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Ancient DNA reconstructs the genetic legacies of pre-contact Puerto Rico communities

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34 **Abstract**

35 Indigenous peoples have occupied the island of Puerto Rico since at least 3000 B.C. Due to the
36 demographic shifts that occurred after European contact, the origin(s) of these ancient populations, and
37 their genetic relationship to present-day islanders, are unclear. We use ancient DNA to characterize the
38 population history and genetic legacies of pre-contact Indigenous communities from Puerto Rico. Bone,
39 tooth and dental calculus samples were collected from 124 individuals from three pre-contact
40 archaeological sites: Tibes, Punta Candelerero and Paso del Indio. Despite poor DNA preservation, we used
41 target enrichment and high-throughput sequencing to obtain complete mitochondrial genomes (mtDNA)
42 from 45 individuals and autosomal genotypes from two individuals. We found a high proportion of
43 Native American mtDNA haplogroups A2 and C1 in the pre-contact Puerto Rico sample (40% and 44%,
44 respectively). This distribution, as well as the haplotypes represented, support a primarily Amazonian
45 South American origin for these populations, and mirrors the Native American mtDNA diversity patterns
46 found in present-day islanders. Three mtDNA haplotypes from pre-contact Puerto Rico persist among
47 Puerto Ricans and other Caribbean islanders, indicating that present-day populations are reservoirs of pre-
48 contact mtDNA diversity. Lastly, we find similarity in autosomal ancestry patterns between pre-contact
49 individuals from Puerto Rico and the Bahamas, suggesting a shared component of Indigenous Caribbean
50 ancestry with close affinity to South American populations. Our findings contribute to a more complete
51 reconstruction of pre-contact Caribbean population history and explore the role of Indigenous peoples in
52 shaping the biocultural diversity of present-day Puerto Ricans and other Caribbean islanders.

53

54 **Introduction**

55 Puerto Rico is the smallest of the Greater Antilles (Figure 1), the northernmost island grouping of the
56 Caribbean archipelago. The archaeological record of the island's prehistoric communities suggests a
57 dynamic population history with multiple peopling events, frequent migration, and expanding population
58 settlements (Rouse 1992; Rodríguez Ramos 2010). However, different lines of evidence produce
59 conflicting results about the origins and number of these migrations and the role of genetic admixture in
60 pre-contact Caribbean population history (Chanlatte Baik 2003; Rodríguez Ramos, et al. 2013).

61 Archaeological evidence indicates humans first arrived in the Antilles by approximately 7000 B.C.,
62 reaching Puerto Rico before 3000 B.C. (Burney, et al. 1994; Rodríguez Ramos, et al. 2013). Due to the
63 abundance of stone tools at these early sites, early settlers are known as the first representatives of the
64 Caribbean Lithic Age, also referred to as Archaic or pre-Arawak. The origins of these first peoples and
65 the route(s) they used to enter the Antilles are largely unknown. Based on similarities in material culture,
66 Lithic Age populations may have originated in South America, Central America or the Isthmo-Colombian
67 region (Rouse 1992; Rodríguez Ramos 2010; Rodríguez Ramos, et al. 2013).

68 Around 500 B.C., a new group of peoples with elaborate ceramic technology and large-scale
69 agriculture entered the Antilles, arriving into Puerto Rico by 200 B.C. Archaeological and ethnohistoric
70 evidence suggests these were likely Arawak-speakers from the Orinoco River delta region in South
71 America (Chanlatte Baik 2003). Their arrival and the technological and cultural changes they introduced
72 launched the Caribbean Ceramic Age (CA). Although it was initially thought these migrants displaced
73 pre-existing Lithic/Archaic populations (Rouse 1992), more recent archaeological evidence suggests
74 admixture occurred, and both groups contributed to the ancestry of Late Ceramic Age (LCA) peoples who
75 expanded and diversified across the Antilles after A.D. 600 (Wilson 1999; Keegan and Rodriguez Ramos
76 2005). On Puerto Rico and other islands, the LCA was characterized by increased social stratification, the
77 possible rise of organized chiefdoms, and the emergence of regional technological and artistic traditions
78 (Rouse 1992; Chanlatte Baik 2003; Curet, et al. 2004). Material and isotopic evidence suggest that inter-
79 island interaction and mobility expanded during the LCA (Laffoon and Hoogland 2012; Mol 2013). By
80 the time of European contact in the 15th century, multiple ethnic groups with varying levels of social and
81 political complexity existed in the Antilles (Wilson 2007). In Puerto Rico, and other islands, these groups
82 are known as the “Taino” (although see Curet (2014) for discussion of the problematic Taino ethnonym).

83 European colonialism altered the demography of Puerto Rico and the Caribbean. Forced relocations,
84 disease, and slavery decimated native populations. The establishment of plantation and mining economies
85 increased migration from Europe, and systems of forced labor brought peoples from Africa, Asia, and
86 other parts of the Americas to the Antilles (Rogozinski 2008). Despite extensive ethnohistorical research
87 (Anderson-Córdova 2005; Anderson-Córdova 2010), the extent to which Puerto Rico’s native Indigenous
88 communities resisted, survived and were transformed by colonization is unclear. Their biocultural
89 connection to present-day islanders is also disputed. Historical claims of extinction, based largely on
90 colonial era censuses, are strongly opposed by islanders who assert cultural affiliation and direct
91 descentance from native pre-contact communities (Haslip-Viera 2001; Forte 2006; Benn-Torres 2014).

92 Previous research has attempted to address these debates by characterizing Native American genome
93 segments found in present-day, admixed Puerto Ricans (Martínez-Cruzado, et al. 2001; Martínez-Cruzado
94 2002; Martínez-Cruzado, et al. 2005; Martínez-Cruzado 2010; Gravel, et al. 2013; Moreno-Estrada, et al.
95 2013; Vilar, et al. 2014). Caribbean islanders have varying proportions of African, European, Native
96 American, and Asian genetic ancestry. These patterns differ among populations and reflect the sex-biased
97 nature of colonial and post-colonial demographic processes (Bryc, et al. 2010; Moreno-Estrada, et al.
98 2013). For instance, Puerto Ricans have high proportions of European and African ancestry in autosomal
99 and Y-chromosome loci, but large proportions of Native American ancestry in the mitochondrial genome
100 (Martínez-Cruzado, et al. 2005; Via, et al. 2011; Vilar, et al. 2014). This Native American ancestry

101 component, presumed to originate from ancient island populations, has been used as a proxy to
102 reconstruct pre-contact genetic variation (Martínez-Cruzado 2010; Gravel, et al. 2013).

103 However, present-day Caribbean genomes may not fully represent pre-contact genetic diversity.
104 Evolutionary forces such as drift and natural selection affect lineage survival in descendant populations
105 and bias reconstructions of ancient demography. Recent population replacements can also mask the
106 genetic signal of ancient groups (Pickrell and Reich 2014). In Puerto Rico for example, historical
107 documents indicate that Indigenous peoples from other parts of the Americas were imported as slave
108 labor during the early 16th century (Anderson-Córdova 2005; Anderson-Córdova 2010), yet their
109 contribution to the gene pool of present-day Puerto Ricans is currently unknown (Martínez-Cruzado
110 2010). Many of these gaps could be filled with ancient DNA (aDNA), but poor preservation in pre-
111 contact Caribbean archaeological contexts has limited aDNA research to partial fragments of
112 mitochondrial DNA (mtDNA) (Lalueza-Fox, et al. 2001; Lalueza-Fox, et al. 2003; Mendisco, et al. 2015)
113 or genome-wide data from few individuals (Schroeder, et al. 2015; Schroeder, et al. 2018).

114 To address these limitations, in this study we use paleogenomics to examine directly the genetic
115 diversity of pre-contact populations from Puerto Rico. Using target enrichment and high-throughput
116 sequencing, we recovered 45 complete mtDNA genomes and two partial autosomal genomes from 124
117 individuals sampled from three pre-contact sites. To draw inferences on the origins of these communities,
118 and their relationship to coeval Caribbean populations, we compare these sequences to genetic data from
119 ancient and present-day populations from the Caribbean islands and continental Americas. Contextualized
120 within the framework of previous genetic and archaeological research, our findings prove instrumental for
121 reconstructing the population history of pre-contact Puerto Rico and lead to a better understanding of the
122 biocultural links between ancient Indigenous Caribbean communities and present-day islanders.

123

124 **Results**

125 **Preservation and ancient DNA recovery.** We sampled 124 human skeletal remains excavated from CA
126 and LCA archeological contexts at three sites in Puerto Rico: Punta Candelero (n=34), Tibes (n=46) and
127 Paso del Indio (n=44) (Figure 1; Figures S1-S2). Direct radiocarbon dates previously obtained for 81 of
128 the individuals ranged between A.D. 500-1300 (Pestle 2010) (Table S1; Figure S3). The adverse
129 preservation conditions of the Caribbean severely restricted aDNA recovery. Samples from Tibes had the
130 worst mtDNA preservation (Figure S4). After enrichment and Illumina sequencing, complete mtDNA
131 genomes with $\geq 5X$ average read depth and $\geq 98\%$ genome coverage were recovered from 45 individuals
132 (36%) (Table S2-S3). Five of these mtDNA genomes were obtained through multiple sequencing runs.

133 In addition to mitochondrial enrichment, 35 samples were shotgun sequenced and 22 of these were
134 subjected to whole genome enrichment (WGE). Endogenous DNA content was extremely low for all

135 samples even after WGE. Analyses of autosomal genotypes were performed solely on two samples from
136 Paso del Indio with the highest average read depth and genome coverage after multiple rounds of
137 enrichment and sequencing: PI-51 (0.16X, 13.89% genome coverage) and PI-420a (0.27X, 19.16%
138 genome coverage) (Table S4-S5). Reads obtained for all 45 samples selected for mtDNA analyses and
139 both samples selected for autosomal DNA analyses had damage patterns characteristic of authentic aDNA
140 such as short fragments (<200 bp) and high rates of C-to-T and A-to-G transitions at 5' and 3' DNA
141 fragment ends, respectively (Briggs, et al. 2007) (Figure S5-S6). For two mtDNA enriched samples with
142 evidence of contamination (PC-443 and PC-448), endogenous reads were recovered after additional
143 filtering with PMDtools (Skoglund, et al. 2014) (Table S6; Figure S7). Average DNA fragment length
144 was approximately 65 bp and estimated contamination ranged between 1-9% for all analyzed samples.

145
146 **MtDNA diversity.** Three of the five characteristic Native American mtDNA haplogroups were found in
147 the pre-contact Puerto Rico (PC-PR) sample: A2, C1 and D1. When considering complete mtDNA
148 variants, we identified 29 haplotypes in 45 individuals (Table S7). 84% were classified into haplogroups
149 A2 and C1. The most frequent sub-haplogroup was C1b2, accounting for 33% of all mtDNA lineages
150 (Figure 2). When considering only HVR-1 sequences, lineage variation is collapsed into 18 haplotypes.

151 We tested for differences in complete mtDNA haplotype diversity between the three pre-contact sites
152 by calculating an exact test of population differentiation and pairwise Φ_{st} measures. We found no
153 significant differences ($p > 0.05$), suggesting no genetic structure existed between site communities (Table
154 S8-S9, Figure S8). We also found no significant relationship between genetic and temporal distance in the
155 complete PC-PR sample ($z = 182.65$, $p = 0.880$) (Figure S9). Thus, subsequent analyses were conducted
156 assuming a panmictic pre-contact island population.

157 Intra-population summary statistics calculated with complete mtDNA sequences indicate that the PC-
158 PR sample has low haplotype ($Hd = 0.902$) and nucleotide diversity ($\pi = 0.001$) compared to most
159 reference populations, except for several groups with known low diversity such as the Surui and Karitiana
160 of Amazonia (Wang, et al. 2007) (Table S10-S11, Figure S10A-B). A second test restricted to HVR-1 for
161 comparison with available comparative datasets, found that PC-PR had low haplotype ($Hd = 0.942$) and
162 nucleotide diversity ($\pi = 0.009$) relative to other Caribbean populations (Table S12, Figure S10C-D).

163
164 **MtDNA inter-population differentiation.** We measured inter-population genetic distance and
165 differentiation by comparing complete mtDNA haplotypes between PC-PR and 46 reference populations
166 from the Americas (Table S10). Exact tests found significant differences in haplotype frequencies
167 between PC-PR and 17 populations, but the null hypothesis of panmixia could not be rejected for the
168 remaining 29 comparisons, including between PC-PR and present-day Puerto Ricans (Table S13).

169 Pairwise comparisons of Φ_{st} measures calculated with complete mtDNA sequences found the lowest
170 subdifferentiation values between PC-PR and Indigenous populations from northwest Amazonia and the
171 Andes (Figure S11). For ten of these comparisons, we were unable to reject the null hypothesis of no
172 differentiation (Table S14). Φ_{st} inter-population distances are visually represented with non-metric
173 multidimensional scaling (MDS) in Figure 3. The MDS patterns are broadly recapitulated in the
174 correspondence analysis plot in Figure 4 which clusters populations based on haplogroup frequency. PC-
175 PR falls in the upper left quadrant of the plot, clustering with Amazonian populations carrying high
176 frequencies of haplogroup C. We note that Φ_{st} distances between PC-PR and Puerto Ricans were low (Φ_{st}
177 $=0.0441$, $p=0.018$), but significantly different. This suggests that although haplotype frequencies are
178 similar between them, some differentiation exists.

179 We repeated this analysis comparing PC-PR to eight Caribbean populations. The exact test found no
180 significant difference in HVR-1 haplotype frequencies between PC-PR and most islanders, including
181 Puerto Ricans, Cubans, Dominicans and the Trinidad First People's Community (Table S15). However,
182 pairwise comparisons of Φ_{st} measures calculated with direct haplotype sequences (Table S16) found the
183 lowest distances were between PC-PR and PC-Guadeloupe, Puerto Ricans and the St. Vincent Garifuna
184 (Native American lineages only) (Figure S12-S13; Table S15). When plotting two dimensions of non-
185 metric MDS, PC-PR clusters closest to present-day Puerto Ricans (Figure 5). This pattern is repeated in
186 the correspondence plot of haplogroup frequencies shown in Figure S14. Overall, these comparisons
187 suggest Native American haplogroup frequencies are similar throughout the Caribbean, but specific
188 haplotypes differ between pre-contact and present-day populations, and among island groups.

189
190 **MtDNA network analysis.** We constructed haplotype networks with complete mtDNA sequences from
191 PC-PR, and reference populations from the Americas (Native American lineages only). We found that
192 present-day Puerto Ricans were the only population that shared mtDNA haplotypes with PC-PR (Figure
193 S15-S17). Thus, network analysis was repeated with only ancient and present-day Puerto Rican complete
194 mtDNA haplotypes. We also conducted a second round of network analysis including HVR-1 haplotypes
195 from the Caribbean. These networks suggest diverging histories for Caribbean A2 and C1 lineages.

196 The Puerto Rican complete mtDNA C1 network shows a large cluster of identical haplotypes found at
197 high frequency in both the ancient and present-day populations (Figure 6). This clade is sub-haplogroup
198 C1b2, the most common C1 lineage in both pre and post-contact Puerto Rico. C1b2 exhibits a star-like
199 phylogenetic pattern, consistent with a history of lineage expansion and subsequent in-situ differentiation
200 of derived haplotypes (Bandelt, et al. 1995). This pattern is mirrored in the HVR-1 Caribbean C1 network
201 (Figure S18), although diversity is reduced, and previously distinct haplotypes are collapsed into a central
202 C1 founder lineage. The C1 founder, and derived lineages, are found at high frequencies in most

203 Caribbean populations (Lalueza-Fox, et al. 2001; Lalueza-Fox, et al. 2003; Mendizabal, et al. 2008;
204 Martínez-Cruzado 2010; Vilar, et al. 2014; Benn-Torres, et al. 2015; Mendisco, et al. 2015). This pattern
205 of reduced diversity is consistent with a strong founder effect of pre-contact C1 lineages in the peopling
206 of the Caribbean islands and has been noted previously (Martínez-Cruzado 2010; Vilar, et al. 2014).

207 In contrast, the complete mtDNA Puerto Rican A2 network exhibits a diversity of low and mid-
208 frequency haplotype clusters (Figure 6). Five sub-haplogroups (A2, A2+16218, A2+64, A2e, A2z) are
209 represented in 14 PC-PR individuals. Two haplotypes belonging to sub-haplogroups A2+16218 and A2z
210 are found at low frequencies in both PC-PR and present-day Puerto Ricans. Additionally, four HVR-1
211 haplotypes seen in PC-PR are also present in other Caribbean populations, including Cubans,
212 Dominicans, and pre-contact individuals from Guadeloupe (Figure S18). However, many low frequency,
213 derived A2 haplotypes have a restricted distribution, found on only one island or two neighboring islands.
214 This topology is consistent with patterns noted in previous research that suggest multiple independent
215 introductions of A2 lineages into Puerto Rico and the Caribbean, with subsequent expansion of some
216 derived haplotypes across island populations (Martínez-Cruzado 2010; Vilar, et al. 2014).

217 Lastly, the HVR-1 network for haplogroup D1 demonstrates the high diversity of this clade despite its
218 low frequency in the Antilles (e.g. six unique haplotypes in six PC-PR individuals) (Figure S18). This
219 topology is inconsistent with expansion of a single founder and instead suggests derived D1 haplotypes
220 may have also arrived independently to the Antilles. No complete mtDNA sequences matching PC-PR D1
221 haplotypes were found in comparative datasets, or in PhyloTree build 17 (van Oven and Kayser 2009).

222
223 **MtDNA demographic models and estimation of effective population size.** The pre-contact
224 demographic history of Puerto Rico and the Caribbean was further analyzed through an approximate
225 Bayesian computation (ABC) approach. We used Bayesian Serial SimCoal (BayeSSC) to simulate
226 samples based on the available HVR-1 data for pre-contact individuals from Cuba, Dominican Republic,
227 Puerto Rico and Guadeloupe, under eight possible demographic scenarios (M1-M8 with several variants
228 a,b,c, and d, as shown in Figure S19). In each scenario, we varied the amount and direction of migration
229 and gene flow. The best goodness-of-fit value (AIC = 80.59) and highest relative likelihood value
230 ($\omega=0.87$) were associated with model M3b, which represented the peopling of the Antilles as an initial
231 population split followed by low levels of inter-island migration in all directions (Table S17). All other
232 models had low relative likelihood values, including those that varied the direction of gene flow.
233 Therefore, we find that with the resolution provided by the available data, we cannot confidently estimate
234 the direction or timing of pre-contact Caribbean migrations. We also used BayeSSC to test if neutral
235 processes of drift and mutation sufficiently explain the observed genetic distances between pre and post-
236 contact Native American mtDNA lineages in Puerto Rico. We simulated 10,000 mtDNA genome datasets

237 with samples sizes matching the observed data under a model of population continuity (Reynolds, et al.
238 2015). Comparing our empirical genetic distances to the simulated distribution suggests we cannot reject
239 the null hypothesis of population continuity over time ($p=0.0804$).

240 We estimated female effective population size (N_e) over time in Puerto Rico by constructing an
241 extended Bayesian Skyline Plot (eBSP) in BEAST using 127 complete mtDNA sequences from ancient
242 and present-day individuals as input (Native American lineages only) (Figure 7). The eBSP shows an
243 increase in female N_e around 17,000 years before present (YBP) corresponding to population expansion
244 associated with the settlement of the Americas (Mulligan, et al. 2008; Llamas, et al. 2016). After the
245 peopling of Puerto Rico, approximately 5,000 YBP, N_e declines slowly until European contact, about 500
246 YBP, when a sharp reduction occurs. Similar contractions are seen in the demographic histories of other
247 Indigenous populations impacted by European colonization (O'Fallon and Fehren-Schmitz 2011; Lindo,
248 et al. 2016; Llamas, et al. 2016). We also detected some evidence of different demographic histories for
249 the two major Puerto Rican mtDNA clades: A2 and C1 (Figure S20-S24). Specifically, we were unable to
250 reject the null hypothesis of constant population size for the Puerto Rican C1 clade, but we detect a slight
251 decline in N_e over time for haplogroup A2. The BEAST tree in Figure S21 shows an acceleration of the
252 molecular rate of evolution for C1b2 compared to other Puerto Rican clades (Table S18-S19).

253
254 **Autosomal genotypes.** The two newly sequenced partial genomes recovered from Paso del Indio were
255 analyzed alongside the genome of a pre-contact individual from the Preacher's Cave site in the Bahamas:
256 PC537, previously sequenced by Schroeder, et al. (2018). The ancient samples were intersected with a
257 reference panel of 597,573 genome-wide SNPs collected from 967 present-day and ancient individuals in
258 37 worldwide populations (Table S20). Initial analyses conducted with all overlapping sites suggested the
259 PC-PR individuals had a component of non-Native American ancestry (Figure S25-S26). However, this
260 pattern disappeared after removing transitions, suggesting it was an artifact of post-mortem damage
261 (Briggs, et al. 2007). At the best fit value of $K=7$, ADMIXTURE analysis conducted on the no-transitions
262 dataset shows that the two PC-PR individuals have ancestry proportions similar to PC537, and to present-
263 day Amazonian populations such as the Yukpa, Piapoco and Surui (Figure 8). Similarly, in the Principal
264 Components Analysis (PCA), ancient Caribbean individuals cluster with South American populations
265 from Amazonia and the Andes (Figure S27). These results echo the findings of our mtDNA analyses and
266 suggest a close relationship between PC-PR communities and Indigenous South American populations.

267 To determine the genome-wide affinities of these individuals, Outgroup f_3 statistics were calculated
268 in the form $f_3(\text{ancient}, X; \text{Yoruba})$ (Raghavan, et al. 2014), where 'ancient' represented one of the two
269 PC-PR ancient genomes and X was a set of 22 Americas populations or the PC537 individual (Figure
270 S28). However, results were inconsistent and affected by the low number of overlapping sites recovered

271 in the PC-PR samples (Table S21). In our first analysis including all overlapping positions, the two PC-
272 PR individuals were closest first to PC537, and second to South American populations. But, upon
273 removing transitions the closest similarities were between PC-PR and both South American and
274 Mesoamerican groups. Thus, with the low resolution provided by the available data, we cannot
275 confidently estimate genome-wide affinities. Additional analysis with higher coverage genomes is
276 necessary to draw further conclusions about the autosomal ancestry and affinities of PC-PR communities.
277

278 **Discussion**

279 **Preservation and ancient DNA recovery in Puerto Rican archaeological sites.** Endogenous aDNA
280 was poorly preserved in the samples included in this study, consistent with previous Caribbean
281 paleogenomics research (Schroeder, et al. 2015; Nieves-Colón, et al. 2018; Schroeder, et al. 2018).
282 Shotgun sequencing demonstrated that endogenous DNA was found at low quantities in the PC-PR
283 sample. But, through target enrichment, we successfully recovered medium to high coverage mtDNA
284 genomes and low coverage partial autosomal genomes. Thus, our findings show that target enrichment
285 and high-throughput sequencing are essential approaches for maximizing aDNA recovery in Caribbean
286 and tropical archaeological contexts. We also found that DNA preservation varied across sites, with Tibes
287 having the worst preservation. This is consistent with previous reports of poor organic preservation at
288 Tibes relative to other sites in Puerto Rico (Pestle and Colvard 2012). Our results suggest that site-
289 specific processes may play a larger part in aDNA decay than island or region-wide environmental
290 conditions. Future human paleogenomics research at Tibes may benefit from assessing endogenous
291 aDNA preservation in dense skeletal tissues, such as the petrous bone (Gamba, et al. 2014).

292
293 **MtDNA diversity in pre-contact Puerto Rico communities.** Genetic relationships at an intra-island
294 scale were evaluated by testing for significant differences in mtDNA diversity between individuals from
295 the three studied sites. Archaeological evidence indicates a trend towards cultural differentiation and
296 regionalization across Caribbean communities during the LCA. Within Puerto Rico, this is visible in
297 distinctive material culture, settlement patterns and ceramic traditions that differentiated communities
298 along an East-West divide after A.D. 600 (Rouse 1992; Curet, et al. 2004). In this study, we found no
299 evidence for genetic structure or significant differences in mtDNA diversity between PC-PR
300 communities. This suggests that cultural diversification during the LCA may not have been accompanied
301 by genetic isolation or inter-site restrictions on female-mediated gene flow. This interpretation contrasts
302 with (Martínez-Cruzado, et al. 2005) who reported a geographic gradient in the distribution of C1 mtDNA
303 lineages among present-day Puerto Ricans. However, our results are consistent with subsequent work
304 conducted with higher-resolution markers (Gravel, et al. 2013; Vilar, et al. 2014). Thus, we infer that

305 biogeographic differentiation patterns in the Native American ancestry of present-day Puerto Ricans do
306 not date to the pre-contact period, but arose later, due to recent demographic processes. However, we
307 acknowledge that these results may be biased by small sample sizes per site (especially for Tibes) and that
308 future research may reveal more complex patterns of intra-island diversity.

309 Archaeological and isotopic evidence indicate that inter-island interaction and mobility increased in
310 the Antilles during the LCA (Laffoon and Hoogland 2012; Mol 2013). But, dental biodistance studies
311 found few shared morphological traits among pre-contact island groups, suggesting that little gene flow
312 occurred between them (Coppa, et al. 2008). Here, we find genetic evidence for both migration and
313 contact, as well as isolation and differentiation in the population history of the ancient Antilles. We
314 identified a shared mtDNA component among pre-contact groups, mainly represented by high frequencies
315 of shared C1 haplotypes, and similar haplogroup frequencies. But, we also observed several private or
316 island-specific mtDNA haplotypes, which differentiate groups in inter-island comparisons. Further, with
317 the available HVR-1 data, our best fit demographic model supports a scenario where island populations
318 diverge from each other after initial settlement with limited subsequent gene flow. These findings suggest
319 that female-mediated gene flow and matrilineal kinship was not essential for the maintenance of Pan-
320 Caribbean interaction networks during the CA and LCA. Therefore, inter-island connectivity and mobility
321 patterns may respond primarily to other factors such as trade, residency patterns or patrilineal kin
322 networks (Keegan and Maclachlan 1989; Laffoon and Hoogland 2012; Mol 2013). Future research with a
323 more comprehensive genomic sampling of the pre-contact Antilles is needed to study this in more detail.

324
325 **MtDNA lineages and the origins of Ceramic Age Caribbean populations.** MtDNA haplogroup
326 distribution in PC-PR fits a broader Caribbean-wide pattern of high frequencies of haplogroups A2 and
327 C1 and low frequencies of D1 (Martínez-Cruzado, et al. 2005; Benn-Torres, et al. 2007; Mendizabal, et
328 al. 2008; Vilar, et al. 2014; Benn-Torres, et al. 2015; Schurr, et al. 2016). Similar distributions in several
329 ancient Caribbean populations suggest this pattern was common throughout the pre-contact era (Lalueza-
330 Fox, et al. 2003; Mendisco, et al. 2015). We did not find haplogroup B2 in the PC-PR sample. This is
331 consistent with previous research which suggests that B2 was rare in the pre-contact period and that most
332 B2 lineages in present-day Puerto Rico arrived during the final centuries of the LCA or after European
333 contact (Martínez-Cruzado 2010; Vilar, et al. 2014; Benn-Torres, et al. 2015; Mendisco, et al. 2015).
334 However, PC537, the pre-contact individual from the Bahamas, carried a B2 haplotype (Schroeder, et al.
335 2018), so this pattern may not extend to other Antilles.

336 We found similarities in mtDNA variation and haplotype frequencies between PC-PR and Indigenous
337 populations from northwest Amazonia and the Andes. Specifically, the closest relationships were
338 observed between PC-PR and Eastern Tukanoan speakers from the regions surrounding the Orinoco and

339 Rio Negro rivers. These include groups such as the Siriano, Desano and Wanano. We also found close
340 similarities between PC-PR, Yekuana and Kamentsa groups living in Venezuela and Colombia; as well as
341 between PC-PR, Pasto and Quechua speakers from the Andean foothills of Colombia and northern Peru
342 (Barbieri, et al. 2011; Lee and Merriwether 2015; Arias, et al. 2018). These findings are consistent with
343 previous genetics research (Lalueza-Fox, et al. 2001; Lalueza-Fox, et al. 2003; Martínez-Cruzado, et al.
344 2005; Martínez-Cruzado 2010; Gravel, et al. 2013; Moreno-Estrada, et al. 2013; Vilar, et al. 2014;
345 Schroeder, et al. 2018), biodistance studies (Ross 2004; Coppa, et al. 2008) and archaeological evidence
346 (Rouse 1992; Chanlatte Baik 2003; Rodríguez Ramos, et al. 2013) all of which suggest that Caribbean
347 CA populations originated in the Orinoco River delta region of northern South America.

348 The high frequency of sub-haplogroup C1b2 we found in PC-PR (33%) also supports a strong genetic
349 link with South America. Coalescent analyses indicate C1b2 arose in that continent approximately 2,000
350 YBP (Perego, et al. 2010; Gomez-Carballa, et al. 2015). It has been identified in Amazonian populations
351 including the Yanomamö, Kraho and Pasto from Colombia, Brazil and Venezuela, and in communities of
352 Indigenous-descent from Uruguay (Torrioni, et al. 1993; Williams, et al. 2002; Noguera-Santamaría, et al.
353 2015; Sans, et al. 2015). C1b2 is also the most frequent C1 lineage found in present-day Puerto Ricans
354 (Martínez-Cruzado 2010; Vilar, et al. 2014). Previous research estimated that it likely arrived in Puerto
355 Rico after the CA expansions, between 647 ± 373 YBP (estimated with mtDNA control region sequences)
356 and $1,195 \pm 690$ YBP (estimated with HVR-1 sequences) (Martínez-Cruzado 2010; Vilar, et al. 2014). In
357 our network analysis, the Puerto Rican C1b2 clade exhibits a star-like signature suggestive of a strong
358 founder effect with subsequent expansion and differentiation. This is also supported by the BEAST tree
359 for haplogroup C1, which shows that the Puerto Rican C1b2 clade has an abnormally high rate of
360 evolution relative to other clades. Given the large number of identical C1b2 haplotypes we identified, and
361 its consistently high frequency over time, we infer that this lineage has had a continuous presence on the
362 island from the pre-contact period until the present-day. HVR-1 polymorphisms typed in most other
363 Caribbean populations lack sufficient resolution to distinguish C1b2 from the C1 New World founder
364 lineage. Thus, we cannot conclusively determine its frequency over time in other islands. However, C1
365 and C1b haplotypes are found at high frequency throughout the region (Lalueza-Fox, et al. 2001; Lalueza-
366 Fox, et al. 2003; Mendizabal, et al. 2008; Benn-Torres, et al. 2015; Mendisco, et al. 2015; Schurr, et al.
367 2016). Taken together, these data suggest that the Caribbean C1b2 clade arrived originally from South
368 America during the expansion of Arawak speaking populations into the Antilles. We thus support the
369 conclusions of previous research and designate this clade as a characteristic lineage of CA Antillean
370 populations (Martínez-Cruzado 2010; Vilar, et al. 2014).

371 Haplogroup A2 was the second most common haplogroup in our sample, accounting for 40% of all
372 PC-PR sequences. In contrast with the patterns observed for lineage C1b2, the topology of haplotype A2

373 networks suggest multiple, independent lineage introductions into Puerto Rico and other Antilles during
374 the pre-contact period. Previous research proposed that some of these lineages may have originated in
375 Mesoamerica and arrived in the Caribbean during the Lithic Age (Martínez-Cruzado 2002, 2010; Vilar, et
376 al. 2014). This hypothesis is supported by similarities in material culture between Lithic Age Caribbean
377 groups and coeval populations in Belize and Honduras (Wilson and Hester 1998). Additionally,
378 Mesoamerican populations have the highest frequency and diversity of A2 haplotypes (Perego, et al.
379 2010; Gonzalez-Martin, et al. 2015) and similarities have been identified between Caribbean A2 HVR-1
380 types and continental lineages (Vilar, et al. 2014). However, none of the A2 haplotypes in PC-PR were
381 represented in a reference database of over 1,600 complete mtDNA genomes from the Americas, or in
382 broad surveys of Mesoamerican mtDNA diversity (Kumar, et al. 2011; Gorostiza, et al. 2012; Perego, et
383 al. 2012; Mizuno, et al. 2014; Gonzalez-Martin, et al. 2015; Söchtig, et al. 2015). Thus, we cannot trace a
384 direct genetic link between PC-PR and Mesoamerican populations.

385 Lastly, we find that some mtDNA lineages in the PC-PR sample, are not found outside of the Antilles
386 and as such may represent locally differentiated variation. For instance, three PC-PR individuals had
387 haplotypes belonging to sub-haplogroups A2+16218 and A2z. A2+16218 was first reported in self-
388 identified communities of Indigenous descent from Indiera Alta, Maricao, Puerto Rico (Martínez-
389 Cruzado, et al. 2001; Martínez-Cruzado 2010). Afterwards, it was found in broad samples of Cubans and
390 Puerto Ricans (Mendizabal, et al. 2008; Vilar, et al. 2014) and in one individual from Grande Anse, a pre-
391 contact site in Guadeloupe (Mendisco, et al. 2015). Martinez-Cruzado (2010) proposed that A2+16218
392 could be a derived Caribbean-specific lineage, with a proximate origin in pre-contact communities from
393 Mona Island, an island off the southwestern coast of Puerto Rico. Similarly, A2z may also be a Caribbean
394 lineage, with a possible origin in Cuba, where it is found at high frequencies today (Vilar, et al. 2014).

395 That said, genetic drift and the population bottlenecks caused by European contact have led to loss of
396 mtDNA diversity in Native American populations. Genetic discontinuity between pre-contact populations
397 and their descendants has been observed previously in aDNA studies in the Americas (Lindo, et al. 2016;
398 Llamas, et al. 2016). Additionally, some sub-haplogroups identified in PC-PR, such as C1d, C1c, D1 and
399 basal A2 types, are New World founding lineages and have a Pan-American distribution (Perego, et al.
400 2010; Kumar, et al. 2011). These lineages are not informative for tracing sub-continental origins within
401 the Americas. Thus, with the available data, we cannot exclude potential genetic contributions from
402 Mesoamerica or other regions of the Americas to the ancestry of pre-contact Puerto Rican communities.

403
404 **MtDNA diversity and effective population size.** MtDNA diversity in PC-PR is low relative to most
405 comparative populations; with the exception of several Amazonian groups such as the Surui and
406 Karitiana. Low genetic diversity has previously been reported for Amazonian and Eastern South

407 American Indigenous groups due to small historical effective population sizes, isolation and repeated
408 genetic bottlenecks (Lewis, et al. 2007; Hunley, et al. 2016). Similarly, low diversity in PC-PR
409 communities may stem from genetic drift and serial founder effects during the original peopling of the
410 Americas, the initial peopling of the Antilles or the CA expansions. Previous studies of mtDNA and
411 autosomal loci in Puerto Ricans noted a pattern consistent with strong effects of drift and at least one pre-
412 contact population bottleneck (Martínez-Cruzado, et al. 2005; Gravel, et al. 2013). Estimates of long-term
413 historical N_e gleaned from autosomal genome fragments suggest that the Native American ancestors of
414 present-day Puerto Ricans had an effective breeding population size of approximately 1,922 individuals;
415 32 times smaller than the estimated size of coeval populations in Mexico (Gravel, et al. 2013). Strong
416 effects of genetic drift have also been described in previous studies of mtDNA diversity in ancient and
417 present-day Cuban and Dominican populations (Lalueza-Fox, et al. 2001; Lalueza-Fox, et al. 2003;
418 Mendizabal, et al. 2008). In contrast, based on the ancient genome of one individual, Schroeder, et al.
419 (2018) estimated a relatively high N_e of around 1,600 breeding individuals for pre-contact communities in
420 the Bahamas. The results of our diversity estimates, haplotype network and eBSP analyses are consistent
421 with low mtDNA N_e for PC-PR. Our findings support a scenario where a reduced number of mtDNA
422 haplotypes became isolated from their ancestral population after settling in Puerto Rico. This led to a
423 reduction in female N_e , increased susceptibility to genetic drift and loss of mtDNA diversity over time,
424 with the largest reduction occurring after European contact.

425
426 **Autosomal ancestry of PC-PR communities.** Here we report the first recovery of human autosomal
427 genomes from pre-contact Puerto Rico. Our analyses of these data show similar ancestry patterns between
428 two PC-PR individuals and PC537, a pre-contact individual from the Bahamas. Schroeder, et al. (2018)
429 found the genome-wide ancestry of PC537 to be closely related to present-day Arawakan speakers from
430 the Amazon and Orinoco regions of Northern South America, in agreement with archaeological and
431 genetic evidence. The results of our ADMIXTURE analysis are broadly consistent with these findings as
432 we identify similar ancestry proportions between PC-PR individuals and Indigenous populations from the
433 Colombian, Venezuelan and Brazilian Amazon, including the Yukpa, Piapoco, Karitiana and Surui. These
434 similarities suggest that pre-contact Caribbean populations had a shared component of autosomal ancestry
435 with close affinity to South American populations. However, due to poor aDNA preservation and limited
436 overlap between our samples and available reference panels, our genome-wide data lack resolution for
437 confident estimation of allele frequency-based statistics such as Outgroup f_3 , and for direct estimation of
438 sub-continental origins. Thus, we refrain from drawing further inferences regarding the autosomal
439 ancestries or genome-wide affinities of PC-PR communities. Future research with higher coverage
440 genomes and finer-scale analyses of genetic structure is necessary to further address this question.

441 **Indigenous genetic legacies in Puerto Rico.** Through demographic modeling and lineage sharing
442 analysis, we find evidence of direct mtDNA ancestry and partial genetic continuity between pre-contact
443 Indigenous communities and present-day Puerto Ricans. Specifically, three mtDNA haplotypes from PC-
444 PR persist in the modern population. Martínez-Cruzado (2010) predicted that nine Native American
445 mtDNA lineages found in Puerto Ricans had their proximate origin in pre-contact Caribbean populations.
446 We find two of these lineages in PC-PR: C1b2 and A2+16218. However, we also identified multiple
447 haplotypes that are not shared between pre and post contact Puerto Rico. This differentiation reflects both
448 the neutral processes of drift and lineage loss, and the demographic shifts brought by European contact.

449 Extensive historical evidence documents the decline of Indigenous Caribbean populations during the
450 first decades of European colonization. Just before contact, in the 15th century, estimates place the
451 population of Puerto Rico between 30,000 to 70,000 individuals (Anderson-Córdova 2005; Anderson-
452 Córdova 2010). By the census of 1530, the native population was reportedly 1,543 individuals. This count
453 included native communities as well as Indigenous people forcibly relocated from other islands or from
454 the Circum-Caribbean basin (Anderson-Córdova 2005; Wilson 2007). Despite this decline, in a database
455 of over 1,600 complete mitochondrial genomes from the Americas, the only identical matches to PC-PR
456 haplotypes were found in Puerto Rico. This indicates that admixed Puerto Rican genomes are at least a
457 partial reservoir of pre-contact mtDNA diversity. As publicly available genomic datasets become more
458 representative of the diversity of Native American and Latino populations (Bustamante, et al. 2011), it is
459 possible we may find other populations, in the Caribbean or elsewhere, that are also closely related to pre-
460 contact Puerto Rican communities. Beyond its anthropological importance, better characterization of the
461 genetic diversity of ancient populations that contributed to present-day Puerto Rican ancestry may guide
462 future efforts at rare variant discovery in Caribbean biomedical cohorts (Belbin, et al. 2017).

463 Our findings do not support historical narratives of complete population replacement or genetic
464 extinction of Indigenous communities in Puerto Rico. Indigenous heritage is an important component of
465 Puerto Rican national and ethnic identity (Haslip-Viera 2001; Veran 2003). However, cultural claims of
466 indigeneity and Native American ancestry coexist with narratives of Indigenous extinction (Benn-Torres
467 2014). For the most part, these narratives are rooted in interpretations of the written historical record and
468 colonial era censuses. But these documents under-represent the number of Indigenous people living in
469 Puerto Rico and other islands during Spanish occupation (Anderson-Córdova 2010; Benn-Torres 2014).
470 Other historical sources as well as ethnographic research note that Indigenous peoples in Puerto Rico and
471 the Antilles resisted European colonization and persisted, albeit in small numbers, well into the 16th
472 century (Anderson-Córdova 2010). Moreover, oral histories of Indigenous survival are common in Puerto
473 Rico and other islands (Forte 2006). Some of these oral narratives are reinforced by genetic research
474 which has found reservoirs of Native American genetic ancestry in communities that self-identify with

475 Indigenous or Maroon descent in Puerto Rico, Jamaica and other islands (Martínez-Cruzado, et al. 2001;
476 Madrilejo, et al. 2015; Schurr, et al. 2016; Fuller and Benn Torres 2018; Benn Torres, et al. 2019).
477 Furthermore, self-identified and government-recognized Indigenous communities are found throughout
478 the Caribbean and the international Caribbean diaspora (Forte 2006).

479
480 **Conclusions** In conclusion, this research characterized the genetic diversity of pre-contact communities
481 in Puerto Rico and tested hypotheses about their origins and relationships to other Native American and
482 Caribbean populations. Our findings support a primarily South American contribution to the genetic
483 ancestry of pre-contact Puerto Rican peoples, in agreement with previous genetics and archaeological
484 research. However, we cannot reject the possibility that additional migrations from other parts of the
485 Americas also contributed to the peopling of Puerto Rico. Future research with more ancient genomes
486 from the Antilles, and higher coverage genome-wide data will provide added resolution for detecting
487 ancient admixture events in the Caribbean and elucidating the genetic relationships between island
488 communities and continental Native American populations. We also found evidence that at least some of
489 the mtDNA diversity of present-day Puerto Ricans, can be directly traced to pre-contact Puerto Rican
490 communities. Thus, our study adds to a growing corpus of research documenting the persistence of
491 cultural and biological elements from pre-contact Indigenous Caribbean peoples into the present day. We
492 hope our findings lead to a critical and interdisciplinary reassessment of historical narratives of
493 Indigenous extinction in Puerto Rico, while informing future study of Indigenous responses to European
494 colonization, and of the complex role of native peoples in shaping the biocultural diversity of the Antilles.

495 496 **Materials and Methods**

497 **Sampling, DNA extraction and library preparation.** The human skeletal remains included in this study
498 are patrimony of the people of the Commonwealth of Puerto Rico. Permits for destructive sampling and
499 DNA analysis were obtained from three government agencies in Puerto Rico (Figure S1). We sampled
500 124 individuals excavated from three pre-contact sites: Punta Candeleró (n=34), Tibes (n=46) and Paso
501 del Indio (n=44) (Figure 1; Figure S2). Direct radiocarbon dates for 81 individuals were previously
502 obtained by (Pestle 2010) (Table S1; Figure S3). DNA was extracted from tooth roots, bone or dental
503 calculus using silica-based extraction methods optimized for aDNA (Table S2) (Rohland and Hofreiter
504 2007; Dabney, et al. 2013; Nieves-Colón, et al. 2018). DNA libraries were constructed, double-indexed
505 and amplified following (Meyer and Kircher 2010; Kircher, et al. 2012; Seguin-Orlando, et al. 2015). The
506 optimal number of PCR cycles was determined by real-time PCR (qPCR). Libraries were purified with
507 the Qiagen® MinElute PCR kit. DNA fragment sizes were assessed with the Agilent 2100 Bioanalyzer.

508 **Target enrichment and Illumina sequencing.** Targeted enrichment for the complete mtDNA genome
509 was performed following (Maricic, et al. 2010; Ozga, et al. 2016). MtDNA enriched libraries were
510 sequenced on multiple runs of the Illumina MiSeq. Thirty-five libraries were additionally screened by
511 shotgun sequencing on several runs of the Illumina NextSeq 500 and HiSeq 2500. Twenty-two of these
512 libraries were selected for WGE performed following (Carpenter, et al. 2013), with slight modifications
513 (see Supplementary Materials & Methods) or with the MYbaits Human Whole Genome Capture Kit
514 (Arbor Biosciences), following manufacturer's instructions. After WGE, libraries were amplified, and
515 purified as detailed above, then sequenced on several runs of the Illumina NextSeq 500 and HiSeq 2500.

516
517 **Sequence read processing.** Illumina sequence reads were merged, and adapters trimmed with SeqPrep
518 (<https://github.com/jstjohn/SeqPrep>). Mapping was performed using BWA v.0.7.5 with seed disabled (Li
519 and Durbin 2009; Schubert, et al. 2012). For mtDNA enriched libraries, reads were mapped to the revised
520 Cambridge Reference Sequence (rCRS) (Andrews, et al. 1999). For shotgun and WGE libraries, reads
521 were mapped to the GRCh37 (hg19) assembly with the mitochondrial sequence replaced by the rCRS.
522 BAM files from samples sequenced over multiple sequencing runs were merged with SAMtools v.0.1.19
523 (Li, et al. 2009). Filtering and duplicate removal were also performed with SAMtools, keeping reads with
524 quality $\geq Q30$ and no multiple mappings. Damage patterns were characterized and read quality scores
525 rescaled with mapDamage v.2.0.2 (Figure S5-S7) (Jónsson, et al. 2013). Contamination estimates were
526 generated for mtDNA reads with contamMix (Fu, et al. 2013) and schmutzi (Renaud, et al. 2015). Reads
527 were further contamination filtered with PMDtools (Skoglund, et al. 2014) (Tables S3-S4).

528
529 **MtDNA variant calling, haplogroup assignment and data analyses.** MtDNA variants were called
530 using SAMtools *mpileup* on the rescaled BAM files. Haplogroup assignment was performed in
531 HaploGrep 2.0 (Weissensteiner, et al. 2016) and confirmed manually with reference to Phylotree mtDNA
532 tree Build 17 (van Oven and Kayser 2009). MtDNA consensus sequences were generated with schmutzi
533 and curated manually in Geneious v.7.0.6 (Biomatters). MtDNA reference data collected from the
534 literature included 1,636 complete mtDNA genomes from ancient and present-day individuals from the
535 Americas and Caribbean. For comparative analyses, this dataset was restricted to 1,403 sequences
536 grouped into 47 populations. A second comparative dataset included 391 mtDNA HVR-1 sequences from
537 ancient and present-day Caribbean islanders (see Table S10).

538 We performed a Mantel test to evaluate the relationship between temporal and genetic distance in the
539 PC-PR sample. For radiocarbon dated individuals ($n=35$), this test compared a Euclidean distance matrix
540 of median calibrated radiocarbon dates and a Tamura-Nei genetic distance matrix. Intra-population
541 diversity measures such as number of haplotypes (h), number of segregating sites (S), nucleotide (π) and

542 haplotype diversity (Hd) were calculated for PC-PR and all reference populations. We calculated exact
543 tests of genetic differentiation to compare haplotype frequencies between the three PC-PR sites, between
544 PC-PR and continental Native American populations (complete mtDNA) and between PC-PR and
545 Caribbean populations (HVR-1). We also calculated pairwise population Φ_{st} measures using complete
546 mtDNA and HVR-1 sequences (Excoffier, et al. 1992). The resulting matrix was used as input for non-
547 metric MDS scaling. Population haplogroup frequencies (e.g. A, B, C, D, X) were estimated by direct
548 counting and used as input for correspondence analysis. These analyses were performed and plotted using
549 R v. 3.6.1 (R Core Team). For additional details see Supplementary Materials & Methods. Lastly, median
550 joining haplotype networks were constructed in popART with default parameters (Bandelt, et al. 1999;
551 Leigh and Bryant 2015). Complete mtDNA and HVR-1 networks were constructed per haplogroup (A2,
552 C1, D1) comparing PC-PR with populations from the Americas and Caribbean.

553 We used BayeSSC (Excoffier, et al. 2000; Anderson, et al. 2005) to model eight possible
554 demographic scenarios (Figure S19: M1-M8) that could explain the HVR-1 mtDNA diversity reported for
555 ancient Caribbean populations (Lalueza-Fox, et al. 2001; Lalueza-Fox, et al. 2003; Mendisco, et al. 2015).
556 To determine which model most likely explained the observed data, we calculated Euclidean distances
557 between simulated and empirical datasets following (Beaumont, et al. 2002; Duggan, et al. 2017).
558 Goodness of fit was determined using the Akaike information criterion (AIC) (Akaike 1974). The model
559 with the lowest AIC value and the highest relative likelihood value was chosen as the best fit. We also
560 used BayeSSC simulations to evaluate if the observed Fst genetic distances between the complete
561 mtDNA genomes collected from ancient and present-day Puerto Rico (Native American haplotypes only)
562 were consistent with a model of population continuity. This model was simulated 10,000 times to produce
563 a distribution of possible Fst values for comparison with observed Fst values (Reynolds, et al. 2015).

564 Demographic history was further reconstructed with eBSP in BEAST 1.8.4 (Drummond, et al. 2012)
565 using partitioned complete mtDNA sequences from ancient and present-day Puerto Rico as input.
566 PartitionFinder v.1.1.1 (Lanfear, et al. 2012) was used to determine the best partitioning scheme and
567 substitution model for the data. Median calibrated radiocarbon date estimates were used as tip calibrations
568 to reconstruct the phylogeny. Individuals without radiocarbon dates were assigned a prior date range
569 based on archaeological context, and posterior dates were estimated based on the empirically calculated
570 molecular rate (Table S19). Analyses were conducted under a strict clock model, which could not be
571 rejected after testing other models. A total of 3 chains of 100 million generations were executed for three
572 datasets: All sequences, Haplogroup A, and Haplogroup C. Parameters were sampled every 10,000
573 generations, with the initial samples discarded as burn-in. Generation time was set as 25 years.

574

575 **Autosomal genome genotype calling and data analysis.** Two PC-PR samples with the highest read
576 depth and endogenous content (PI-420a and PI-51) were sequenced across multiple runs to increase
577 genome coverage. Reads were combined, and filtering was repeated as detailed above. Chromosomal sex
578 was estimated following (Skoglund, et al. 2013) and X-chromosome contamination was estimated with
579 ANGSD (Korneliussen, et al. 2014) (Table S5). Autosomal genotypes from PC-PR were analyzed
580 alongside the genome of a pre-contact individual from the Bahamas: PC537 (Schroeder, et al. 2018).
581 These samples were intersected with a reference panel of 597,573 SNPs from 37 worldwide populations
582 compiled from the literature (N=967), which included 656 individuals from the Americas (see Table
583 S20). Haploid genotype calls in the ancient Caribbean individuals were generated by randomly sampling
584 one read per overlapping positions with the reference panel. PCA was performed with Eigensoft 6.0.1
585 (Patterson, et al. 2006) using the *lsqproject* option. Outgroup- f_3 analysis was performed with *qp3pop*
586 within Admixtools (Patterson, et al. 2012) in the form $f_3(\text{ancient}, X; \text{Yoruba})$ (Raghavan, et al. 2014),
587 where ‘ancient’ represented one of the ancient genomes from PC-PR and X was a reference population or
588 individual. For admixture analysis, a genotype likelihood approach was implemented with FastNGSadmix
589 (Jørsboe, et al. 2017). Genotype likelihoods in the ancient samples were estimated for all reference panel
590 overlapping positions using ANGSD. We then conducted ten runs of ADMIXTURE for each value of K3
591 to K7 (Alexander, et al. 2009) using only the reference panel individuals. We retained the run with the
592 highest likelihood per K to calculate the proportion of the ancestral components in the ancient genomes
593 (Figure S26). Analyses were performed both with and without transitions to account for aDNA damage.
594

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604

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887 **Figure Legends**

888

889 **Figure 1. Map of Puerto Rico and the Antilles.** Triangles are approximate location of pre-contact sites.

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891

892 **Figure 2. MtDNA sub-haplogroups in the PC-PR sample.**

893

894 **Figure 3. Non-metric MDS plot of complete mtDNA Φ_{ST} distances between PC-PR and 46 ancient**
895 **and present-day populations from the Americas**

896

897 **Figure 4. Correspondence analysis plot of haplogroup frequencies between PC-PR and 46 ancient**
898 **and present-day populations from the Americas.**

899

900 **Figure 5. Non-metric MDS plot of HVR-1 Φ_{ST} distances between PC-PR and eight ancient and**
901 **present-day Caribbean populations.**

902

903 **Figure 6. Median joining network of complete mtDNA haplotypes in PC-PR and present-day**
904 **Puerto Rico. A) Haplogroup A, B) Haplogroup C. Major sub-haplogroup clades are labeled.**

905

906 **Figure 7. Extended Bayesian skyline plot (eBSP) of female effective population size, based on a**
907 **generation time of 25 years. Bottom panel is zoomed in to 5,000 YBP.**

908

909 **Figure 8. ADMIXTURE analysis at K=7 including autosomal genotypes from two pre-contact**
910 **individuals from PC-PR (PI-420A, PI-51), one pre-contact individual from the Bahamas (PC537),**
911 **and 37 worldwide reference population**

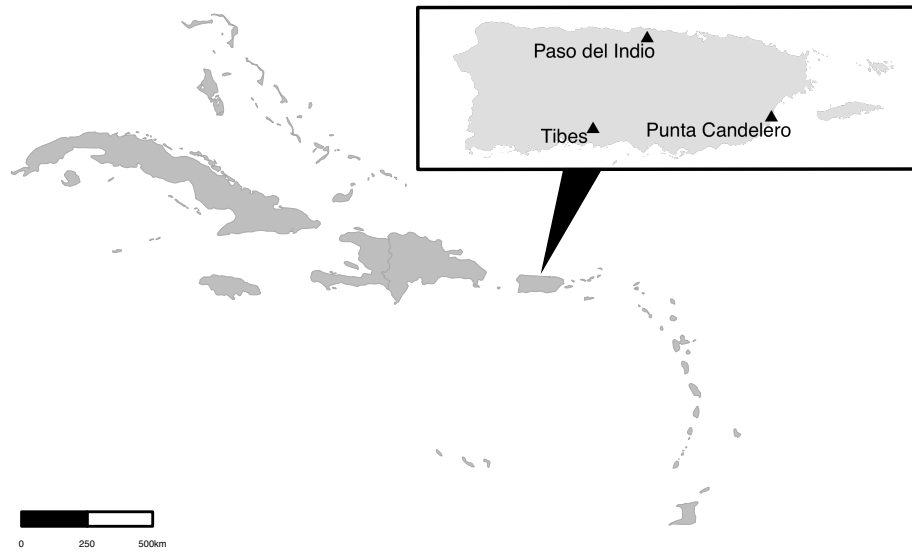


Figure 1. Map of Puerto Rico and the Antilles. Triangles are approximate location of pre-contact sites.

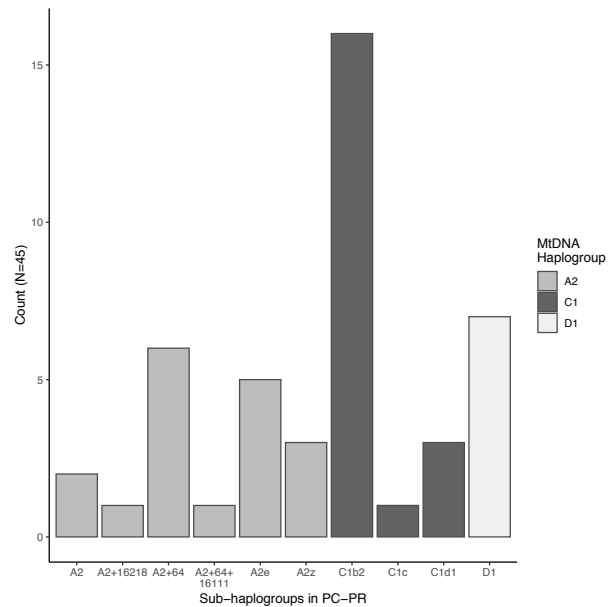


Figure 2. MtDNA sub-haplogroups in the PC-PR sample.

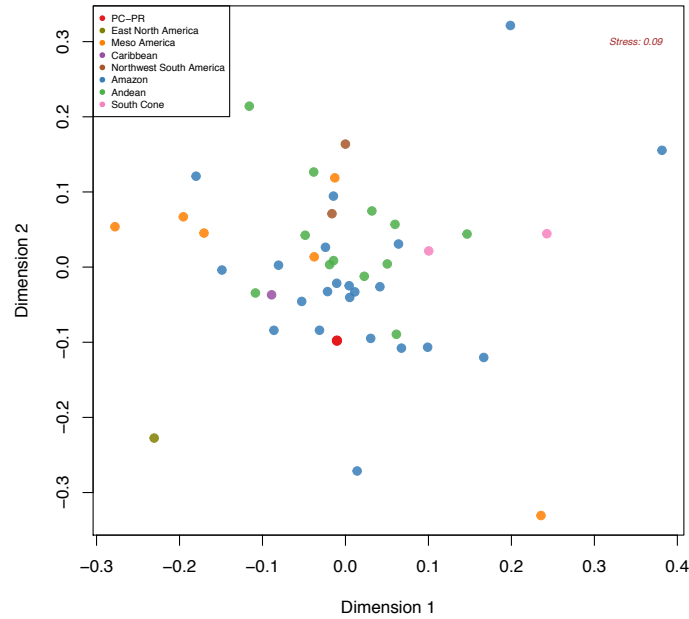


Figure 3. Non-metric MDS plot of complete mtDNA Φ_{ST} distances between PC-PR and 46 ancient and present-day populations from the Americas

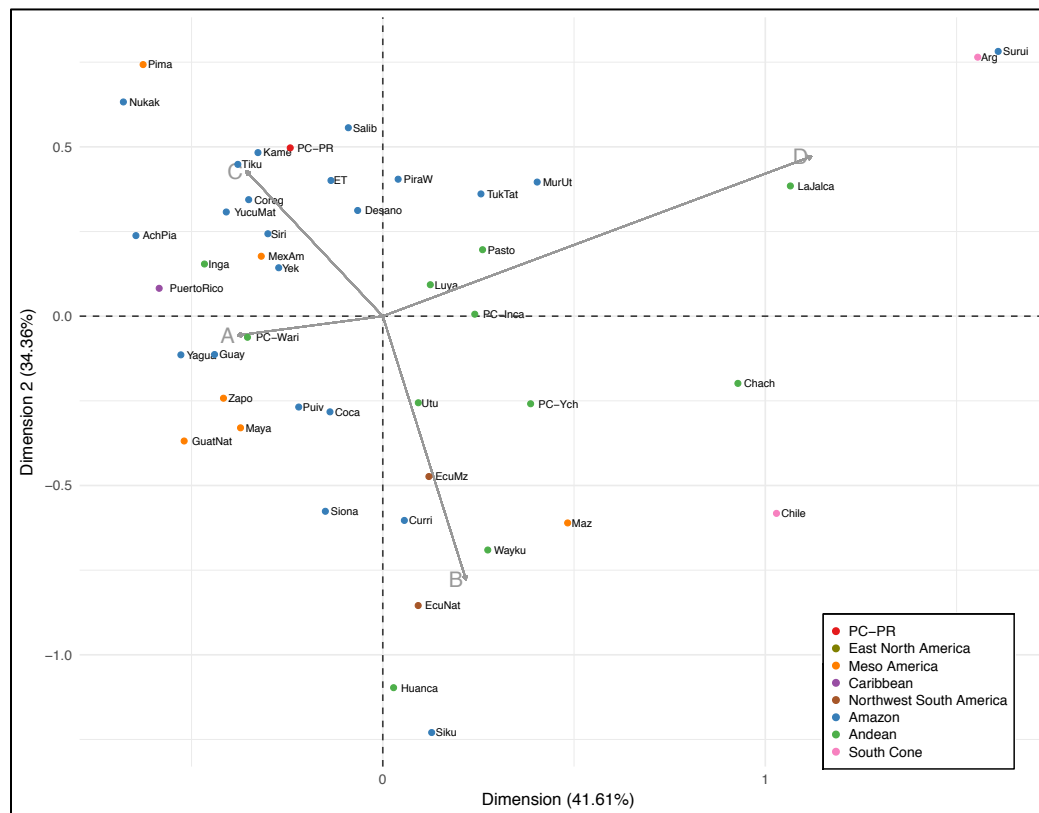


Figure 4. Correspondence analysis plot of haplogroup frequencies between PC-PR and 46 ancient and present-day populations from the Americas

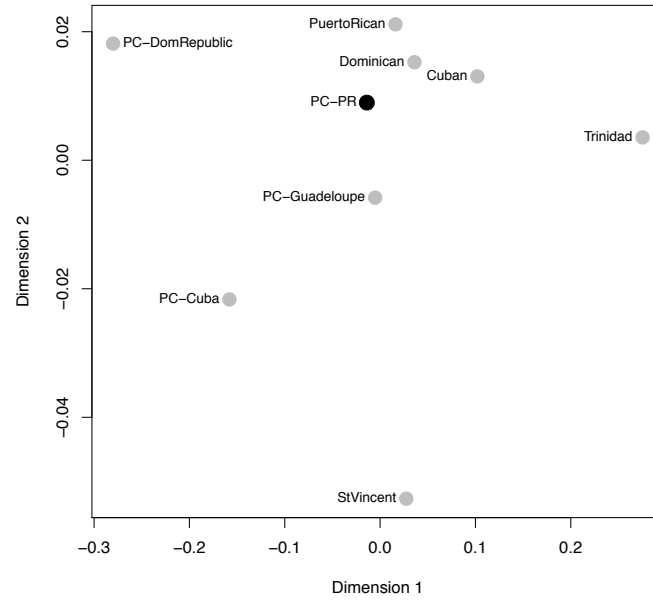


Figure 5. Non-metric MDS plot of HVR-1 Φ_{ST} distances between PC-PR and eight ancient and present-day Caribbean populations.

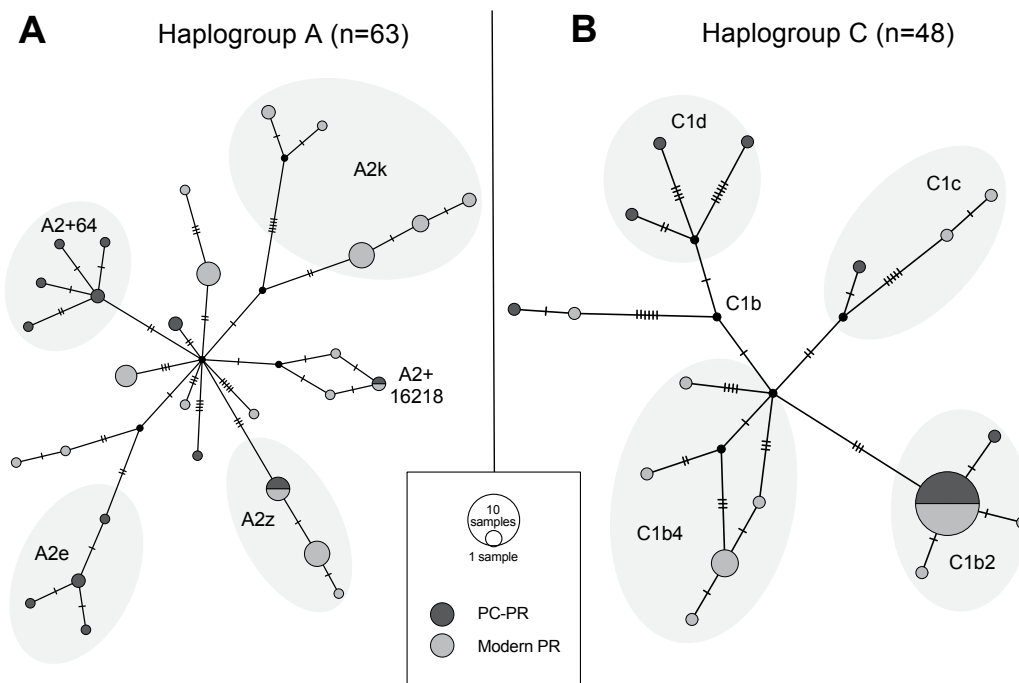


Figure 6. Median joining network of complete mtDNA haplotypes in PC-PR and present-day Puerto Rico. A) Haplogroup A, B) Haplogroup C. Major sub-haplogroup clades are labeled

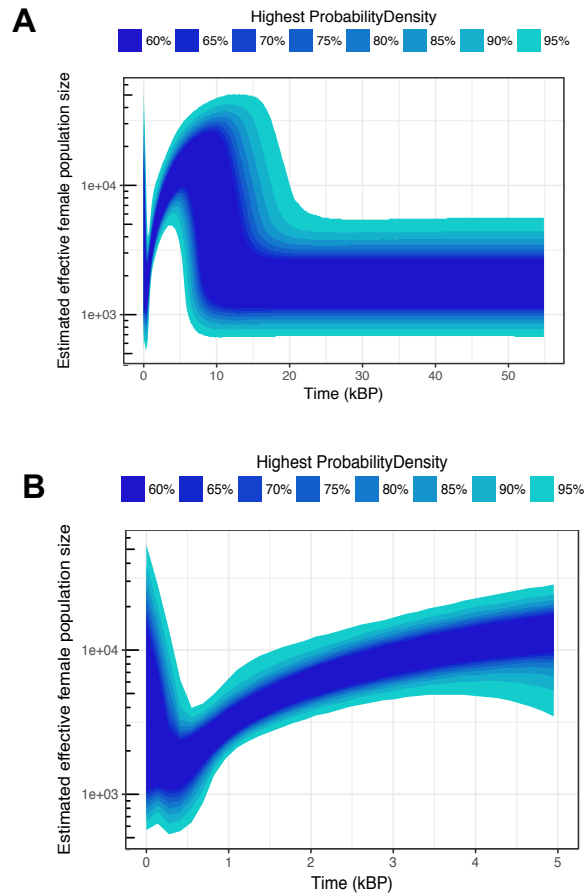


Figure 7. Extended Bayesian skyline plot (eBSP) of female effective population size, based on a generation time of 25 years. Bottom panel is zoomed in to 5,000 YBP.

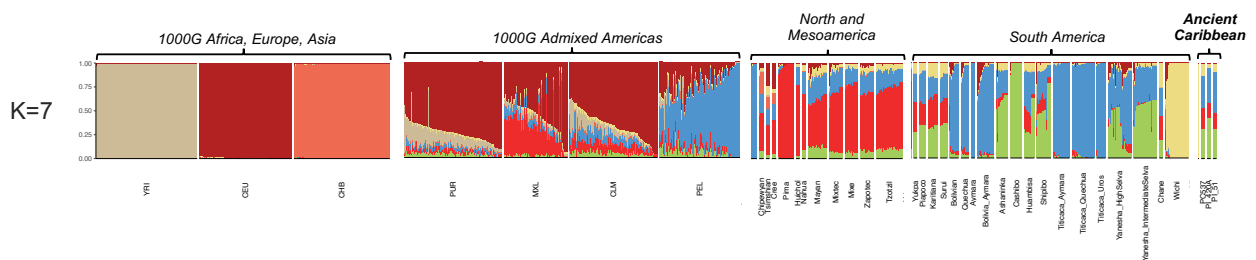


Figure 8. ADMIXTURE analysis at K=7 including autosomal genotypes from two pre-contact individuals from PC-PR (PI-420A, PI-51), one pre-contact individual from the Bahamas (PC537), and 37 worldwide reference populations.