reduce the p-value of the 1-way models to under 0.05 (but still within the buffer zone above 0.01), the 2-way models with the Ceramic-associated source plus *GreaterAntilles_Archaic produce proportions of ancestry of the latter consistent with or below 0 (Table S9). As we could not clearly support their outlier status, we merged individuals I7969 and I16520 back into their sub-clades for all subsequent analyses and renamed the admixed individual I16539 as *SECoastDR_Ceramic16539.

**Table S9: Modeling the Ceramic- and Archaic-related ancestries of *SECoastDR_Ceramic16520, *SECoastDR_Ceramic16539 and *EasternGreaterAntilles_Ceramic7969.**

| *Test* | A | B | Sources on "Right" | p-value | Anc_A | Anc_B | SE_A | SE_B |
|---|---|---|---|---|---|---|---|---|
| *SECoastDR_Ceramic 16520 | *SECoastDR_Ceramic | - | - | 0.237 | 1.000 | - | 0.000 | - |
| *SECoastDR_Ceramic 16520 | *SECoastDR_Ceramic | - | *GreaterAntilles_Archaic | 0.045 | 1.000 | - | 0.000 | - |
| *SECoastDR_Ceramic 16520 | *SECoastDR_Ceramic | *GreaterAntilles_Archaic | - | 0.179 | 1.000 | -0.000 | 0.113 | 0.113 |
| *SECoastDR_Ceramic 16539 | *SECoastDR_Ceramic | - | - | 0.900 | 1.000 | - | 0.000 | - |
| *SECoastDR_Ceramic 16539 | *SECoastDR_Ceramic | - | *GreaterAntilles_Archaic | 7.12E-06 | 1.000 | - | 0.000 | - |
| *SECoastDR_Ceramic 16539 | *SECoastDR_Ceramic | *GreaterAntilles_Archaic | - | 0.951 | 0.871 | 0.129 | 0.103 | 0.103 |
| *SECoastDR_Ceramic 16539 | *SECoastDR_Ceramic | *Cuba_Archaic | *Dominican_Andres_Archaic | 0.922 | 0.853 | 0.147 | 0.030 | 0.030 |
| *SECoastDR_Ceramic 16539 | *SECoastDR_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.876 | 0.882 | 0.118 | 0.019 | 0.019 |
| *EasternGreaterAntilles _Ceramic7969 | *EasternGreaterAntilles _Ceramic | - | - | 0.737 | 1.000 | - | 0.000 | - |
| *EasternGreaterAntilles _Ceramic7969 | *EasternGreaterAntilles _Ceramic | - | *GreaterAntilles_Archaic | 0.034 | 1.000 | - | 0.000 | - |
| *EasternGreaterAntilles _Ceramic7969 | *EasternGreaterAntilles _Ceramic | *GreaterAntilles_Archaic | - | 0.680 | 1.041 | -0.041 | 0.104 | 0.104 |

***LesserAntilles_Ceramic**

We started by identifying the lowest-rank valid models for *LesserAntilles_Ceramic using the three major clades as sources, and then individually added each of the remaining clades to the "Right." As above, if those clades contributed ancestry to the test population, it is expected that the previously-working model would now fail. The 1-way model for the pool of the other *Caribbean_Ceramic sub-clades was found to be valid (p=0.083), but the inclusion of either *GreaterAntilles_Archaic or *Venezuela_Ceramic on the "Right" caused that model to fail (Table S10). However, all non-Ceramic-related ancestry proportion estimates on these 2-way models were small (<1.7%) and always at least four times smaller than the corresponding standard errors, suggesting that the 1-way model with only *Caribbean_Ceramic ancestry cannot be ruled out. Statistics of the form $f_4$(Mbuti, *GreaterAntilles_Archaic/*Venezuela_Ceramic; *LesserAntilles_Ceramic, *OtherCeramicSubClades) also suggest no specific attraction of *LesserAntilles_Ceramic to *GreaterAntilles_Archaic or *Venezuela_Ceramic when compared to the remaining *Caribbean_Ceramic sub-clades (Table S11). Therefore, *LesserAntilles_Ceramic was kept as one of the sub-clades composing *Caribbean_Ceramic.

**Table S10: Modeling *LesserAntilles_Ceramic with three major ancestry components: *Caribbean_Ceramic (composed of the sub-clades from the Greater Antilles), *GreaterAntilles_Archaic, and *Venezuela_Ceramic.**

| A | B | C | Sources on "Right" | p-value | Anc_A | Anc_B | Anc_C | SE_A | SE_B | SE_C |
|---|---|---|---|---|---|---|---|---|---|---|
| *Caribbean_Ceramic_excl_LesserAntilles | - | - | - | 0.083 | 1.000 | - | - | 0.000 | - | - |
| *Caribbean_Ceramic_excl_LesserAntilles | - | - | *GreaterAntilles_Archaic | 0.001 | 1.000 | - | - | 0.000 | - | - |
| *Caribbean_Ceramic_excl_LesserAntilles | - | - | *Venezuela_Ceramic | 3.94E-06 | 1.000 | - | - | 0.000 | - | - |
| *Caribbean_Ceramic_excl_LesserAntilles | *GreaterAntilles_Archaic | - | - | 0.068 | 0.983 | 0.017 | - | 0.042 | 0.042 | - |
| *Caribbean_Ceramic_excl_LesserAntilles | *GreaterAntilles_Archaic | - | *Venezuela_Ceramic | 3.35E-06 | 0.993 | 0.007 | - | 0.044 | 0.044 | - |
| *Caribbean_Ceramic_excl_LesserAntilles | *GreaterAntilles_Archaic | *Venezuela_Ceramic | - | 0.042 | 0.974 | 0.046 | -0.020 | 0.042 | 0.075 | 0.052 |
| *Caribbean_Ceramic_excl_LesserAntilles | *Venezuela_Ceramic | - | - | 0.047 | 0.993 | 0.007 | - | 0.028 | 0.028 | - |
| *Caribbean_Ceramic_excl_LesserAntilles | *Venezuela_Ceramic | - | *GreaterAntilles_Archaic | 0.002 | 0.998 | 0.002 | - | 0.029 | 0.029 | - |
| *GreaterAntilles_Archaic | - | | - | 2.64E-28 | 1.000 | - | - | 0.000 | - | - |
| *GreaterAntilles_Archaic | *Venezuela_Ceramic | - | - | 4.67E-14 | 2.474 | -1.474 | - | 0.535 | 0.535 | - |
| *Venezuela_Ceramic | - | | - | 8.39E-69 | 1.000 | - | - | 0.000 | - | - |

**Table S11: Testing the affinities of *GreaterAntilles_Archaic and *Venezuela_Ceramic to the different *Caribbean_Ceramic sub-clades.**

| Pop X | Pop Y | Pop W | Pop Z | f4 | SE | Z | SNPs |
|---|---|---|---|---|---|---|---|
| Mbuti | *GreaterAntilles_Archaic | *LesserAntilles_Ceramic | *BahamasCuba_Ceramic | 0.000212 | 0.000149 | 1.422 | 1072438 |
| Mbuti | *GreaterAntilles_Archaic | *LesserAntilles_Ceramic | *SECoastDR_Ceramic | 0.000229 | 0.000135 | 1.702 | 1075649 |
| Mbuti | *GreaterAntilles_Archaic | *LesserAntilles_Ceramic | *EasternGreaterAntilles_Ceramic | 0.000242 | 0.000134 | 1.805 | 1075254 |
| Mbuti | *Venezuela_Ceramic | *LesserAntilles_Ceramic | *BahamasCuba_Ceramic | 0.000259 | 0.000134 | 1.931 | 952025 |
| Mbuti | *Venezuela_Ceramic | *LesserAntilles_Ceramic | *SECoastDR_Ceramic | 0.000526 | 0.000124 | 4.232 | 951776 |
| Mbuti | *Venezuela_Ceramic | *LesserAntilles_Ceramic | *EasternGreaterAntilles_Ceramic | 0.000418 | 0.000125 | 3.334 | 952688 |

***Haiti_Ceramic**

A 1-way model for *Haiti_Ceramic with *Caribbean_Ceramic as the source was initially valid, but the addition of *GreaterAntilles_Archaic to the "Right" caused it to fail, suggesting the presence of Archaic-related ancestry in *Haiti_Ceramic (Table S12). Therefore, we added *GreaterAntilles_Archaic as a source together with *Caribbean_Ceramic and tested a 2-way model, which we found to be valid even with *Venezuela_Ceramic on the "Right." The final admixture model for *Haiti_Ceramic based on the three major clades was between 85.4±8.6% and 87.1±8.5% *Caribbean_Ceramic-related ancestry and 14.6±8.6% and 12.9±8.5% *GreaterAntilles_Archaic-related ancestry (p=0.180; see below for more precise mixture proportion estimates). The presence of admixture explains why they do not form a clade with any other group during the *qpWave* analysis.

**Table S12: Modeling *Haiti_Ceramic with three major ancestry components - *Caribbean_Ceramic, *GreaterAntilles_Archaic, and *Venezuela_Ceramic.**

| A | B | Sources on "Right" | p-value | Anc_A | Anc_B | SE_A | SE_B |
|---|---|---|---|---|---|---|---|
| *Caribbean_Ceramic | - | - | 0.169 | 1.000 | - | 0.000 | - |
| *Caribbean_Ceramic | - | *GreaterAntilles_Archaic | 2.11E-26 | 1.000 | - | 0.000 | - |
| *Caribbean_Ceramic | - | *Venezuela_Ceramic | 0.125 | 1.000 | - | 0.000 | - |
| *Caribbean_Ceramic | *GreaterAntilles_Archaic | - | 0.288 | 0.854 | 0.146 | 0.086 | 0.086 |
| *Caribbean_Ceramic | *GreaterAntilles_Archaic | *Venezuela_Ceramic | 0.180 | 0.871 | 0.129 | 0.085 | 0.085 |
| *GreaterAntilles_Archaic | - | - | 3.29E-13 | 1.000 | - | 0.000 | - |
| *Venezuela_Ceramic | - | - | 1.30E-21 | 1.000 | - | 0.000 | - |

We then explored the possibility of improving model fit using the specific sub-clades within the two major clades identified as sources for *Haiti_Ceramic (*Caribbean_Ceramic and *GreaterAntilles_Archaic). Two-way models using the sub-clades *BahamasCuba_Ceramic, *EasternGreaterAntilles_Ceramic, *SECoastDR_Ceramic, and *LesserAntilles_Ceramic within *Caribbean_Ceramic and either *Cuba_Archaic or *Dominican_Andres_Archaic, produced valid models with p>0.080 (Table S13). The addition of the four unused sources to the "Right" of the working 2-way models caused the p-values to decrease to below the threshold of 0.05, but still above 0.01 for the models with *BahamasCuba_Ceramic/*EasternGreaterAntilles_Ceramic and *Cuba_Archaic as sources. Standard errors were also substantially reduced when unused sources were added to the "Right." The model with *BahamasCuba_Ceramic as the Ceramic-related source, for example, had an almost five-times decrease in the standard errors and the highest p-value (0.033). We therefore consider these model competition results as successful but with the caveat that the p-value <0.05 might reflect less optimal models. In Fig. 1b we report the averages of the two model competition models above 0.01 (81.50±2.1% and 18.50±2.1%), as they both constitute the *Caribbean_Ceramic clade.

**Table S13: Modeling *Haiti_Ceramic with groups from within the two major clades identified in Table S12.**

| A | B | Sources on "Right" | p-value | Anc_A | Anc_B | SE_A | SE_B |
|---|---|---|---|---|---|---|---|
| *BahamasCuba_Ceramic | *Cuba_Archaic | - | 0.328 | 0.861 | 0.139 | 0.096 | 0.096 |
| *EasternGreaterAntilles _Ceramic | *Cuba_Archaic | - | 0.287 | 0.866 | 0.134 | 0.091 | 0.091 |
| *SECoastDR_Ceramic | *Cuba_Archaic | - | 0.340 | 0.841 | 0.159 | 0.089 | 0.089 |
| *LesserAntilles_Ceramic | *Cuba_Archaic | - | 0.367 | 0.863 | 0.137 | 0.098 | 0.098 |
| *BahamasCuba_Ceramic | *Dominican_Andres_Archaic | - | 0.155 | 0.891 | 0.109 | 0.104 | 0.104 |
| *EasternGreaterAntilles _Ceramic | *Dominican_Andres_Archaic | - | 0.080 | 0.886 | 0.114 | 0.104 | 0.104 |
| *SECoastDR_Ceramic | *Dominican_Andres_Archaic | - | 0.110 | 0.870 | 0.130 | 0.100 | 0.100 |
| *LesserAntilles_Ceramic | *Dominican_Andres_Archaic | - | 0.118 | 0.889 | 0.111 | 0.104 | 0.104 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *BahamasCuba_Ceramic | *Cuba_Archaic | *EasternGreaterAntilles _Ceramic, *SECoastDR_Ceramic, *LesserAntilles_Ceramic, *Dominican_Andres_Archaic | 0.033 | 0.813 | 0.187 | 0.020 | 0.020 |
| *BahamasCuba_Ceramic | *Dominican_Andres_Archaic | *EasternGreaterAntilles _Ceramic, *SECoastDR_Ceramic, *LesserAntilles_Ceramic, *Cuba_Archaic | 0.005 | 0.818 | 0.182 | 0.014 | 0.014 |
| *EasternGreaterAntilles _Ceramic | *Cuba_Archaic | *BahamasCuba_Ceramic, *SECoastDR_Ceramic, *LesserAntilles_Ceramic *Dominican_Andres_Archaic | 0.021 | 0.817 | 0.183 | 0.021 | 0.021 |
| *EasternGreaterAntilles _Ceramic | *Dominican_Andres_Archaic | *BahamasCuba_Ceramic, *SECoastDR_Ceramic, *LesserAntilles_Ceramic *Cuba_Archaic | 0.003 | 0.818 | 0.182 | 0.014 | 0.014 |
| *SECoastDR_Ceramic | *Cuba_Archaic | *BahamasCuba_Ceramic, *EasternGreaterAntilles _Ceramic, *LesserAntilles_Ceramic, *Dominican_Andres_Archaic | 0.007 | 0.815 | 0.150 | 0.021 | 0.021 |
| *SECoastDR_Ceramic | *Dominican_Andres_Archaic | *BahamasCuba_Ceramic, *EasternGreaterAntilles _Ceramic, *LesserAntilles_Ceramic *Cuba_Archaic | 0.001 | 0.817 | 0.183 | 0.014 | 0.014 |
| *LesserAntilles_Ceramic | *Cuba_Archaic | *BahamasCuba_Ceramic, *EasternGreaterAntilles _Ceramic, *SECoastDR_Ceramic, *Dominican_Andres_Archaic | 1.70E-06 | 0.855 | 0.145 | 0.024 | 0.024 |
| *LesserAntilles_Ceramic | *Dominican_Andres_Archaic | *BahamasCuba_Ceramic, *EasternGreaterAntilles _Ceramic, *SECoastDR_Ceramic, *Cuba_Archaic | 2.09E-05 | 0.832 | 0.169 | 0.015 | 0.015 |

***Curacao_Ceramic**

No 1-way models were valid for *Curacao_Ceramic, but a 2-way model using *Caribbean_Ceramic (68.0±5.1%) and *Venezuela_Ceramic (32.0±5.1%) produced a good fit (p=0.298) (Table S14). The addition of the unused source, *GreaterAntilles_Archaic, to the "Right" reduced the model's *p*-value to between 0.01 and 0.05, possibly due to excess allele sharing and/or gene flow with one of the source populations. This is shown by the highly positive *gendstats* results from *qpAdm*'s output for *GreaterAntilles_Archaic (Z-score average=2.290, median=2.269, maximum=3.371, minimum=0.661), based on statistics of the form $f_4$(*Curacao_Ceramic, *Caribbean_Ceramic+*Venezuela_Ceramic; Right1, *GreaterAntilles_Archaic), where Right1 are the 13 modern populations from the base "Right" set, and *Caribbean_Ceramic+*Venezuela_Ceramic represents a modeled mixture of the two sources.

**Table S14: Modeling *Curacao_Ceramic with three major ancestry components - *Caribbean_Ceramic, *GreaterAntilles_Archaic, and *Venezuela_Ceramic.**

| A | B | Sources on "Right" | p-value | Anc_A | Anc_B | SE_A | SE_B |
|---|---|---|---|---|---|---|---|
| *Caribbean_Ceramic | - | - | 1.29E-07 | 1.000 | - | 0.000 | - |
| *Caribbean_Ceramic | *GreaterAntilles_Archaic | - | 1.64E-05 | 0.693 | 0.307 | 0.108 | 0.108 |
| *Caribbean_Ceramic | *Venezuela_Ceramic | - | 0.298 | 0.680 | 0.320 | 0.051 | 0.051 |
| *Caribbean_Ceramic | *Venezuela_Ceramic | *GreaterAntilles_Archaic | 0.048 | 0.686 | 0.314 | 0.051 | 0.051 |
| *GreaterAntilles_Archaic | - | - | 9.55E-13 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | *Venezuela_Ceramic | - | 1.54E-13 | 1.031 | -0.031 | 0.262 | 0.262 |
| *Venezuela_Ceramic | - | - | 8.16E-22 | 1.000 | - | 0.000 | - |

To investigate this suggestion of an imperfect model fit further, we used the *Caribbean_Ceramic sub-clades to improve model fits. While we find that all fit equally well without model competition (Table S15)—consistent with the genetic homogeneity observed within the *Caribbean_Ceramic* clade—with model competition, the only working model is the one for *LesserAntilles_Ceramic (p=0.362), and we reported these admixture proportions in the manuscript. We note that this final model also works with *GreaterAntilles_Archaic on the "Right" (p=0.345).

**Table S15: Modeling *Curacao_Ceramic with groups from within the two major ancestry clades identified in Table S14.**

| A | B | Sources on "Right" | p-value | Anc_A | Anc_B | SE_A | SE_B |
|---|---|---|---|---|---|---|---|
| *Bahamas_Ceramic | *Venezuela_Ceramic | - | 0.330 | 0.688 | 0.312 | 0.052 | 0.052 |
| *EasternGreaterAntilles_Ceramic | *Venezuela_Ceramic | - | 0.230 | 0.681 | 0.319 | 0.053 | 0.053 |
| *SECoastDR_Ceramic | *Venezuela_Ceramic | - | 0.334 | 0.679 | 0.321 | 0.052 | 0.052 |
| *LesserAntilles_Ceramic | *Venezuela_Ceramic | - | 0.371 | 0.684 | 0.316 | 0.054 | 0.054 |
| *Bahamas_Ceramic | *Venezuela_Ceramic | *SECoastDR_Ceramic, *EasternGreaterAntilles_Ceramic | 7.06E-04 | 0.519 | 0.481 | 0.026 | 0.026 |
| *EasternGreaterAntilles_Ceramic | *Venezuela_Ceramic | *Bahamas_Ceramic, *SECoastDR_Ceramic | 9.09E-04 | 0.526 | 0.474 | 0.026 | 0.026 |
| *SECoastDR_Ceramic | *Venezuela_Ceramic | *Bahamas_Ceramic, *EasternGreaterAntilles_Ceramic | 1.84E-04 | 0.493 | 0.507 | 0.025 | 0.025 |
| *LesserAntilles_Ceramic | *Venezuela_Ceramic | *Bahamas_Ceramic, *EasternGreaterAntilles_Ceramic, *SECoastDR_Ceramic | 0.362 | 0.745 | 0.255 | 0.037 | 0.037 |

**Distal modeling with literature samples**

For the distal analysis we used the following previously published individuals/populations (Lindo et al. 2018; Moreno-Mayar et al. 2018; Scheib et al. 2018; Posth et al. 2018) with more than 100,000 SNPs as possible sources for the three major *qpWave* clades:

Argentina_ArroyoSeco2_7700BP, Argentina_LagunaChica_6800BP, Belize_MayahakCabPek_9300BP, Belize_SakiTzul_7400BP, Brazil_LapaDoSanto_9600BP, Brazil_Laranjal_6700BP, Brazil_Moraes_5800BP, Brazil_Sumidouro_10100BP, Chile_LosRieles_12000BP, Chile_LosRieles_5100BP, Chile_PuntaSantaAna_7300BP, Chile_Ayayema_5100BP, Peru_Cuncaicha_4200BP, Peru_Cuncaicha_9000BP, Peru_LaGalgada_4100BP, Peru_Lauricocha_3500BP, Peru_Lauricocha_5800BP, Peru_Lauricocha_8600BP, USA_CA_Early_SanNicolas

We first identified which source populations were involved in passing models of the lowest possible rank (i.e., minimum number of sources) with a threshold of p>0.05. We then re-ran the same tests moving the unused sources to the "Right" to increase the power of the tests (Table S16).

For *GreaterAntilles_Archaic, the shared ancestry between Brazil_Moraes_5800BP and Brazil_Laranjal_6700BP meant that their previously working 1-way models failed during model competition. Thus, we removed the lower coverage individual (Brazil_Moraes_5800BP) and repeated

these analyses. In this new model competition step, the 1-way model for Brazil_Laranjal_6700BP (p=0.271) was the only model with p>0.05, which in principle would be consistent with an affinity between *GreaterAntilles_Archaic and ancient Brazilian populations (Table S16). However, statistics of the form $f_4$(Mbuti, *GreaterAntilles_Archaic, Brazil_Laranjal_6700BP, Test), with Test as the five other sources, do not support this finding (|Z|<1.736), as expected if the fit was simply a reflection of the limited data from Brazil_Laranjal_6700BP (making it difficult to reject a model that was successfully rejected in the other cases) (Table S17).

For *Caribbean_Ceramic and *Venezuela_Ceramic we found no valid models.

**Table S16: Results of *qpAdm* modeling using ancient published data for the major clades defined in this work.**

| Test | A | B | Sources on "Right" | p-value | Anc_A | Anc_B | SE_A | SE_B |
|---|---|---|---|---|---|---|---|---|
| *GreaterAntilles_Archaic | Belize_SakiTzul_7400BP | - | - | 0.052 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | Brazil_LapaDoSanto _9600BP | - | - | 0.060 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | Brazil_Laranjal_6700BP | - | - | 0.262 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | Brazil_Moraes_5800BP | - | - | 0.666 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | Chile_Ayayema_5100BP | - | - | 0.522 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | Chile_PuntaSantaAna _7300BP | - | - | 0.172 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | Belize_SakiTzul_7400BP | - | Brazil_LapaDoSanto_9600BP, Brazil_Laranjal_6700BP, Chile_Ayayema_5100BP, Chile_PuntaSantaAna_7300BP | 0.004 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | Brazil_LapaDoSanto_9600BP | - | Belize_SakiTzul_7400BP, Brazil_Laranjal_6700BP, Chile_Ayayema_5100BP, Chile_PuntaSantaAna_7300BP | 0.001 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | Brazil_Laranjal_6700BP | - | Brazil_LapaDoSanto_9600BP, Belize_SakiTzul_7400BP, Chile_Ayayema_5100BP, Chile_PuntaSantaAna_7300BP | 0.271 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | Chile_Ayayema_5100BP | - | Brazil_LapaDoSanto_9600BP, Brazil_Laranjal_6700BP, Belize_SakiTzul_7400BP, Chile_PuntaSantaAna_7300BP | 1.47E-16 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | Chile_PuntaSantaAna _7300BP | - | Brazil_LapaDoSanto_9600BP, Brazil_Laranjal_6700BP, Chile_Ayayema_5100BP, Belize_SakiTzul_7400BP | 3.57E-19 | 1.000 | - | 0.000 | - |

**Table S17: Results of statistics of the form $f_4$(Mbuti, *GreaterAntilles_Archaic, Brazil_Laranjal_6700BP, Test), with *Test* as the five other possible sources for *GreaterAntilles_Archaic as in Table S16.**

| Pop X | Pop Y | Pop W | Pop Z | f4 | SE | Z | SNPs |
|---|---|---|---|---|---|---|---|
| Mbuti | *GreaterAntilles_Archaic | Brazil_Laranjal_6700BP | Belize_SakiTzul_7400BP | -0.000294 | 0.000495 | -0.593 | 379662 |
| Mbuti | *GreaterAntilles_Archaic | Brazil_Laranjal_6700BP | Brazil_LapaDoSanto_9600BP | -0.000630 | 0.000363 | -1.736 | 558436 |
| Mbuti | *GreaterAntilles_Archaic | Brazil_Laranjal_6700BP | Brazil_Moraes_5800BP | 0.000313 | 0.000541 | 0.578 | 204716 |
| Mbuti | *GreaterAntilles_Archaic | Brazil_Laranjal_6700BP | Chile_Ayayema_5100BP | -0.000473 | 0.000498 | -0.948 | 580448 |
| Mbuti | *GreaterAntilles_Archaic | Brazil_Laranjal_6700BP | Chile_PuntaSantaAna_7300BP | -0.000339 | 0.000485 | -0.700 | 454336 |

**Distal modeling using present-day populations**

We also ran *qpAdm* to study the ancestry of the three major clades using present-day populations representing different language groups and regions. As we had been using these populations as the "Right" of all previous *qpAdm* and *qpWave* analyses, here we tested all 1-, 2-, and 3-way models of the populations from the "Right", moving them to the "Left" as necessary. We did not use Chipewyan in these models as a source due to their distance from ancient Caribbean groups (as shown in previous *f*-statistics results), and also excluded Wayuu and Yukpa due to evidence of admixture. We present all fitting results for both 1- and 2-way models; although some of our three major clades had working 1-way models, the models from the rank above can provide insights into the validity of the lower ranks in poorly modeled situations (Table S18). We also confirmed the results by using the same but unmasked/unadmixed populations from the Illumina dataset (Reich et al. 2012), to the best possible match; only Apalai and Mixtec are missing from this dataset (Table S19).

**Table S18: Results of 1- and 2-way *qpAdm* modeling using present-day populations from the Human Origins dataset for the three major clades defined in this work.**

| Test | A | B | p-value | Anc_A | Anc_B | SE_A | SE_B |
|---|---|---|---|---|---|---|---|
| *Caribbean_Ceramic | Piapoco | - | 0.525 | 1.000 | - | 0.000 | - |
| *Caribbean_Ceramic | Piapoco | Quechua | 0.573 | 0.930 | 0.070 | 0.050 | 0.050 |
| *GreaterAntilles_Archaic | Karitiana | Quechua | 0.130 | 0.225 | 0.775 | 0.048 | 0.048 |
| *GreaterAntilles_Archaic | Surui | Quechua | 0.091 | 0.177 | 0.823 | 0.046 | 0.046 |
| *Venezuela_Ceramic | Cabecar | - | 0.144 | 1.000 | - | 0.000 | - |
| *Venezuela_Ceramic | Cabecar | Apalai | 0.165 | 0.911 | 0.089 | 0.064 | 0.064 |
| *Venezuela_Ceramic | Cabecar | Arara | 0.122 | 0.931 | 0.069 | 0.053 | 0.053 |
| *Venezuela_Ceramic | Cabecar | Karitiana | 0.095 | 0.933 | 0.067 | 0.060 | 0.060 |
| *Venezuela_Ceramic | Cabecar | Surui | 0.134 | 0.924 | 0.076 | 0.049 | 0.049 |

*Caribbean_Ceramic could be modeled as a 1-way model with the Arawak-speaking Piapoco (p=0.525). Upon looking at the working 2-way models, we find support for this result as Piapoco still contributes the great majority of ancestry to *Caribbean_Ceramic (93.0±5.0%). The results were unchanged when we ran the same tests on the unmasked and unadmixed populations from the Illumina dataset (Reich et al. 2012) (Table S19). These results are consistent with the hypothesis of an origin of *Caribbean_Ceramic in Arawak-speaking groups from northeastern South America, as is also suggested by the *TreeMix* and *qpGraph* results. When considered in parallel with the results from outgroup $f_3$-statistics (Fig. 2a; Supplementary Information section 11; Supplementary Data 10) it is possible that a more coastal Arawak-speaking population, such as the Palikur, is more closely related to the true source, although the Piapoco do occupy inland regions of the Orinoco basin.

Two working 2-way models involving a major component of Quechua-related ancestry (77.5±4.8% to 82.3±4.6%) were found for *GreaterAntilles_Archaic, with an Amazonian source such as Suruí or Karitiana as the second source. The results on the Illumina dataset identify valid 1-way models for both the Quechua and Cabécar, and 2-way models with these populations as one of the sources (Table S19). These results are consistent with the lack of affinity between *GreaterAntilles_Archaic and

populations from a particular language family (Fig. 2a, 2b; Supplementary Information section 11; Supplementary Data 10 and 11) and with *TreeMix* results (Extended Data Fig. 3), all of which point to an early split position relative to other South and Central American populations.

*Venezuela_Ceramic could be modeled successfully with *Cabécar as the single source, a result that is supported by additional analyses, as 1) all 2-way models require most ancestry to be derived from the Chibchan-speaking *Cabécar (91.1±6.4% to 93.3±6.0%), 2) the same result was found on the Illumina dataset populations (Table S19), and 3) *Venezuela_Ceramic clearly shares more drift with Chibchan-speaking groups, as shown by *f*-statistics (Fig. 2a, 2b; Supplementary Information section 11; Supplementary Data 10 and 11).

**Table S19: Results of *qpAdm* models using present-day populations from the Illumina dataset for the three major clades defined in this work.**

| Test | A | B | p-value | Anc_A | Anc_B | SE_A | SE_B |
|---|---|---|---|---|---|---|---|
| *Caribbean_Ceramic | *Piapoco | - | 0.281 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | *Cabecar | - | 0.094 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | *Quechua | - | 0.624 | 1.000 | - | 0.000 | - |
| *GreaterAntilles_Archaic | *Cabecar | *Piapoco | 0.068 | 0.814 | 0.186 | 0.146 | 0.146 |
| *GreaterAntilles_Archaic | *Cabecar | *Karitiana | 0.140 | 0.852 | 0.148 | 0.073 | 0.073 |
| *GreaterAntilles_Archaic | *Cabecar | *Arara | 0.076 | 0.892 | 0.108 | 0.087 | 0.087 |
| *GreaterAntilles_Archaic | *Quechua | *Surui | 0.575 | 0.916 | 0.084 | 0.077 | 0.077 |
| *Venezuela_Ceramic | *Cabecar | - | 0.460 | 1.000 | - | 0.000 | - |

**Testing for Differences in Archaic-related Ancestry in the Ceramic Clades**

We used the statistic $f_4$(*Mbuti, *GreaterAntilles_Archaic, Sub_Clade1, Sub_Clade2) to directly test if any specific *Caribbean_Ceramic sub-clade had more Archaic-related ancestry than another, but found that no comparisons produced a Z-score above 2.8, with the exception of ones involving I16539 (Table S20), an individual who has evidence of admixture between Archaic- and Ceramic-related ancestry (shown in Table S9). The strongest signals that we observe that do not involve I16539 are only in the suggestive range of Z=-1.5 to -1.8; these signals consist of all the comparisons of *LesserAntilles_Ceramic to the subclades of *Caribbean_Ceramic, with *GreaterAntilles_Archaic sharing fewest alleles with *LesserAntilles_Ceramic.

**Table S20: Comparing the affinities of *GreaterAntilles_Archaic to the *Caribbean_Ceramic sub-clades, after excluding the identified genetic outlier individual I16539.**

| Pop X | Pop Y | Pop W | Pop Z | f4 | SE | Z | SNPs |
|---|---|---|---|---|---|---|---|
| Mbuti | *GreaterAntilles_Archaic | *Bahamas_Ceramic | *SECoastDR_Ceramic | -0.000037 | 0.000098 | -0.373 | 1099930 |
| Mbuti | *GreaterAntilles_Archaic | *Bahamas_Ceramic | *SECoastDR_Ceramic16539 | 0.002051 | 0.000394 | 5.21 | 699455 |
| Mbuti | *GreaterAntilles_Archaic | *Bahamas_Ceramic | *EasternGreaterAntilles_Ceramic | -0.000078 | 0.000103 | -0.754 | 1098814 |
| Mbuti | *GreaterAntilles_Archaic | *Bahamas_Ceramic | *LesserAntilles_Ceramic | -0.000262 | 0.000145 | -1.808 | 1087064 |
| Mbuti | *GreaterAntilles_Archaic | *SECoastDR_Ceramic | *SECoastDR_Ceramic16539 | 0.002111 | 0.000384 | 5.502 | 699782 |
| Mbuti | *GreaterAntilles_Archaic | *SECoastDR_Ceramic | *EasternGreaterAntilles_Ceramic | -0.000037 | 0.000081 | -0.448 | 1104504 |
| Mbuti | *GreaterAntilles_Archaic | *SECoastDR_Ceramic | *LesserAntilles_Ceramic | -0.000225 | 0.000132 | -1.703 | 1091659 |
| Mbuti | *GreaterAntilles_Archaic | *SECoastDR_Ceramic16539 | *EasternGreaterAntilles_Ceramic | -0.002096 | 0.000385 | -5.448 | 699724 |
| Mbuti | *GreaterAntilles_Archaic | *SECoastDR_Ceramic16539 | *LesserAntilles_Ceramic | -0.002209 | 0.000394 | -5.609 | 697547 |
| Mbuti | *GreaterAntilles_Archaic | *EasternGreaterAntilles_Ceramic | *LesserAntilles_Ceramic | -0.0002 | 0.000132 | -1.512 | 1090797 |

To further investigate this suggestive signal of lower Archaic-related ancestry in *LesserAntilles_Ceramic relative to other Ceramic-associated individuals, we used *qpAdm* to test each pair of *Caribbean_Ceramic sub-clades as consistent with deriving most ancestry from the other sub-clades, using our standard "Right" set of reference populations supplemented with both *Cuba_Archaic and *Dominican_Andres_Archaic to increase our sensitivity to subtle differences in Archaic-related ancestry. Table S21 shows the possible comparisons of the four *Caribbean_Ceramic subclades with each other. The 1-way models involving *LesserAntilles_Ceramic *and* the Greater Antilles Ceramic sub-clades did not pass a threshold of 0.05 or 0.01, providing strong motivation to examine 2-way mixture models for the Greater Antilles Ceramic subclades.

To model each of the *Caribbean_Ceramic sub-clades as one of the others plus a source of Archaic-related ancestry we chose *Dominican_Andres_Archaic over *Cuba_Archaic, because it provided lower standard errors in previous model explorations and was plausibly (due to geography) more closely related to the true source of the Archaic-related ancestry in Hispaniola, Puerto Rico, and The Bahamas. While in 1-way (clade test) models, only *BahamasCuba_Ceramic, *EasternGreaterAntilles_Ceramic, and *SECoastDR_Ceramic can be modeled with each other as a single source, models for *LesserAntilles_Ceramic do not fit as a clade with the others. Table S21 shows that they can be used as fitting sources to fit the Greater Antilles Ceramic subclades, however, when the target is *BahamasCuba_Ceramic (p=0.086, 1.9±0.7% Archaic), *EasternGreaterAntilles_Ceramic (p=0.03, 1.5±0.6% Archaic), or *SECoastDR_Ceramic (p=0.072, 1.6±0.6% Archaic).

**Table S21: Results of *qpAdm* assessing Archaic-related ancestry in the *Caribbean_Ceramic sub-clades.**

| Test | A | B | Base "Right" plus... | p-value | Anc_A | Anc_B | SE_A | SE_B |
|---|---|---|---|---|---|---|---|---|
| *BahamasCuba_Ceramic | *EasternGreaterAntilles_Ceramic | | *Cuba_Archaic, *Dominican_Andres_Archaic | 0.934 | 1.000 | - | 0.000 | - |
| *BahamasCuba_Ceramic | *EasternGreaterAntilles_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.986 | 0.996 | 0.004 | 0.004 | 0.004 |
| *BahamasCuba_Ceramic | *LesserAntilles_Ceramic | | *Cuba_Archaic, *Dominican_Andres_Archaic | 0.008 | 1.000 | - | 0.000 | - |
| *BahamasCuba_Ceramic | *LesserAntilles_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.086 | 0.981 | 0.019 | 0.007 | 0.007 |
| *BahamasCuba_Ceramic | *SECoastDR_Ceramic | | *Cuba_Archaic, *Dominican_Andres_Archaic | 0.792 | 1.000 | - | 0.000 | - |
| *BahamasCuba_Ceramic | *SECoastDR_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.967 | 0.999 | 0.001 | 0.004 | 0.004 |
| *EasternGreaterAntilles_Ceramic | *BahamasCuba_Ceramic | | *Cuba_Archaic, *Dominican_Andres_Archaic | 0.934 | 1.000 | - | 0.000 | - |
| *EasternGreaterAntilles_Ceramic | *BahamasCuba_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.986 | 1.004 | -0.004 | 0.004 | 0.004 |
| *EasternGreaterAntilles_Ceramic | *LesserAntilles_Ceramic | | *Cuba_Archaic, *Dominican_Andres_Archaic | 0.001 | 1.000 | - | 0.000 | - |
| *EasternGreaterAntilles_Ceramic | *LesserAntilles_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.031 | 0.985 | 0.015 | 0.006 | 0.006 |
| *EasternGreaterAntilles_Ceramic | *SECoastDR_Ceramic | | *Cuba_Archaic, *Dominican_Andres_Archaic | 0.387 | 1.000 | - | 0.000 | - |
| *EasternGreaterAntilles_Ceramic | *SECoastDR_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.586 | 1.004 | -0.004 | 0.003 | 0.003 |
| *SECoastDR_Ceramic | *BahamasCuba_Ceramic | | *Cuba_Archaic, *Dominican_Andres_Archaic | 0.792 | 1.000 | - | 0.000 | - |
| *SECoastDR_Ceramic | *BahamasCuba_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.967 | 1.001 | -0.001 | 0.004 | 0.004 |
| *SECoastDR_Ceramic | *EasternGreaterAntilles_Ceramic | | *Cuba_Archaic, *Dominican_Andres_Archaic | 0.387 | 1.000 | - | 0.000 | - |
| *SECoastDR_Ceramic | *EasternGreaterAntilles_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.585 | 0.996 | 0.004 | 0.003 | 0.003 |
| *SECoastDR_Ceramic | *LesserAntilles_Ceramic | | *Cuba_Archaic, *Dominican_Andres_Archaic | 5.62E-04 | 1.000 | - | 0.000 | - |
| *SECoastDR_Ceramic | *LesserAntilles_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.072 | 0.982 | 0.018 | 0.006 | 0.006 |
| *LesserAntilles_Ceramic | *BahamasCuba_Ceramic | | *Cuba_Archaic, *Dominican_Andres_Archaic | 0.008 | 1.000 | - | 0.000 | - |
| *LesserAntilles_Ceramic | *BahamasCuba_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.088 | 1.02 | -0.02 | 0.007 | 0.007 |
| *LesserAntilles_Ceramic | *EasternGreaterAntilles_Ceramic | | *Cuba_Archaic, *Dominican_Andres_Archaic | 0.001 | 1.000 | - | 0.000 | - |
| *LesserAntilles_Ceramic | *EasternGreaterAntilles_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.032 | 1.015 | -0.015 | 0.006 | 0.006 |
| *LesserAntilles_Ceramic | *SECoastDR_Ceramic | | *Cuba_Archaic, *Dominican_Andres_Archaic | 5.62E-04 | 1.000 | - | 0.000 | - |
| *LesserAntilles_Ceramic | *SECoastDR_Ceramic | *Dominican_Andres_Archaic | *Cuba_Archaic | 0.075 | 1.019 | -0.019 | 0.006 | 0.006 |

Taken together, these results are consistent with the archaeological evidence for a lack of Archaic presence in the Lesser Antilles (Keegan and Hofman 2017), and suggests *LesserAntilles_Ceramic is potentially genetically continuous with (direct descendants with minimal later admixture of) earlier first Ceramic-associated populations that moved into the Greater Antilles and admixed with local populations at a minimal level (up to ~2%).

## SI10 - Uniparental haplogroups

### Mitochondrial (mtDNA) haplogroups

We determined mtDNA haplogroups for the 174 newly-reported individuals included in this study (described in Methods, with data provided in Supplementary Data 9). Figure S29 illustrates the observed distribution of mtDNA haplogroups across the Caribbean and Venezuela. Here we discuss these new haplogroup calls along with those reported in Nägele et al. (2020).

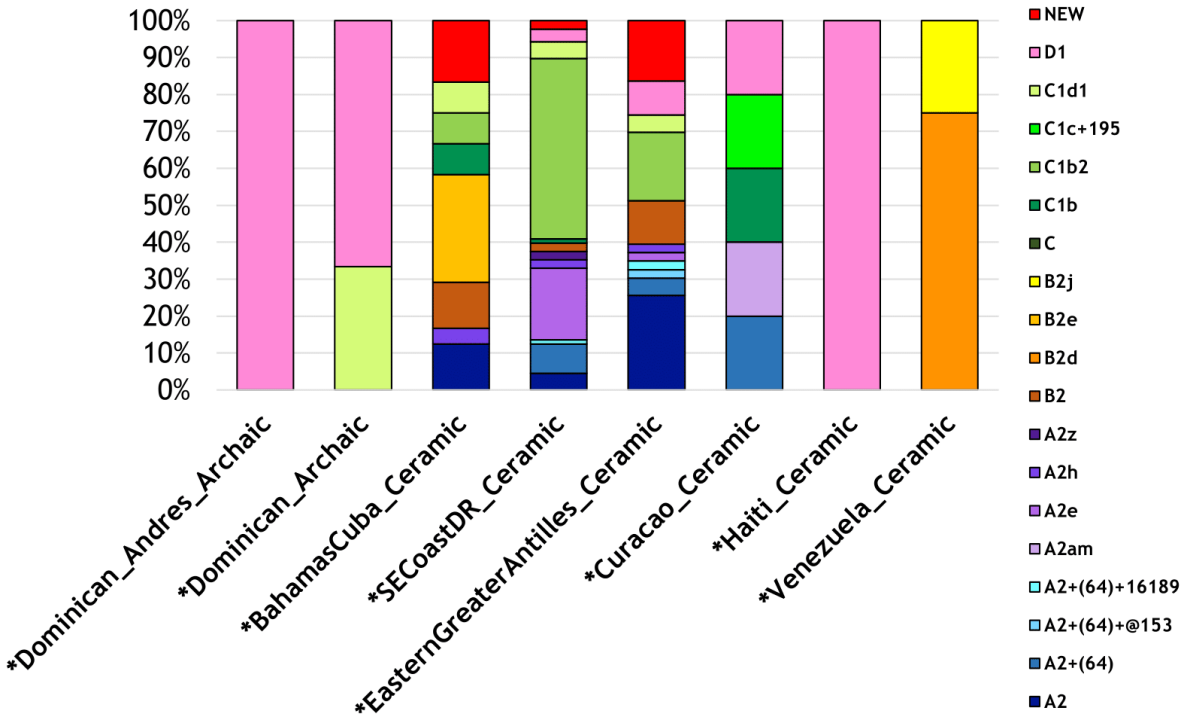



**Figure S29: Distribution of mitochondrial haplogroups for newly-reported individuals across the Caribbean and Venezuela.** Three individuals from the Archaic-associated site of Cueva Roja (Dominican Republic) were not included in the clading analysis due to low coverage, and so are presented here as *Dominican_Archaic.

We find evidence of all four major pan-American haplogroups (A2, B2, C1, and D1; Schurr and Shery 2004; Achilli et al. 2008) among the 174 newly-reported individuals in our dataset, consistent with Nägele et al. Lineages from all four of these haplogroups are present in the 162 Caribbean Ceramic-associated individuals (the earliest of which dates to ~1800 calBP), and are not differentially distributed within the *Caribbean_Ceramic sub-clades defined in this work. Also consistent with Nägele et al., the four Archaic-associated individuals for whom we report data belong to haplogroups D1 and C1d1; these lineages then reduce in frequency among individuals from Ceramic-associated contexts. Interestingly, two admixed individuals from the Ceramic-associated site of Diale 1 in Haiti belong to haplogroup D1, raising the possibility that this mtDNA lineage is associated with the Archaic-related ancestry in these individuals.

As in Nieves-Colón et al. (2020), the most common haplogroup to which the individuals in our dataset belong to is C1b2, with this haplogroup restricted to Ceramic-associated individuals and almost one-third of them belonging to it (in contrast, this haplogroup is called for only six out of 93 individuals reported in Nägele et al.). C1b haplogroups identified in the Greater Antilles have been previously reported to represent biological links to South America (Vilar et al. 2014), with C1b2 identified in Amazonian populations from interior South America as well as Colombia (Williams et al. 2002; Noguera-Santamaría et al. 2015). It has been proposed that the C1b2 lineage has been present in Puerto Rico from the pre-contact era through the present day (Nieves-Colón et al. 2020) where it is currently the most common C1 lineage found (Vilar et al. 2014), and we confirm its ancient presence on the island through its detection in an individual (I13538) from the Ceramic-associated site of Santa Elena. Despite its high frequency, the C1b2 haplogroup is not found in any admixed individuals, including those from Haiti and I16539 who have Archaic-related ancestry, or those from Curaçao who have ancient Venezuela-related ancestry. We note that C1c+195 and A2am lineages are unique in our dataset to individuals from Curaçao, represented in one individual each.

Ten individuals from our dataset belong to the B2 haplogroup, which we find to be absent in the Caribbean prior to the Ceramic Age in both the new data reported here and that reported in Nägele et al. Furthermore, it is not identified in any Archaic-associated people who lived during the Ceramic Age reported in the latter dataset, suggesting that this haplogroup could be associated specifically with ceramic users and was plausibly introduced into the Caribbean during the spread of ceramic technology. While Schroeder and colleagues (2018) report that this haplogroup is rare in the Caribbean today and propose that it may also have been relatively rare in the Caribbean in the past, our finding of this haplogroup in ten Ceramic Age individuals - as well as its identification in Ceramic-associated individuals from The Bahamas and Cuba in Nägele et al. (2020)- suggests that it is more likely that limited pre-Columbian haplotypes persist into the present day (Moreno-Estrada et al. 2013). One interesting observation is that the haplogroup B2e is found in The Bahamas, specifically in the Abaco Islands, Long Island, and Andros Island, but not on Crooked Island or Eleuthera, and also in St. Lucia in the Lesser Antilles, where it is called for three out of the 12 individuals from this site reported in Nägele et al. (2020).

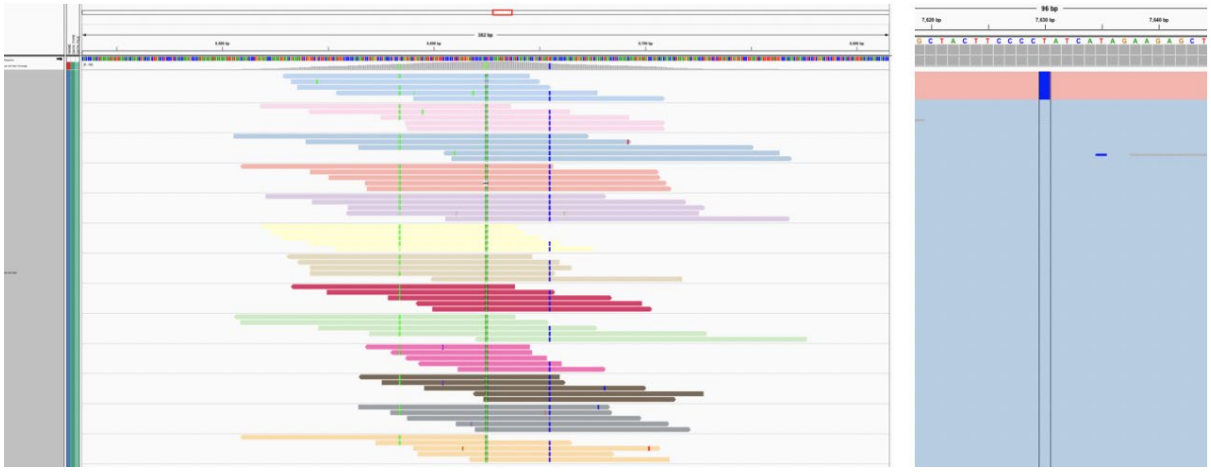
The B2d and B2j lineages are unique in our dataset to Venezuela, and are identified there ~2350 calBP at the beginning of the Caribbean Ceramic Age; however, we do not find evidence of their introduction into the Caribbean. Haplogroup B2j has been previously reported to have evolved in Venezuela ~3,900 years ago (Gómez-Carballa et al. 2012), consistent with its presence in six out of eight individuals from Las Locas.

Our data broadly mirror a present-day pattern in the Caribbean characterized by high frequencies of haplogroups A2 and C1 and lower frequencies of D1 (Mendizabal et al. 2008; Vilar, et al. 2014; Benn-Torres, et al. 2015), with A2 accounting for ~10% of haplogroup calls in our dataset, and lineages of A2 accounting for over 30%, while C1 lineages (C1b, C1b2, C1c+195, and C1d1) accounted for an additional ~40%; however, we do find that D1 is represented at a frequency of ~7%, a non-negligible presence of this haplogroup in the ancient Caribbean. Therefore, it is possible that its scarcity in the

present-day Caribbean is again a consequence of limited pre-Columbian haplotypes persisting into the present day or persisting in different proportions than in the past.

Out of the 174 newly-reported individuals for whom we called mtDNA haplogroups, 13 were ambiguous in their haplogroup assignment (I7974, I7976, I7973, I7972, I14991, I13206, I14990, I14992, I15600, I14879, I13738, I14920, I14921). Therefore, we applied more stringent quality control, restricting to reads with mapping quality ≥ 30 and base quality ≥ 30, and trimming two base pairs to remove deamination artifacts. Further investigation showed that these 13 individuals all supported mutations which were not present in any other individuals in our dataset: G5237A, G6429A, T7630C, C8625A, C11509T, C12346T, G13578A, C15381T, A16335G. Of these, three mutations (T7630C C8625A C11509T; shown in Figure S30) were not only exclusive to these 13 individuals but were also absent from Phylotree version 17. In our ancient individuals, these mutations showed good support from multiple reads and were resilient to aggressive filtering. Further, these mutations are not associated with known differences between rCRS or RSRS mitogenomes, suggesting this is not a mitochondrial reference bias artifact.

T7630C



C8625A

C11509T

**Figure S30: Details of three mutations exclusive to 13 out of 174 individuals in our dataset and not found in Phylotree.** For each of the three mutations, two panels are shown. On the left, a subset of five reads for each of the thirteen individuals carrying the novel haplogroup are shown, each colored uniquely. Unambiguous support for the proposed mutations is shown, not associated with indels, proximity to the ends of the reads or other potential confounders. On the right, mitogenomes for all individuals newly-reported in this study are shown (picking alleles based on a majority rule of pass-filter bases); the thirteen individuals are shown in pink; other individuals are shown in blue.

These 13 individuals were assigned by HaploGrep2 as belonging to haplogroup C, with C1d as the second position call based on the presence of the A16051G mutation diagnostic of this haplogroup. Considering this diagnostic mutation with the additional unique mutations, these data provide evidence of a previously unobserved haplogroup which is a variant of C1d.

We also identify these three mutations in three samples from Nägele et al. (2020) which were undetected in their analyses. These three individuals are all associated with Ceramic contexts and were assigned in Nägele et al. (2020) to the following haplogroups:

- PCV001 (Preachers Cave, The Bahamas): called C1d;
- CDE002 (Cueva de los Esqueletos, Cuba): called C;
- CDE004 (Cueva de los Esqueletos, Cuba): called C

The detection of this lineage in two individuals from Cueva de los Esqueletos expands our understanding of its geographic distribution to include Ceramic Age Cuba as well as The Bahamas and the Dominican Republic. This haplogroup is now documented at four different sites on three Bahamian islands, six different sites in the Dominican Republic, and one site in Cuba, suggesting that it was likely widespread and common throughout the Ceramic Age Caribbean. We did not find this haplogroup in the 52 Archaic-associated individuals from Nägele et al. (2020) or in the four Archaic-associated individuals first reported in this study, providing further support that this is a Ceramic-specific haplogroup that was likely introduced into the Caribbean during the spread of people during the Ceramic Age. Providing additional support for the persistence of pre-contact Indigenous ancestry in present-day Caribbean people, we identified a single Puerto Rican individual from the 1000

Genomes Project dataset (HG01248) that also had these three mutations. This haplogroup remains undocumented outside of the Caribbean.

**Y chromosome haplogroups**

We determined Y chromosome haplogroups for the 96 newly-reported male individuals included in this study (described in Methods, with data provided in Supplementary Data 9). Figure S31 illustrates the observed distribution of Y chromosome haplogroups across the sub-clades in the Caribbean and Venezuela. Here we discuss these new haplogroup calls along with those reported in Nägele et al. (2020).

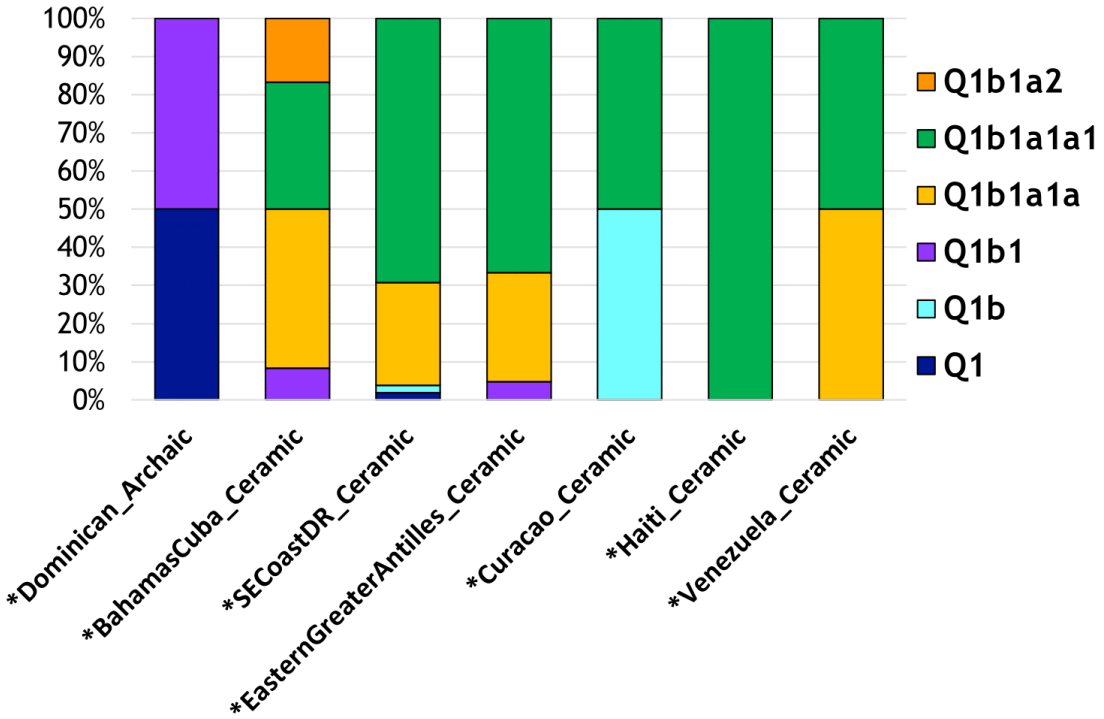



**Figure S31: Distribution of Y chromosome haplogroups across sub-clades in the Caribbean and Venezuela.** Two male individuals from the Archaic-associated site of Cueva Roja (Dominican Republic) were not included in the clading analysis due to low coverage, and so are presented here as *Dominican_Archaic.

Out of 96 Y chromosome haplogroups called for the newly-reported individuals in this study, 87 individuals were assigned to the Q-M3 lineage, with 28 male individuals belonging to Q1b1a1a and 59 to Q1b1a1a1; this is consistent to Nägele et al. (2020) who call Y chromosome haplogroups for 42 individuals and find that six belong to Q1b1a1a and 25 to Q1b1a1a1. Q-M3 is strongly and deeply linked to Indigenous peoples of the Americas (Underhill et al. 1996; Kivisild 2017), where it is still found today (Bortolini et al. 2003; Battaglia et al. 2013; Benn Torres et al. 2015), showing the persistence of Y chromosome ancestry despite significant population turnover that resulted from European contact.

Two individuals, both from the Bahamas, belong to Q1b1a2, with one individual sharing the same derived mutation as found in Anzick (Q-M971; Rasmussen et al. 2014; Kivisild 2017). This suggests that not only did this Anzick-related lineage reach South America, but that it also spread into the Caribbean as well. Nägele et al. identify the Q1b1a2 haplogroup in three individuals from Cuba, all from Archaic-associated contexts.

## SI11 - *f*-statistics and relatedness to modern language groups

The Ceramic migration into the Caribbean has been attributed to populations related to modern Indigenous groups from the Arawakan language family (Supplementary Information section 1). We investigated the affinities of the ancient individuals from this study to present-day populations representing the different language families from Central and South America. Based on the results of outgroup-$f_3$ analysis for each of the three major clades, we identified the top 10 populations with whom they shared the most drift, and their corresponding language family (Supplementary Data 10). We then performed symmetry tests on the populations from these language families using statistics of the form $f_4$(Mbuti, Test; LanguageGroupA_Pop, LanguageGroupB_Pop), with Test as the *qpWave* clades, and LanguageGroupA/B_Pop as all pairwise combinations of the populations identified in outgroup-$f_3$ (listed in Supplementary Data 5). The individual test results are presented in Supplementary Data 11.

The modern Indigenous populations with which ancient Caribbean people shared the highest drift belong to seven main language families: Arawakan, Cariban, Chibchan, Chocoan, Guajiboan, Mataco-Guaicuru, and Tupian (Fig. 2). For *GreaterAntilles_Archaic we found no specific affinities to any language group. *Venezuela_Ceramic showed clear shared ancestry with Chibchan-speaking populations. Finally, *Caribbean_Ceramic was found to have a significantly closer relationship to Arawakan/Guajiboan/Tupian/Cariban-speakers than the other groups.

## SI12- *qpGraph*

We fit the three major clades along with the admixed populations *Curacao_Ceramic and *Haiti_Ceramic into an admixture graph using *qpGraph* and excluding CpG sites. To provide further insights into the movement of Ceramic-associated people into the Caribbean we separated *Caribbean_Ceramic into *LesserAntilles_Ceramic and *GreaterAntillesBahamas_Ceramic and also investigated where the present-day Piapoco would fit. As a base, we used an edited version of the skeleton tree of ancient American populations previously published by Posth and colleagues (2018), removing some of the populations at the base of the tree (Fig. 2c).

The final graph had a worst |Z|-score of 3.583. We make several observations about the final graph:

- The source nodes of admixture for *Haiti_Ceramic and *Curacao_Ceramic produce the best "final score" among the tested variations.
- All Caribbean populations fit better on a sister branch with ancient Brazilian and Argentinian populations, with ancient Andeans from Chile and Peru on a different branch.
- All studied Caribbean populations stemmed from a single branch, with *GreaterAntilles_Archaic as the first diverging group, just before Ceramic-associated groups and Piapoco. *LesserAntilles_Ceramic was best fit before *GreaterAntillesBahamas_Ceramic, in suggestive agreement of a stepping-stone migration into the Caribbean.
- In agreement with *qpAdm* results, we found that the best admixture source for *Curacao_Ceramic as a population between the Ceramic-associated populations from Greater and Lesser Antilles, whereas *Haiti_Ceramic was best modeled with a population closer to *GreaterAntillesBahamas_Ceramic.
- Also in agreement with *qpAdm*, we are able to model up to 5% of Archaic-related ancestry in *GreaterAntillesBahamas_Ceramic.
- Lastly, we found that *Venezuela_Ceramic was best modeled as mixed between the Caribbean Ceramic-associated branch and a population related to Andean groups.

## SI13 - Ability to detect a Carib migration into the Caribbean: simulations and *qpWave*

Using facial morphology, Ross and colleagues (2020) found support for a movement of Carib peoples originating in the northwestern Amazon basin into the Greater Antilles around 800 CE. We therefore looked for evidence of a specific Carib-related genetic contribution to Ceramic Age individuals. As possible proxies for this ancestry we used the present-day Arara, a Carib-speaking population from northern South America; our *Venezuela_Ceramic individuals from Las Locas, as they inhabited the center of the region suggested by Ross and colleagues (2020) as the source of Carib-related ancestry, and predate the hypothetical arrival of ancestry from this region in the Greater Antilles; and *LesserAntilles_Ceramic, as possible representative of the indigenous Island Caribs of the Lesser Antilles. Using admixture simulations (as described in the Methods section of the manuscript) we found that *qpWave* is able to detect ancestry from these Carib-related proxies when they are present at levels that range between at least 2 and 8% in the genome of a *Caribbean_Ceramic individual (Figure S32). We therefore cannot completely rule out a possible migration of individuals with these ancestries into the Greater Antilles at around 800 CE; however, we can attest that if this migration did occur, the contribution of this specific ancestry to peoples of the Greater Antilles analyzed here was likely equal to or less than 8%. An important caveat to this analysis is the requirement that the proxy populations used closely represent the ancient Carib ancestry.

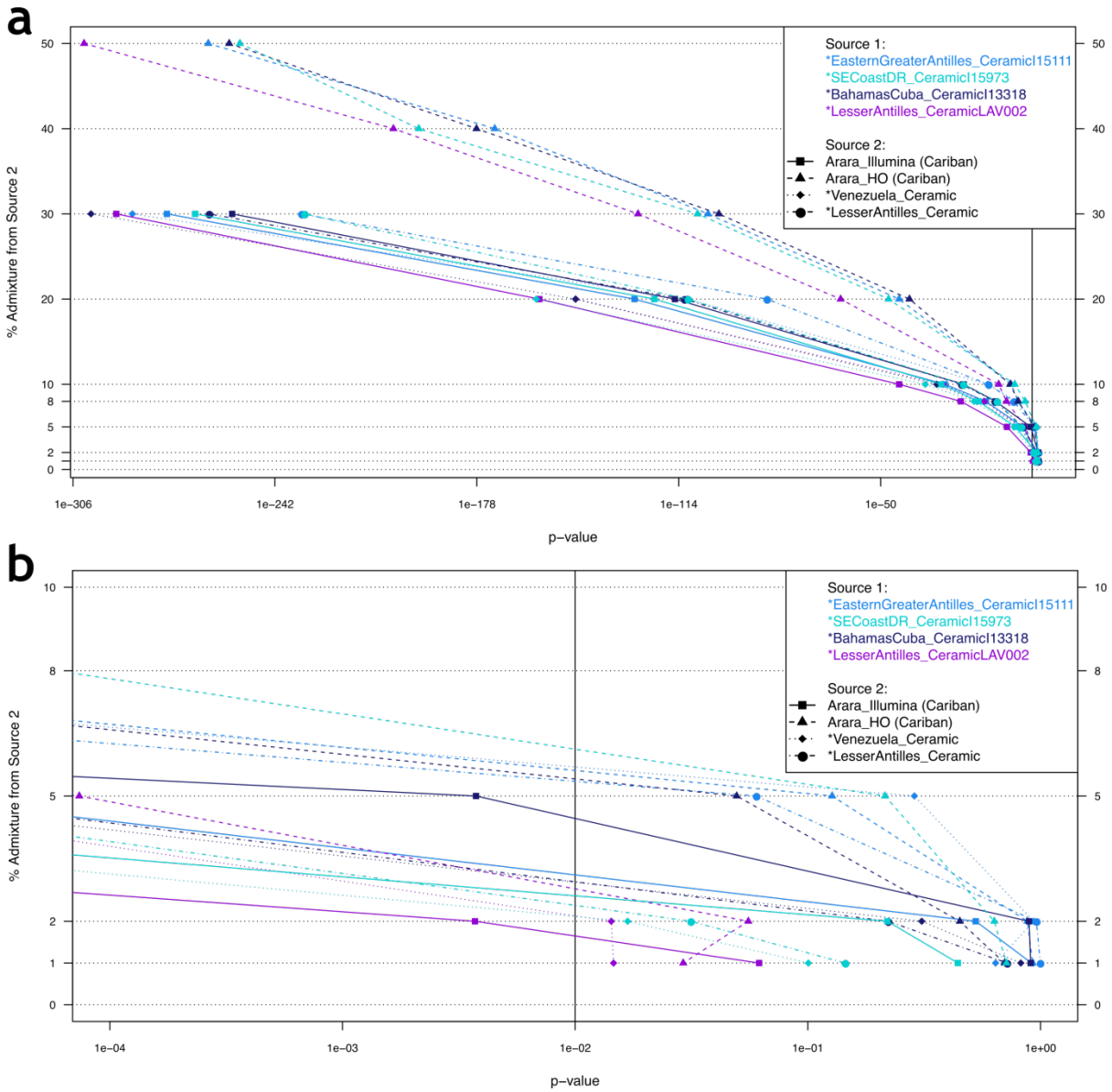



**Figure S32: Minimum admixture detection threshold for Arara-, LesserAntilles_Ceramic-, and *Venezuela_Ceramic-related ancestries (Source 2) using *qpWave* and simulated admixed individuals with primarily *Caribbean_Ceramic ancestry (Source 1).** The *qpWave* p-value threshold used was 0.01. Complete (**a**) and zoomed (**b**) plots shown. Logarithmic scale used for the X axis.

## SI14- *DATES*

For individuals identified as admixed, we attempted to estimate the time of admixture using *DATES* (Narasimhan et al. 2019; Chintalapati, M., Neel, A., Patterson, N. & Moorjani, P. Reconstructing the spatio-temporal patterns of admixture in human history. *In Preparation*). We used *Cuba_Archaic and *EasternGreaterAntilles_Ceramic as a reference pair, prioritizing high-quality data over the precisely correct ancestry sources (as recommended) by restricting to individuals with more than 500,000 SNPs for increased resolution and running *DATES* with 'maxdis: 1.0' to ensure that we detected even relatively long LD blocks indicating more recent admixture.

For the two admixed individuals from the site of Diale 1 in Haiti, we estimated that admixture occurred an average of ~16±3 generations (or ~470±90 years; Z=5.0) before the individuals from Haiti lived, assuming a generation time of 28 years (Moorjani et al. 2016) (Table S22). Using an average context date of 950 years BP for *Haiti_Ceramic, this places admixture as occurring ~1500-1350 years BP, though we note that the dates obtained with this method are based on a model of a single pulse of admixture and thus reflect an intermediate value if the true history includes multiple waves or continuous admixture. Two hypotheses exist for explaining admixed ancestry in Hispaniola (the island that includes the present-day country of Haiti): one (Veloz Maggiolo 1972, 1993; Zucchi 1985) posits direct migration from west of the middle Orinoco that introduced Meillacoid pottery to the Caribbean around 500 CE (citing stylistic consistencies with pottery styles in western Venezuela), while the other (Rouse 1992) posits movement of some Saladoid-associated individuals across the Mona passage (the strait that separates Puerto Rico and Hispaniola) prior to the widespread introduction of Ostionoid pottery around 600 CE. In each scenario, ceramic users and Archaic-associated people admixed with each other. While our estimated admixture dates do not clearly support one hypothesis over the other, our genetic data suggest that if a separate Meillacoid migration did occur, the population was genetically very similar to the people who carried Saladoid pottery into other parts of the Caribbean. Additional data from more Meillacoid-associated sites in Haiti and northern South America (specifically identifying the ancient source population(s) for the Ceramic-associated migration(s) into the Caribbean) will further resolve these scenarios in the future.

We were unable to obtain feasible dates for the Ceramic-associated individuals from Curaçao or the admixed individual I16539.

**Table S22: Admixture date estimation with DATES for *Haiti_Ceramic.**

| Admixed Pop | Reference Pop 1 | Reference Pop 2 | Estimate (generations) | Std Err (generations) | Z-score |
|---|---|---|---|---|---|
| *Haiti_Ceramic | *Cuba_Archaic | *EasternGreaterAntilles_Ceramic | *16.474* | 3.301 | 4.991 |

## SI15– Relatedness of ancient individuals to present-day admixed Caribbean populations

After computing relative allele sharing between present-day admixed Caribbean populations and the ancient individuals, we performed an empirical power analysis for the present-day Cubans by leveraging our ancient data to study whether we would expect to be able to detect differentiation between Archaic-related and Ceramic-related Indigenous ancestry. As a baseline, we had observed a significance level of Z = 0.7 for the statistic $f_4$(European, Cuban; *Cuba_Archaic, *Caribbean_Ceramic). We then merged the Archaic-associated individual I10126 into the present-day Cuban test population and recomputed the statistic, obtaining a significantly lower value (Z = -2.5). Thus, we can conclude that we have sufficient power to detect one full genome from the *GreaterAntilles_Archaic clade when added to the second position in the $f_4$-statistic, even though I10126 has incomplete sequencing coverage and is plausibly more differentiated from *Cuba_Archaic than persisting Archaic-related ancestry in Cuba would be.

We estimated 2.9-4.2% (±0.2%) Indigenous ancestry in the present-day Cuban individuals using qpAdm (proxy sources consisting of *Caribbean_Ceramic, Europeans [1000 Genomes CEU], and West Africans [1000 Genomes YRI], and reference groups drawn either from 1000 Genomes [PEL, PJL, JPT, and MSL] or Mallick et al. (2016) [Karitiana, Mixe, Yakut, Ulchi, Papuan, Mursi, and Mbuti]) (Extended Data Table 3). For 55 individuals, this equates to a total of 1.6-2.3 genomes' worth of Indigenous ancestry. Given that we observe sufficient power with a single genome under conservative assumptions, we expect that if the actual Indigenous ancestry were (all) from an Archaic-related source, we would be able to detect it as well.

We then performed an analogous test with Ceramic-related ancestry in which we combined one Ceramic-associated individual (individual I14992 from Los Muertos) with the present-day Cubans. In this case, the $f_4$-statistic increases slightly to Z = 1.6. The difference from the baseline in this case is not statistically significant, and in fact, even if we extrapolate from a single genome to the high end of the Indigenous ancestry estimate, we would not necessarily expect to have power to detect a significantly greater affinity of the present-day Cubans to *Caribbean_Ceramic (Z ~ 2). Thus, while we can rule out entirely Archaic-related ancestry contributing to the Indigenous ancestry in present-day Cubans, we cannot rule out entirely Ceramic-related ancestry (or a mixture of both).

We note that both provinces and seven of the eight municipalities (Guisa, Media Luna, Manzanillo, Baracoa, Mayari, Puerto Padre, Banes) in Cuba in which we identify weakly Ceramic-related ancestry (Supplementary Data 14; Extended Data Table 3) are located in the eastern portion of the island. Indigenous ancestry is more prevalent in this region of Cuba compared to the central and western parts of the country (Fortes-Lima et al. 2018), allowing our statistics more power.

## SI16 – Analysis of phenotypic SNPs

For 174 newly-reported individuals we examined whether the ancestral or derived allele was present at SNPs known to be relevant to phenotypic traits, providing the first insight into the presence of these traits across the pre-contact Caribbean and in Ceramic-associated Venezuela (see Methods). Here we discuss several results with the clearest signals in our data (all data presented in Supplementary Data 15).

*Diet and Health*

For 172 out of 174 newly-reported individuals in our dataset, all reads covering the rs4988235 variant in *LCT* exhibited the ancestral G allele (individuals I14992 and I16179, both Ceramic-associated individuals from the Dominican Republic, had a single call each of the derived A allele - 1/35 reads and 1/2 reads, respectively). This indicates a clear lack of lactase persistence across the pre-contact Caribbean and suggests that the introduction of the derived allele conferring lactase persistence (today present at ~20% in American 1000 Genomes populations) was likely introduced through extensive European contact and admixture after our studied individuals lived.

We examined two SNPs (rs10246939 and rs1726866) in *TAS2R38* that influence an individual's ability to perceive the taste of the compound phenylthiocarbamide (PTC) as bitter. We find that the alleles that confer the perception of PTC as bitter are present in all of our studied pre-contact Caribbean and Venezuelan individuals that have reads overlapping either or both SNPs. It has been suggested that this polymorphism was present prior to the dispersion of humans throughout the American continents, though it is still unclear precisely how PTC genotype influences food selection (Guo and Reed 2001) and whether both taster and non-taster haplotypes may have been maintained by balancing selection (Risso et al. 2016). Today, tasting alleles for both variants are present at ~70% frequency in 1000 Genomes American populations.

We find that all individuals carry the C allele at rs1229984 in ADHB1 that is not associated with the amino acid change from arginine to histidine in the β subunit of the *ADH* enzyme that has been previously shown to confer a strongly protective effect against alcohol dependence, particularly in East Asians (Bierut et al. 2012). It was previously suggested that the prevalence of the derived variant could be associated with Neolithic rice domestication around 10,000 years ago (Peng et al. 2010), which post-dates the population split that resulted in a group of people migrating from East Asia into the Americas. If so, it is unsurprising that the variant conferring a somewhat reduced rate of alcohol dependence is not found in our ancient Caribbean individuals.

Consistent with previous research on phenotypic SNPs in ancient Americans (e.g., Posth et al. 2018), we find that a variant in a polymorphism (rs174570) in *FADS2* (associated with fatty acid desaturase 2 levels) is derived in all ancient Caribbean and Venezuelan individuals. This provides additional evidence that the selective sweep that drove this allele to near fixation (Fumigalli et al. 2015) occurred in an ancestral population before their entrance into the Americas, and that this group then

spread this adaptive variant across the Americas and into diverse environments (Amorim et al. 2017). Two other variants in *FADS2* (rs74771917 and rs7115739), are also reported to have been under selection in Native American populations, and these two SNPs along with rs174570 have been reported to be part of a putatively selected *FADS* haplotype, where the high frequency of this haplotype and its shared distribution in living and ancient Native Americans is consistent with a scenario of intense selection during the proposed Beringian standstill (Amorim et al. 2017). Out of 166 ancient individuals in our dataset who had at least one call at one of these sites, 124 of them were consistent with the putatively selected *FADS* haplotype; of these, 83 out of 111 had reads consistent with this haplotype at all three positions in *FADS2*. This provides additional support for the spread of this haplotype throughout the ancient Americas following its selection in a deeply ancestral group.

*Physical appearance*

We find that the majority of pre-contact Caribbean and Venezuelan individuals carry an A allele at rs2298080, a variant in the *TBX15* gene that plays a role in the differentiation of brown and brite adipocytes and has been proposed as a candidate for adaptation to life in the Arctic (Racimo et al. 2017). This allele has been previously reported as introgressed from an Archaic (Neandertal or Denisovan) population prior to the introduction of people into the Americas and selected before the diversification of Native Americans (Racimo et al. 2017; Posth et al. 2018).

A derived variant at a polymorphism (rs3827760) in the Ectodysplasin A Receptor (*EDAR*) affecting tooth shape (specifically shovel-shaped incisors), increasing scalp hair thickness, number of eccrine glands, and mammary gland branch density is found predominantly in East Asians and Native Americans (where it occurs presently at nearly 100% frequency) and is nearly absent in Europeans and Africans (Adhikari et al. 2016). Previous research proposed that this variant reached fixation prior to 10,000 years ago in East Asia in response to cold, arid environments (Bryk et al. 2008); however, we find that this variant is present in its ancestral state in a number of the ancient Caribbean individuals. This is consistent with Posth et al. (2018) who detect the ancestral allele in ancient Americans pre-dating our Caribbean individuals and provides additional evidence that the rise in frequency of the derived allele occurred independently in East Asia and in the Americas and that the presence of the derived allele at nearly fixed levels did not occur prior to the migrations of people into the Americas or into the Caribbean.

As expected, we find little to no evidence of derived alleles that conferred lighter skin, hair, and eye pigmentation to European people. The derived allele in variant rs12203592 in *IRF4*, a transcription factor associated with pale skin, eyes, and brown hair in Europeans (Praetorius et al. 2013) is found today at ~7% frequency in 1000 Genomes American populations, but only called for a single read (1/5 reads) in one individual from the pre-contact Caribbean (I13541, a Ceramic-associated individual from Puerto Rico). We also see substantially more individuals with the ancestral G allele at rs1426654 (a variant in SLC24A5 associated with skin pigmentation; Lamason et al. 2005), although four individuals do appear to harbor the derived A allele in between one and five reads. Likewise, we find a predominance of the ancestral A allele (associated with brown eye color) at rs12913832 in *HERC2*

although nine individuals harbor copies of the derived G allele, suggesting that this allele was present in the ancient Caribbean (although if any individuals were homozygous for the G allele at this SNP and thereby had light eye color cannot be determined for certain with the current data). In contrast, the derived T allele for rs11198112 in *EMX2* is associated with darker pigmentation, and today reaches its highest frequencies (~50%) in Amazonians and Melanesians (Adhikhari et al. 2019); it is found in approximately 30% of the ancient Caribbean individuals.

We note that we cannot completely rule out that extremely low levels of contamination influenced these results, making the study of additional ancient individuals essential in helping to more clearly elucidate the phenotypes of ancient American individuals.

## SI17- Evaluation of the proposal of at least two sources of ancestry in Archaic Cuba

Nägele et al. (2020) make the inference that a minimum of two migrations contributed ancestry to Archaic-associated people in Cuba, connecting one to radiation events in North America and another to South America. They propose that ancestry contributions from both dispersals are present in Cuba around 2700-2500 calBP, a period for which they have two directly dated individuals: CIP009 from Cueva del Perico and GUY002 from Guayabo Blanco (another individual from Guayabo Blanco dates to ~1000 years more recent, and the final individual from the site has not been directly dated).

The claim of distinct ancestry contributions associated with multiple geographically-distinct dispersals in Nägele et al. is based on $f_4$-statistics, *qpWave*, and *qpGraph* results. First, they compute the statistic $f_4$(Mbuti, Test; USA_CA_Early_SanNicolas, Bahamas_Taino), where USA_CA_Early_SanNicolas is shotgun sequencing data from individuals from California's Channel Islands Early Period of occupation (Scheib et al. 2018), who represent a population that split off the main Native American lineage prior to the diversification of ancient Central and South Americans, and Bahamas_Taino is shotgun sequencing data from a 1000-year-old individual from the Bahamas (Schroeder et al. 2018). Nägele et al. show that when CIP009 is used as Test, the value of the resulting *f*-statistic indicates non-significantly closer affinity to the individuals from the Channel Islands (Z=-1.3), and when GUY002 is used as Test, the value of the resulting *f*-statistic indicates non-significantly closer affinity to Bahamas_Taino (Z=1.4). A positive statistic (indicating closer affinity to Bahamas_Taino) is also produced when other Archaic-associated individuals/populations from Cuba are used as *Test*. Second, Nägele et al. use *qpWave* to show that a model where CIP009 and a pool of the three individuals from Guayabo Blanco (GUY) are consistent with forming a clade falls below their threshold of $p<0.05$ for evaluating a model as passing ($p=0.013$). Finally, they use *qpGraph* to fit a model in which CIP009 branches off the main Native American lineage with the individuals from California's Channel Islands and prior to the radiation of ancient South and Central Americans. We attempted to replicate these results and to gain additional insight into these inferences by carrying out additional analyses.

We approximately replicated the result presented in Nägele et al. for the statistic $f_4$(Mbuti, Test; USA_CA_Early_SanNicolas, Bahamas_Taino) using either CIP009 or GUY002 as Test, as done in their work (Table S23). We note the relatively low coverage of CIP009, which at ~130K SNPs is substantially below the median coverage of ~490K SNPs for all ancient individuals reported in Nägele et al.; in contrast, GUY002 is covered by ~820K SNPs. We also caution that the shotgun data for the USA_CA_Early_SanNicolas and Bahamas_Taino individuals was generated at different labs with different data generation processes, creating opportunities for artifactual attraction that could bias results.

**Table S23: Replication of $f_4$-statistics for CIP009 and GUY002 reported in Nägele et al. (2020).**

| Pop X | Pop Y | Pop W | Pop Z | f4 | SE | Z | SNPs |
|---|---|---|---|---|---|---|---|
| Mbuti | CIP009 | USA_CA_Early_SanNicolas | Bahamas_Taino | -0.001118 | 0.000887 | -1.260 | 115144 |
| Mbuti | GUY002 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.001007 | 0.000625 | 1.611 | 749385 |

The negative *f*-statistic value for CIP009 (in contrast to positive results for GUY002) is similar in pattern to the findings of Nägele et al., although we emphasize that none of the Z-scores in either their analyses or ours are statistically significant.

We carried out a series of analyses to evaluate this signal further. First, we replaced the outgroup population used by Nägele et al. (Mbuti) with modern Eurasian populations that in principle are just as legitimate as outgroups for this statistic as Mbuti (Table S24**).** Depending on the outgroup population used, we generate positive as well as negative *f*-statistic values for CIP009, although we do not find a case in which the *f*-statistic is negative for GUY002 or GUY (here, we analyze both GUY002 and GUY as *Test*, as the former is used for $f_4$-statistics and the latter for *qpWave* analysis in Nägele et al.). A pattern of inconsistency in the direction of the signal would not be expected if the signal was indeed authentic.

**Table S24: Substituting other present-day populations as outgroups at position X often flips Z-score signs.** The gray cells indicate the statistics reported in Table S5 in Nägele et al. (2020).

| Pop X | Pop Y | Pop W | Pop Z | f4 | SE | Z | SNPs |
|---|---|---|---|---|---|---|---|
| Mbuti | CIP009 | USA_CA_Early_SanNicolas | Bahamas_Taino | -0.001118 | 0.000887 | -1.260 | 115144 |
| Han | CIP009 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.000174 | 0.000856 | 0.204 | 113928 |
| Papuan | CIP009 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.000444 | 0.00089 | 0.499 | 113930 |
| Onge | CIP009 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.001009 | 0.000902 | 1.119 | 113847 |
| Italian_North | CIP009 | USA_CA_Early_SanNicolas | Bahamas_Taino | -0.000259 | 0.000984 | -0.263 | 113682 |
| French | CIP009 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.000243 | 0.000859 | 0.283 | 113927 |
| Itelmen | CIP009 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.000146 | 0.000948 | 0.154 | 113772 |
| Kalash | CIP009 | USA_CA_Early_SanNicolas | Bahamas_Taino | -0.000678 | 0.000923 | -0.735 | 113917 |
| Mbuti | GUY002 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.001007 | 0.000625 | 1.611 | 749385 |
| Han | GUY002 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.00085 | 0.00062 | 1.371 | 740981 |
| Papuan | GUY002 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.000592 | 0.000651 | 0.909 | 740994 |
| Onge | GUY002 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.00139 | 0.000663 | 2.096 | 740540 |
| Italian_North | GUY002 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.001242 | 0.000689 | 1.803 | 739397 |
| French | GUY002 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.001042 | 0.000629 | 1.656 | 740996 |
| Itelmen | GUY002 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.001089 | 0.000709 | 1.537 | 739994 |
| Kalash | GUY002 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.000374 | 0.00065 | 0.575 | 740955 |
| Mbuti | GUY | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.001026 | 0.000539 | 1.903 | 919925 |
| Han | GUY | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.000786 | 0.000537 | 1.464 | 919933 |
| Papuan | GUY | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.000622 | 0.000582 | 1.07 | 919944 |
| Onge | GUY | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.001293 | 0.00058 | 2.23 | 919375 |
| Italian_North | GUY | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.001067 | 0.000617 | 1.729 | 917951 |
| French | GUY | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.000995 | 0.000543 | 1.833 | 919946 |
| Itelmen | GUY | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.000996 | 0.00064 | 1.557 | 918681 |
| Kalash | GUY | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.000319 | 0.000571 | 0.559 | 919894 |

We next computed the *f*-statistic $f_4$(GUY002, CIP009; USA_CA_Early_SanNicolas, Bahamas_Taino). This statistic directly tests for differences in the relatedness of GUY002 and CIP009 to *USA_CA_Early_SanNicolas* and *Bahamas_Taino* - the key signal emphasized in Nägele et al. - without the statistical noise added by an outgroup. We find that the statistic fails to reach significance (Z=-0.913), indicating no significant differentiation between GUY002 and CIP009 in terms of ancestry related to USA_CA_Early_SanNicolas or Bahamas_Taino (Table S25).

**Table S25: Testing differential affinities of GUY002 and CIP009 to USA_CA_Early_SanNicolas and Bahamas_Taino.**

| Pop X | Pop Y | Pop W | Pop Z | f4 | SE | Z | SNPs |
|---|---|---|---|---|---|---|---|
| GUY002 | CIP009 | USA_CA_Early_SanNicolas | Bahamas_Taino | -0.000802 | 0.000879 | -0.913 | 96050 |

We also evaluated possible differences in ancestry between CIP009 and GUY with *qpfstats*, a program in the ADMIXTOOLS suite that pre-computes *f*-statistics independent of the base population used for these statistics (https://github.com/DReichLab/AdmixTools). *qpfstats* accounts for missing data characteristic of ancient DNA better than the 'allsnps: YES' methodology also implemented in ADMIXTOOLS, which is dependent on the base population and is particularly unreliable in cases where the base population is highly diverged (see Lipson 2020). With Mbuti as our base population, we used the "Left" and "Right" population lists from the *qpWave* model presented in Nägele et al. to compute relevant *f*-statistics with *qpfmv*, which computes a single Hotelling $T^2$ statistic to assess the joint fit of the statistics (similar to the *qpWave* methodology). With *qpfmv*, we obtain a value of p=0.20, consistent with CIP009 and GUY forming a clade relative to the populations in the "Right" reference population set. We also applied *qpWave* to the set of *f*-statistics computed using *qpfstats*, and again obtained a p-value of 0.20, again consistent with CIP009 and GUY forming a clade.

We further tested the reliability of the statistic $f_4$(Mbuti, Test; USA_CA_Early_SanNicolas, Bahamas_Taino) by replacing the original shotgun-sequenced Bahamas_Taino individual from Schroeder et al. 2018 used by Nägele et al. with ancient Bahamian data obtained through new whole-genome shotgun sequencing (shotgun versions of the individuals I14879 from South Andros Island, dated ~600 years BP, and I14922 from Abaco Island, dated ~1000 years BP; Supplementary Data 2). The replacement of one individual with another that is temporally-, geographically-, and genetically-similar should clarify if any results are affected by slightly asymmetrical (although non-systematic) attractions between individuals. With these new data, we are unable to replicate the attraction of CIP009 to USA_CA_Early_SanNicolas, and instead show that all statistics lean towards an attraction to Ceramic-associated individuals from the Bahamas (Table S26).

**Table S26: Evidence for differential ancestry in CIP009 weakens when we replace one Bahamas shotgun genome with another.** ".SG" denotes shotgun data, as opposed to the capture data which is also analyzed as part of this work (Supplementary Data 2). CIP and GUY correspond to the respective site without CIP009 and GUY002, respectively.

| Pop X | Pop Y | Pop W | Pop Z | f4 | SE | Z | SNPs |
|---|---|---|---|---|---|---|---|
| Mbuti | CIP009 | USA_CA_Early_SanNicolas | Bahamas_Taino | -0.001096 | 0.000893 | -1.227 | 113926 |
| Mbuti | CIP | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.001306 | 0.00052 | 2.513 | 647954 |
| Mbuti | GUY002 | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.001052 | 0.000623 | 1.689 | 740978 |
| Mbuti | GUY | USA_CA_Early_SanNicolas | Bahamas_Taino | 0.000832 | 0.000609 | 1.366 | 822901 |
| Mbuti | CIP009 | USA_CA_Early_SanNicolas | *Bahamas_AbacoIsl_CeramicI14922.SG | 0.000978 | 0.000996 | 0.982 | 93059 |
| Mbuti | CIP | USA_CA_Early_SanNicolas | *Bahamas_AbacoIsl_CeramicI14922.SG | 0.001224 | 0.000575 | 2.128 | 521450 |
| Mbuti | GUY002 | USA_CA_Early_SanNicolas | *Bahamas_AbacoIsl_CeramicI14922.SG | 0.001770 | 0.000652 | 2.714 | 596919 |
| Mbuti | GUY | USA_CA_Early_SanNicolas | *Bahamas_AbacoIsl_CeramicI14922.SG | 0.000924 | 0.000663 | 1.393 | 661791 |
| Mbuti | CIP009 | USA_CA_Early_SanNicolas | *Bahamas_SouthAndros_CeramicI14879.SG | 0.001366 | 0.000893 | 1.530 | 101228 |
| Mbuti | CIP | USA_CA_Early_SanNicolas | *Bahamas_SouthAndros_CeramicI14879.SG | 0.000969 | 0.000549 | 1.766 | 570021 |
| Mbuti | GUY002 | USA_CA_Early_SanNicolas | *Bahamas_SouthAndros_CeramicI14879.SG | 0.000551 | 0.000646 | 0.853 | 652264 |
| Mbuti | GUY | USA_CA_Early_SanNicolas | *Bahamas_SouthAndros_CeramicI14879.SG | 0.001423 | 0.000607 | 2.343 | 723386 |

Considering these $f_4$-statistics together, we find no statistically significant or even suggestive evidence that CIP009 has different genetic affinities than GUY002 or GUY.

We further probed the signal suggested by Nägele et al. that one of the early dispersal events (working off their conclusion that there was more than one) was connected to radiation events in North America and that this event contributed to CIP009's ancestry. To test the connection between ancestry specifically present in ancient North Americans and the ancestry in CIP009, we replaced USA_CA_Early_SanNicolas by other ancient American individuals/populations as *Test* in the statistic $f_4$(Mbuti, CIP009; Test, Bahamas_Taino), and found that CIP009 shows attraction to at least half of the *Test* populations at a Z-score level similar or stronger than the Z=-1.260 of the test with USA_CA_Early_SanNicolas with no systematic pattern related to geography (Table S27). If we restrict the results to only those based on shotgun data in positions *W* and *Z* (thereby reducing concerns of biases introduced from the co-analysis of capture and shotgun sequencing data), we generate a negative statistic value when ancient individuals from Chile (Chile_PuntaSantaAna_7300BP.SG) and Brazil (Brazil_Sumidouro_10100BP.SG) are used as Test, with the $f_4$-statistic for Chile_PuntaSantaAna_7300BP.SG exhibiting a similar value (around -0.001) to USA_CA_Early_SanNicolas (despite a lower Z-score, which we attribute to a lower number of SNPs). Particularly when the older populations are considered, these results do not support a North American over a South American origin for any alternate ancestry in CIP009.

**Table S27: Affinities of CIP009 to other ancient groups using the same $f_4$-statistic as in Nägele et al. (2020).**

| Pop X | Pop Y | Pop W | Pop Z | f4 | SE | Z | SNPs |
|---|---|---|---|---|---|---|---|
| Mbuti | CIP009 | *Dominican_ElFrances_Ceramic | Bahamas_Taino | -0.003418 | 0.00083 | -4.121 | 100253 |
| Mbuti | CIP009 | *StLucia_Lavoutte_Ceramic | Bahamas_Taino | -0.002562 | 0.000704 | -3.637 | 115665 |
| Mbuti | CIP009 | *Dominican_Atajadizo_Ceramic | Bahamas_Taino | -0.002136 | 0.000679 | -3.145 | 114972 |
| Mbuti | CIP009 | Chile_LosRieles_5100BP | Bahamas_Taino | -0.002576 | 0.000969 | -2.657 | 98470 |
| Mbuti | CIP009 | *Venezuela_LasLocas_Ceramic | Bahamas_Taino | -0.001966 | 0.000758 | -2.595 | 111348 |
| Mbuti | CIP009 | Peru_LaGalgada_4100BP | Bahamas_Taino | -0.002634 | 0.00104 | -2.532 | 106572 |
| Mbuti | CIP009 | *Curacao_deSavaan_Ceramic | Bahamas_Taino | -0.001918 | 0.000823 | -2.33 | 108719 |
| Mbuti | CIP009 | Peru_Lauricocha_8600BP | Bahamas_Taino | -0.001607 | 0.00092 | -1.747 | 110248 |
| Mbuti | CIP009 | Peru_Cuncaicha_9000BP | Bahamas_Taino | -0.001657 | 0.000983 | -1.685 | 95832 |
| Mbuti | CIP009 | Argentina_ArroyoSeco2_7700BP | Bahamas_Taino | -0.001301 | 0.000838 | -1.552 | 109818 |
| Mbuti | CIP009 | Belize_MayahakCabPek_9300BP | Bahamas_Taino | -0.001461 | 0.001209 | -1.208 | 59654 |
| Mbuti | CIP009 | Brazil_Laranjal_6700BP | Bahamas_Taino | -0.001169 | 0.001006 | -1.162 | 77652 |
| Mbuti | CIP009 | Chile_PuntaSantaAna_7300BP.SG | Bahamas_Taino | -0.001006 | 0.001044 | -0.964 | 91397 |
| Mbuti | CIP009 | Argentina_LagunaChica_6800BP | Bahamas_Taino | -0.000924 | 0.000972 | -0.951 | 81499 |
| Mbuti | CIP009 | Brazil_Sumidouro_10100BP.SG | Bahamas_Taino | -0.000363 | 0.000811 | -0.448 | 116209 |
| Mbuti | CIP009 | Chile_LosRieles_12000BP | Bahamas_Taino | -0.000348 | 0.001045 | -0.333 | 111824 |
| Mbuti | CIP009 | Brazil_LapaDoSanto_9600BP | Bahamas_Taino | -0.000182 | 0.000763 | -0.239 | 111815 |
| Mbuti | CIP009 | Belize_SakiTzul_7400BP | Bahamas_Taino | -0.000111 | 0.001104 | -0.1 | 76996 |
| Mbuti | CIP009 | Chile_Ayayema_4700BP.SG | Bahamas_Taino | 0.000171 | 0.000934 | 0.183 | 116197 |
| Mbuti | CIP009 | Brazil_Moraes_5800BP | Bahamas_Taino | 0.000669 | 0.00145 | 0.462 | 40775 |

We attempted to reproduce the *qpWave* analyses reported in Nägele et al. We used the same set of reference groups (Mbuti.DG, Onge.DG, Papuan.DG, Han.DG, Russia_MA1_HG.SG, USA_Ancient_Beringian.SG; USA_Anzick.SG, Mixe.DG, Mexico_Zapotec.DG, Belize_MayahakCabPek_9300, Karitiana.DG, Piapoco.DG) to determine the number of waves of ancestry needed to explain CIP009 and the pool of three Archaic-associated individuals from Guayabo Blanco (GUY). Using the parameters reported in Nägele et al., we obtained a p-value of 0.58 for a clade test between CIP009 and GUY, suggesting that they are consistent with being derived from a common ancestral population since separating from the reference groups (Table S28). This result contrasted with the value of 0.013 reported in Table S6 in Nägele et al., and so we repeated this analysis using the parameter 'allsnps: YES.' With this parameter, we obtained a p-value of 0.027 for the model (Table S28), which is qualitatively similar to the number obtained by Nägele et al. and below the threshold of p>0.05 used to assess whether a qpWave model passes in that study. We conclude that there is no statistically strong signal distinctive ancestry in CIP009 and that signals always weaken from the already at-best marginally significant signal when qualitatively similar statistics are computed.

We tested each individual from GUY separately against CIP009 in order to better understand this result. Carrying out *qpWave* analysis with the 'allsnps: YES' parameter, clade tests p-values for CIP009 and GUY002 or GUY003 were p>0.05, while the result for CIP009 and GUY001 was p=0.02, the only value below the authors' set threshold. This suggests that the evidence for lack of a clade between CIP009 and GUY reported in Nägele et al. may be mostly driven by this single pairing. We note that this result can only be obtained using the 'allsnps: YES' parameter; we obtain a p-value of 0.644 when this parameter is not used; (Table S28). While we focus specifically on this result based on its emphasis in Nägele et al. (2020) we performed pairwise *qpWave* for all Archaic individuals from Nägele et al. with over 100,000 SNPs that we co-analyze as part of this work (45 total) using both the 'allsnps: YES' and 'allsnps: NO' parameters, and provide a heatmap of results as Figure S33. Without the use of 'allsnps: YES', all models for CIP009 have p>0.06.

Nägele et al. make the inference that two distinct ancestries were present in Cuba around 2700-2500 cal. BP represented by the oldest individuals (CIP009 and GUY002), and on this basis suggested multiple early dispersals into the western Caribbean; however, this is inconsistent with our *qpWave* results, where we show that CIP009 and GUY002 can indeed be explained by a single wave of ancestry (p=0.18 with 'allsnps: YES', and 0.61 with 'allsnps: NO').

**Table S28: Affinities of CIP009 to individuals from Guayabo Blanco (GUY) using *qpWave* clade test.** Outgroups used are identical to Nägele et al. (2020).

| Pop X | Pop Y | allsnps: NO | | allsnps: YES | |
|---|---|---|---|---|---|
| | | p-value | SNPs used | p-value | SNPs used |
| *CIP009* | *GUY* | 0.580 | 137524 | 0.027 | 1150346 |
| *CIP009* | *GUY001* | 0.644 | 136446 | 0.022 | 1150346 |
| *CIP009* | *GUY002* | 0.609 | 130100 | 0.181 | 1150344 |
| *CIP009* | *GUY003* | 0.464 | 63897 | 0.361 | 1150343 |

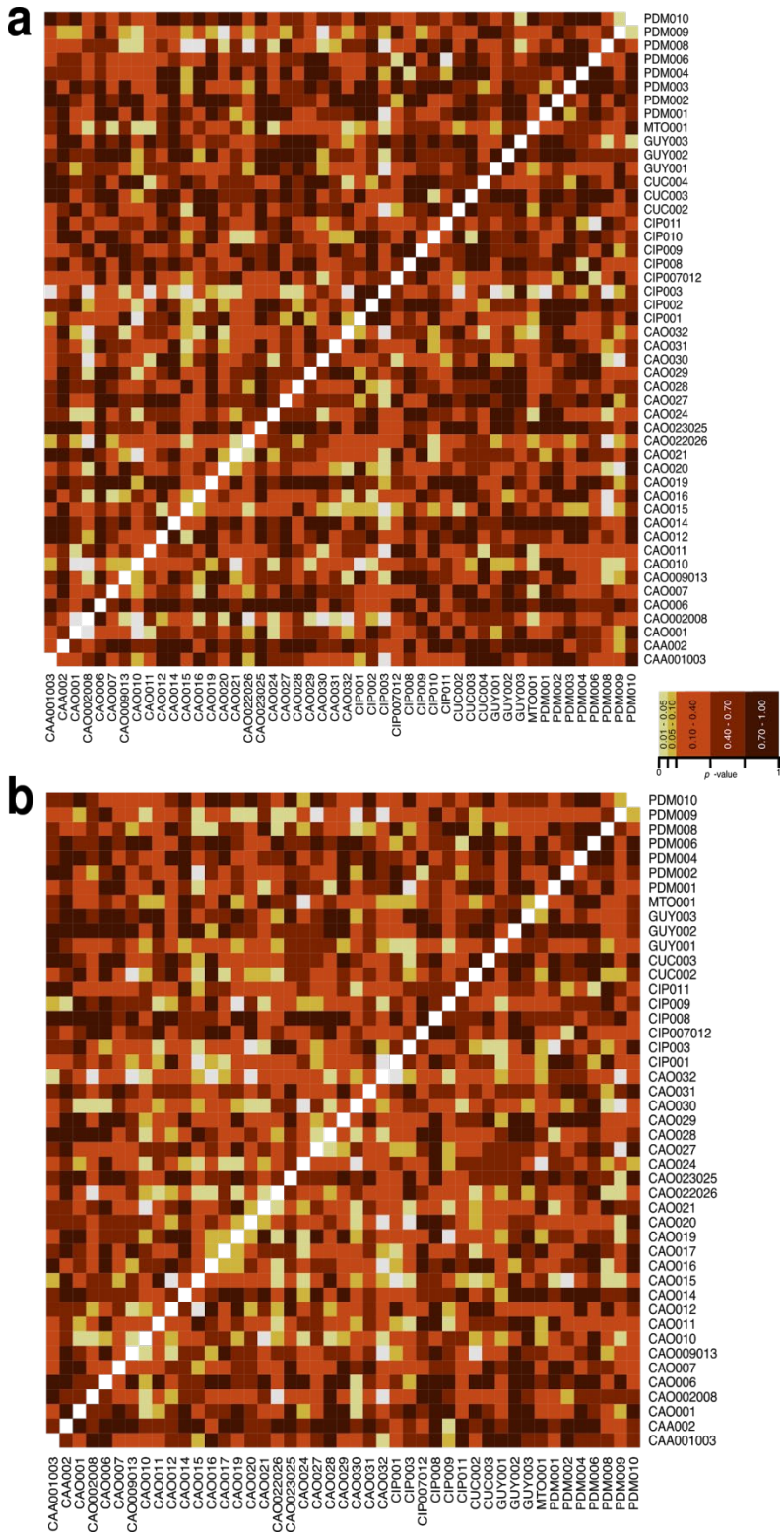



**Figure S33: Heatmap of all *qpWave* pairwise comparisons for the 45 Archaic-associated individuals from Nägele et al. (2020) with >100,000 SNPs included in our co-analysis dataset.** The top heatmap **(a)** presents results when the parameter 'allsnps: NO' is used (this analysis used 59,876-797,463 SNPs, with a median of 316,524 SNPs). The bottom heatmap **(b)** presents results when the parameter 'allsnps: YES' is used (this analysis used 1,150,345 SNPs).

Our re-examination of the statistics reported in Nägele et al. suggest that the signal distinctive ancestry in CIP009 is in fact much weaker even than might be suggested by the *qpWave* p-value of 0.013 reported in that study. In Table S6 of Nägele et al., the authors show that CIP009 in fact forms a clade with many other Archaic-associated individuals (passing models for CIP009 p=0.013-0.19; reprinted as Table S29 below for reference). Correcting for the multiple pairs of Archaic-associated individuals/groups evaluated in this analysis in Table S6 of Nägele et al. makes it clear that there is unlikely to be a significant signal. The authors highlight the CIP009-GUY signal (p=0.013) in the main text; however, it is unclear that this p-value should be prioritized over the other values, including those ranging between p=0.14-0.19 (above their p=0.05 threshold). If the signal were authentic, with the distinctive North American-related migration contributing ancestry only to CIP009, there is no reason to expect that GUY would be the only Cuba Archaic-associated individual yielding significant statistics.

**Table S29: Revisiting the statistics reported in Table S6 of Nägele et al. (2020) for qpWave clade tests of samples from different Archaic-associated sites.** Here we reprint the supplementary table reported in that study and highlight the lines involving CIP009 in gray.

| Group/Individual | Group2/Individual2 | p-value |
|---|---|---|
| CAA | CAO | 0.92 |
| CAO019 | PDM | 0.92 |
| GUY | MTO | 0.80 |
| CAO019 | MTO | 0.77 |
| MTO | PDM | 0.75 |
| CAO | GUY | 0.75 |
| CAO | PDM | 0.74 |
| CAA | PDM | 0.73 |
| CAA | CIP | 0.71 |
| CAA | GUY | 0.64 |
| CAO | MTO | 0.64 |
| CAO019 | CAO | 0.60 |
| CAO019 | GUY | 0.55 |
| GUY | PDM | 0.54 |
| CAA | CAO019 | 0.48 |
| CAA | MTO | 0.42 |
| CUC | PDM | 0.41 |
| CIP | PDM | 0.33 |
| CAA | CUC | 0.28 |
| CIP | MTO | 0.21 |
| CIP009 | MTO | 0.19 |
| CIP009 | PDM | 0.17 |
| CAA | CIP009 | 0.14 |
| CAO019 | CIP | 0.13 |
| CAO | CIP | 0.12 |
| CIP | GUY | 0.065 |
| CUC | MTO | 0.063 |
| CAO | CUC | 0.062 |
| CAO019 | CUC | 0.055 |
| CUC | GUY | 0.055 |
| CIP009 | CIP | 0.040 |
| CIP | CUC | 0.027 |
| CIP009 | CUC | 0.026 |
| CAO019 | CIP009 | 0.018 |
| CIP009 | GUY | 0.013 |

Finally, we looked to fit CIP009 into the *qpGraph* model presented as Fig. 2c, using the same parameters as those described in Supplementary Section 12 (in contrast to Nägele et al., we do not use the 'allsnps: YES' parameter). Due to CIP009's low coverage, removing it from *GreaterAntilles_Archaic and adding it to the graph dramatically dropped the number of SNPs used for analysis (12,045 SNPs). Still, the only place in the graph where CIP009 fit was as a clade with *GreaterAntilles_Archaic (worst Z-score=|3.834|, which is not surprisingly high given the complexity of thee graph), whereas placing CIP009 in a position closer to USA_CA_Early_SanNicolas resulted in a much poorer fit (worst Z-score=|24.504|). To increase the amount of data and power of this analysis, and re-evaluate if we could fit CIP009 in a position that was not within the *GreaterAntilles_Archaic branch, we generated a simplified version of the graph by pooling North American populations, South American populations, leaving USA_CA_Early_SanNicolas unpooled, and excluding other populations that were not relevant to this specific question. Removing CIP009 from within *GreaterAntilles_Archaic and adding it to the graph increased the number of SNPs available for analysis to 50,825, a 4-times increase from the full graph. The best fit identified for CIP009 was again next to *GreaterAntilles_Archaic (Figure S34a; worst Z-score=|1.974|), whereas other positions, namely those closer to USA_CA_Early_SanNicolas as an approximation to Figure 3a of Nägele et al. (2020), produced worse fits (Figure S34b; worst Z-score=|34.596|).

Overall, we do not find significant or consistent evidence of differential ancestry in CIP009 and do not find support for the idea proposed in Nägele et al. that differential ancestry in CIP009 is connected to radiation events in North America. The result reported in Nägele et al. is most likely artifactual or the result of random noise, plausibly due to CIP009 being a single individual with substantially lower coverage than most other Archaic-associated individuals/sites, combined with uncorrected multiple hypothesis testing.

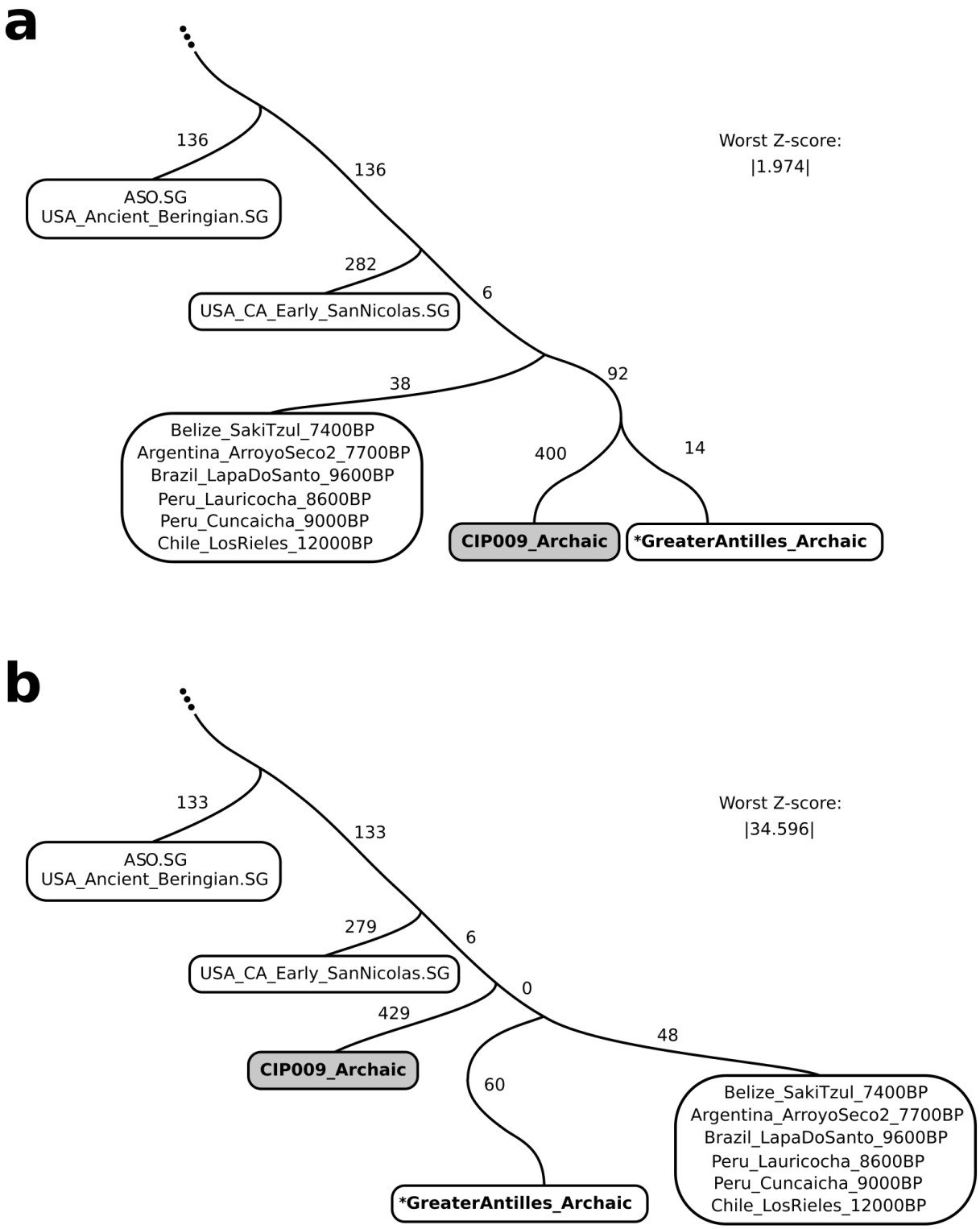



**Figure S34: Fitting CIP009 in a simplified version of the admixture graph presented in Fig. 4.** A position within the Archaic-related branch (a) provided a lower worst Z-score (|1.974|) than when CIP009 preceded South American populations (b) (|34.596|).

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