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HUMAN EVOLUTION

Genomic insights into the early peopling of the Caribbean

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The Caribbean was one of the last regions of the Americas to be settled by humans, but where they came from and how and when they reached the islands remain unclear. We generated genome-wide data for 93 ancient Caribbean islanders dating between 3200 and 400 calibrated years before the present and found evidence of at least three separate dispersals into the region, including two early dispersals into the Western Caribbean, one of which seems connected to radiation events in North America. This was followed by a later expansion from South America. We also detected genetic differences between the early settlers and the newcomers from South America, with almost no evidence of admixture. Our results add to our understanding of the initial peopling of the Caribbean and the movements of Archaic Age peoples in the Americas.

Archaeological evidence suggests that people first moved into the Caribbean around 8000 calibrated years before the present (cal yr B.P.) (*1*, *2*). Apart from Trinidad, which is located closer to the American mainland, the earliest securely dated archaeological sites in the region date to around 5000 cal yr B.P. and are located in Barbados, Cuba, Curaçao, and St. Martin, followed by sites in Hispaniola and Puerto Rico (*2*). The locations of these sites suggest that the early settlers took long and rapid leaps of exploration across the Caribbean Sea. As a result, there is no gradual wave of advance that would point backward to a single point of origin. In the absence of clear chronological clues, some archaeologists have relied on stylistic comparisons of artifact assemblages to suggest possible links between the Caribbean and surrounding mainland (*3*, *4*), and others have studied the prevailing winds and currents to suggest possible dispersal routes (*5*).

Starting around 2800 cal yr B.P., new people began to enter the islands. Their arrival marks the beginning of the Ceramic Age in the Caribbean as a distinctive new style of pottery starts to appear along with more permanent settlements and agricultural practices (*1*). Archaeological and genetic evidence indicates that the new settlers came from South America (*6*, *7*), but how they reached the islands is debated. Two models have been put forward: The traditional model suggests that people gradually moved northward through the Lesser Antilles until they reached Puerto Rico, and then they eventually moved further west into Hispaniola and Cuba (*6*). Alternatively, it has been suggested that the new settlers first reached Puerto Rico, bypassing the Lesser Antilles, before expanding southward (*8*). Whichever way this expansion took place, it seems likely that the newcomers encountered indigenous communities in the islands, but the nature of their interactions is unclear (*9*).

To shed light on the population history of the Caribbean, we retrieved genome-wide data from 93 ancient Caribbean islanders from 16 archaeological sites dating between 3200 and 400 cal yr B.P. (Fig. 1 and tables S1 to S3) (*10*). The skeletal samples derive from two distinct archaeological contexts, which are referred to as Archaic and Ceramic, respectively (*10*). The 52 Archaic-related individuals come from seven sites in Cuba and date to around 3200 to 700 cal yr B.P., whereas the 41 Ceramic-related individuals stem from nine sites in Cuba, the Bahamas, Puerto Rico, Guadeloupe, and St. Lucia and date to around 1500 to 400 cal yr B.P. (Fig. 1). To overcome the challenges posed by poor DNA preservation, we used a hybridization capture method targeting ~1.2 million genome-wide single-nucleotide polymorphisms (SNPs) (*10*). Additionally, we report mitochondrial DNA (mtDNA) haplogroups for 89 of the 93 individuals and Y chromosome haplogroups for 40 of the 47 males (table S1). Contamination estimates were low (on average <1% on both nuclear and mitochondrial estimates) except for five individuals, who were not included in the final dataset (table S4).

The mtDNA data reveal clear differences in haplogroup frequencies between the individuals from the two contexts (fig. S1). Although most of the individuals from Cuba from 3200 to 700 cal yr B.P. carry haplogroups D1 and C1d (with a frequency of 47 and 30%, respectively), these haplogroups are less common among individuals from Ceramic-related contexts, including those reported in previous studies (*11*, *12*). Overall, mtDNA diversity is higher among Ceramic Age individuals, with haplogroups B2, C1b, and C1c specific to this group (fig. S1).

To explore these differences at a genome-wide level, we performed a principal components analysis (PCA) on the capture data using 12 present-day Native American populations as references (*10*) (Fig. 2A), and we found that the individuals fall into two distinct clusters that are consistent with their archaeological contexts. When plotting the ancient Caribbean individuals with other ancient and modern Native Americans (*7*, *13*–*17*), we find that individuals from Ceramic Age contexts, including those from Cuba, cluster with present-day individuals from South America as well as a published 1000-year-old genome from the Bahamas (*7*). By contrast, individuals from Archaic-related contexts in Cuba from 3200 to 700 cal yr B.P. cluster outside present-day Native American variation (fig. S2).

To assess whether the observed clustering reflects different genetic affinities, we grouped individuals by site and computed f_4 statistics of the form f_4(*Mbuti*, *Test*; *Early San Nicolas*, *Preacher's Cave*), measuring the amount of allele sharing between the tested groups (*Test*) and the 1000-year-old individual from the Bahamas (*Preacher's Cave*) (*7*) versus 4900-year-old individuals from California's Channel Islands (*Early San Nicolas*) (*16*), who represent a branch splitting off the main Native American lineage before the diversification of ancient Central and South Americans (Fig. 2B and table S5) (*15*). As expected, the individuals

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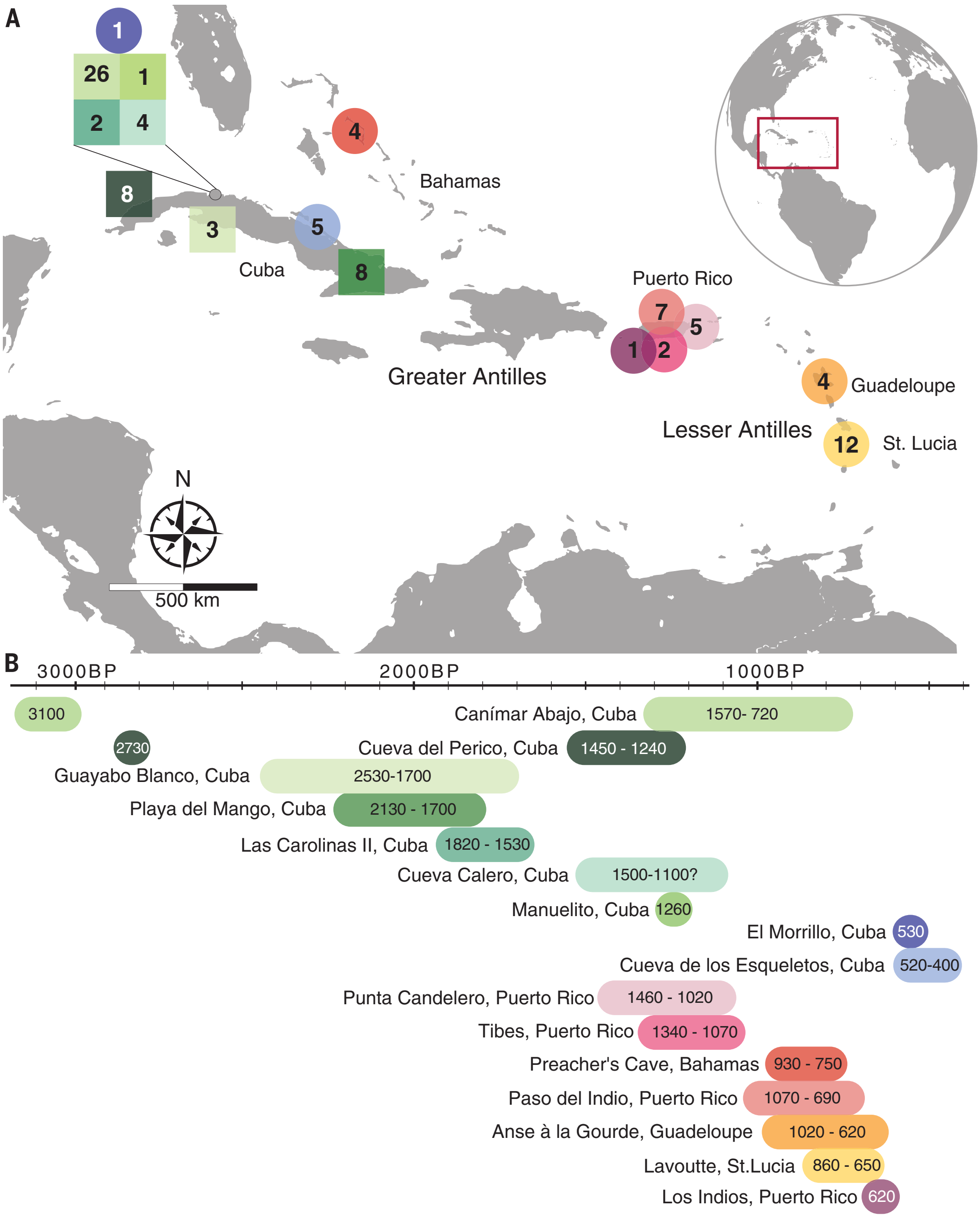

Fig. 1. Sites and samples. (**A**) Map of the Caribbean showing the locations of the sites discussed in the text, including the number of individuals analyzed per site. Squares represent sites with samples from Archaic-related contexts, and circles denote those from Ceramic-related contexts. (**B**) Date ranges for each site are reported in calibrated years before the present (BP). Date ranges derive from directly dated skeletal remains and do not necessarily represent the entire period of occupation of a site. For sites with single individuals, mean point dates are provided. The date ranges for the Cueva Calero individuals are based on archaeological context and indirect radiocarbon dates (*10*).

from Preacher's Cave show the highest affinity to the genome from the same site (*7*), followed by all other Ceramic-related groups. By contrast, all individuals from Cuba from 3200 to 700 cal yr B.P. show less affinity to the Bahamian genome, with one individual from the site of Cueva del Perico (CIP009) being slightly closer to the individuals from California's Channel Islands (*16*). These differences are largely driven by a greater similarity of Ceramic-related groups to present-day populations from northeastern South America (Fig. 2C and figs. S3 and S4) (*7*).

To test whether the two groups derived from the same or distinct ancestral populations, we used *qpWave* (*18*), which estimates the minimum number of sources necessary to explain the genetic composition of an individual or group of individuals (*10*). This analysis was consistent with the groups deriving from at least two separate streams of ancestry (chi-square test, $P = 1.68 \times 10^{-17}$), which demonstrates that the distinction we observe in the PCA cannot be explained by genetic drift alone (table S6). This is also reflected in a supervised clustering analysis, which results in two separate components (fig. S5A) (*10*).

The radiocarbon dates associated with the individuals (Fig. 1B) indicate that both groups were present in the Caribbean at the same time. However, using *qpAdm* (*19*), we do not detect any notable levels of admixture, except for one individual (PDI009) from the Ceramic Age site of Paso del Indio in Puerto Rico, who is dated to 1060 to 910 cal yr B.P. and carries a minor proportion of Archaic-related ancestry (13 ± 7.7%) (table S7). Considering the mounting evidence of the influence of Archaic Age communities on the development of later Caribbean societies (*20*, *21*), it is notable to find so little evidence of admixture between the two groups. However, it is possible that the result is influenced by our limited sampling coverage of the transitional period and of islands such as Hispaniola.

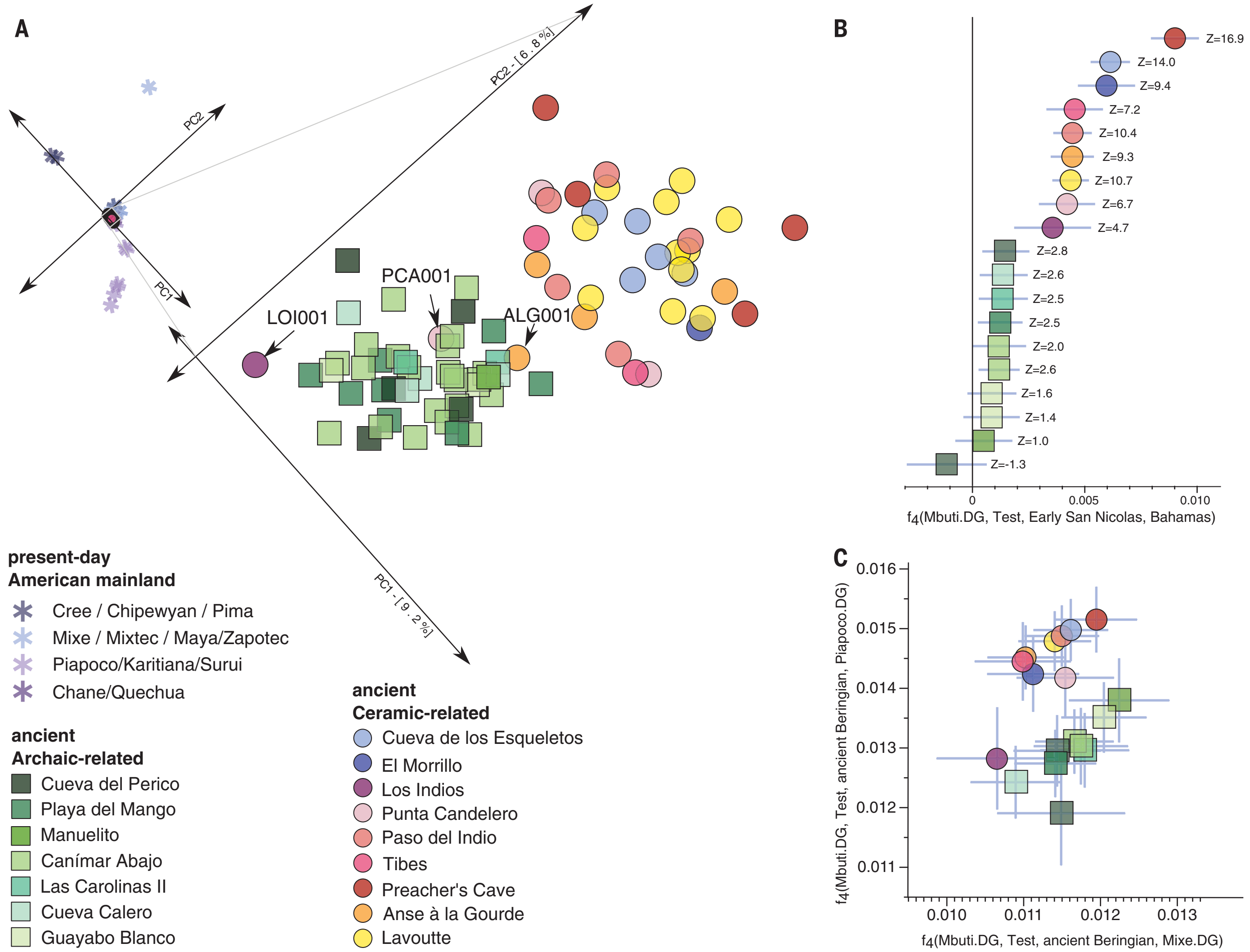

Fig. 2. Population substructure of ancient Caribbean islanders. (**A**) PCA of ancient Caribbean islanders projecting the ancient individuals onto principal components calculated from present-day Native American populations (*10*). Three Ceramic Age individuals (ALG001, LOI001, and PCA001) cluster outside their main grouping, but f_4 statistics indicate that they are more closely related to Ceramic-related than to Archaic-related individuals (table S5). (**B**) f_4 statistics measuring the differential affinities of ancient Caribbean islanders to 4900-year-old individuals from the California Channel Islands (Early San Nicolas) (*16*) and a published 1000-year-old individual from the Bahamas (*7*). The Bahamian genome serves as a proxy for ancient northeastern South American components that are not available from the mainland. (**C**) Differential affinities of ancient Caribbean islanders to present-day Piapoco (y axis) and Mixe (x axis). Light blue lines indicate two standard errors. Squares indicate samples from Archaic-related contexts, and circles denote those from Ceramic-related contexts.

We also detect two distinct ancestries in Cuba around 2700 to 2500 cal yr B.P., represented by the oldest individuals from Cueva del Perico (CIP009) and Guayabo Blanco (GUY002) (Fig. 3, A and B), which suggests multiple early dispersals into the western Caribbean before the arrival of Ceramic Age groups. Using *qpWave* (*18*), we find that some of the oldest individuals in our dataset (i.e., CIP009 and the individuals from Guayabo Blanco) cannot be modeled as descendants of the same ancestral source (chi-square test, $P = 0.013$) (table S6). When we try to model CIP009 alongside other ancient Native American genomes (*14*–*16*) using *qpGraph* (*18*), a model where CIP009 branches off the main Native American lineage with the individuals from California's Channel Islands (*16*) before the radiation of ancient South and Central Americans fits the data best (Fig. 3A). By contrast, all other Archaic-related individuals, including the 2500-year-old individual from Guayabo Blanco (GUY002), require additional gene flow from ancient South Americans to improve the models (Fig. 3B and fig. S6). Together, these results support multiple dispersals into the western Caribbean before the arrival of Ceramic Age groups. Although it is difficult to determine where these early dispersals originated, it seems that at least one of them was connected to radiation events in North America before the diversification of Central and South Americans (*14*, *15*).

After 2800 cal yr B.P., there was another expansion, which originated in South America and is well supported archaeologically (*1*). When we model this expansion using the Ceramic Age genomes in our dataset, we find that a stepping-stone model with people originating in South America and gradually moving northward through the Lesser Antilles fits the data better than a model assuming a southward expansion from Puerto Rico (Fig. 3C and fig. S7). However, because we do not have any individuals with Ceramic-related ancestry from the earliest phase of the Ceramic Age expansion (around 2800 to 2200 cal yr B.P.), it is difficult to model this process accurately. The expansion of Ceramic Age groups

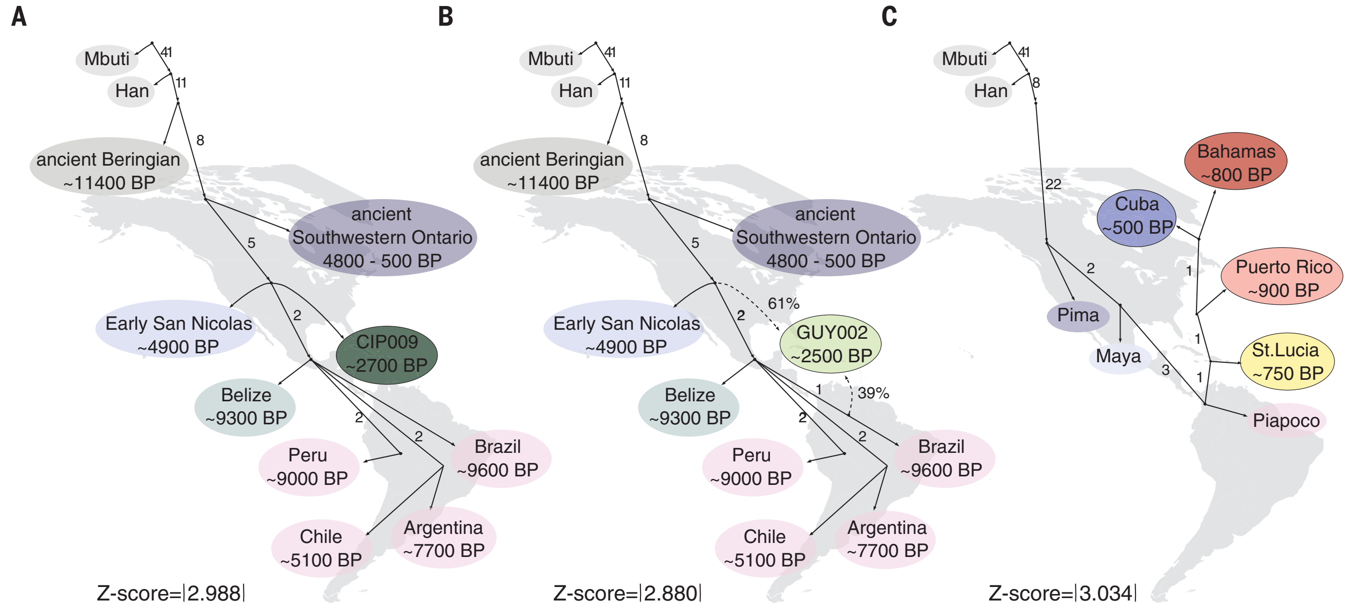

Fig. 3. Admixture graphs modeling the ancestry of ancient Caribbean islanders. (**A** to **C**) We show the best-fitting models for each individual or group as inferred from the final fit score (*10*) for individual CIP009 from the Cueva del Perico (A), individual GUY002 from Guayabo Blanco (B), and several Ceramic Age groups (C). CIP009 (2700 cal yr B.P.) branches off the main Native American lineage along with individuals from the California Channel Islands (*16*) before the diversification of Central and South Americans, whereas GUY002 (2500 cal yr B.P.) requires some South American–related ancestry to make the model fit. The expansion of South American groups after 2800 cal yr B.P. can best be modeled as a stepping-stone process, whereas a southward model results in a worse fit (fig. S7). The geographical positions of ancient groups correspond to their approximate locations. Arrows do not indicate dispersal routes, and node placements do not show the actual geographic regions where the splits took place. Numbers to the right of solid lines are proportional to optimized drift; percentages to the right of dashed lines represent admixture proportions. The label Peru ~9000 BP includes Peru Cunchaicha (9000 cal yr B.P.) and Peru Lauricocha (8600 cal yr B.P.) (*15*). For other groups, see the supplementary materials (*10*).

stalled in Puerto Rico for at least 1000 years before resuming sometime after 1500 cal yr B.P., and it is generally assumed that the advance was halted by the presence of Archaic Age communities in Hispaniola and Cuba (*1*, *6*). Our results are consistent with a temporal gap, as we do not detect any Ceramic-related ancestry in Cuba until 500 cal yr B.P. However, it is still unclear whether we are dealing with a period of genetic turnover (*19*, *22*) or a more-complex history of interaction with intermittent episodes of admixture similar to those that have been observed in other parts of the world (*23*, *24*).

The genetic evidence presented in this work supports the notion that the Caribbean was settled and resettled by successive population dispersals that originated on the American mainland. We find support for at least three separate population dispersals into the region, including two early dispersals, one of which appears to be connected to radiation events in North America. Archaic Age peoples clearly had the seafaring abilities to conquer the Caribbean (*5*). In fact, there is mounting evidence to suggest that, far from being an insuperable barrier, the Caribbean Sea functioned as an aquatic motorway that people crossed frequently, despite its occasional unpredictability (*25*). The initial peopling of the Caribbean was later followed by another expansion from South America. As the newcomers arrived in the islands, they must have encountered descendants of the early settlers, but we find notably little evidence of admixture. This raises questions regarding the nature of their interactions and the role of the early settlers in the development of later Caribbean societies. Additional data and multiple lines of evidence will be needed to explore these questions further and to shed more light on the complex population history of the Caribbean.

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ACKNOWLEDGMENTS

We thank the Comisión Nacional de Monumentos de la República de Cuba; the Consejo para la Protección del Patrimonio Arqueológico Terrestre de Puerto Rico; the Antiquities, Monuments and Museum Corporation of the Bahamas (AMMC); the Direction des affaires culturelles de Guadeloupe; and the St. Lucia Archaeological and Historical Society for supporting this study and providing access to samples. Special thanks go to the staff of the Centro Ceremonial Indígena de Tibes for assistance with sampling and curation at the Tibes site. **Funding:** The research was funded by the Max Planck Society and the European Research Council under the 7th Framework Program (grant agreement no. 319209, ERC Synergy Project NEXUS1492). H.S. was supported by the HERA (Humanities in the European Research Area) Joint Research Program "Uses of the Past" (CitiGen) and the European Union's Horizon 2020 research and innovation program under grant agreement no. 649307. W.J.P. and M.A.N.-C. were supported by the National Science Foundation (BCS-0612727 and BCS-1622479). C.L.-F. was supported by a grant from the Ministry of Science, Innovation and Universities (PGC2018-0955931-B-100, AEI/FEDER, UE). M.R. was supported by the Social Sciences and Humanities Research Council of Canada (435-2016-0529). M.R., Y.C.d.A., U.M.G.H., and S.T.H.G. were supported by the Social Sciences and Humanities Research Council of Canada (standard research grant SSHRC - 410-2011-1179 and SSHRC postdoctoral fellowship - 756-2016-0180) and several University of Winnipeg internal grants (Major grant 2017, 2018; Partnership Development grant 2017, 2018; and Discretionary grant 2017, 2018). **Author contributions:** H.S., K.N., C.P., and J.K. conceived and led the study. M.R. and Y.C.d.A. funded and coordinated excavations in Cuba with administrative and academic support by A.J.M.-F. U.M.G.H. and Y.C.d.A. led the excavation of the Playa del Mango site. S.T.H.G. and Y.C.d.A. led the excavation at the sites of Canímar Abajo. E.C.-T. excavated and curated individuals from the sites of Punta Candelero, Los Indios, and Paso del Indio. C.L.H. and M.H. led the archaeological excavations at the site of Anse à la Gourde and Lavoutte. W.J.P. sampled and M.A.N.-C. extracted DNA from the individuals excavated at Paso del Indio, Punta Candelero, and Tibes. K.N., M.I.O., R.R., M.S.-V., and D.M. processed the rest of the samples. K.N., C.P., and H.S. analyzed the data with input from T.C.L. and J.R.-M. K.N., H.S., C.P., and J.K. interpreted the data with critical input and contextualization from Y.C.d.A., U.M.G.H., S.T.H.G., C.A.A., A.R.R., C.L.-F., I.R., and M.R. for Cuba; W.J.P., M.A.N.-C., and A.C.S. for Puerto Rico; R.S.C., J.S.D., and W.C.S. for the Preacher's Cave; and C.L.H., J.L., and M.H. for the sites in the Lesser Antilles. K.N. and H.S. wrote the manuscript with critical input from C.P., J.K., M.R., C.L.H., and the remaining authors. **Competing interests:** The authors declare no competing interests. **Data and materials availability:** Alignment files of the nuclear and mtDNA sequences for all analyzed individuals are available at the European Nucleotide Archive (ENA) database under the accession no. PRJEB37518.

SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/369/6502/456/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S7
Tables S1 to S7
References (*26–101*)
MDAR Reproducibility Checklist

View/request a protocol for this paper from *Bio-protocol*.

28 February 2020; accepted 18 May 2020
Published online 4 June 2020
10.1126/science.aba8697

Science

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Science **369** (6502), 456-460.
DOI: 10.1126/science.aba8697originally published online June 4, 2020

A complex dispersal into the Caribbean

The settlement of the Caribbean and genetic relationships among pre-European Caribbean people remain a mystery. After examining 93 ancient genomes dating to a range from about 3200 to 400 years ago, Nägele *et al.* suggest that at least three separate colonization events, including a previously unknown wave, were connected to radiation events in North America. The two more ancient lineages coexisted in Cuba but were fully separate genetically, with later movement into the region from a third group from South America. The study not only informs on the settlement of the Caribbean but also lends insights into the broader-scale intercontinental radiation of humans across the American landscape, including across substantial water boundaries.

Science, this issue p. 456