

1 **Population Turnover in Remote Oceania Shortly After Initial Settlement**

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44 **Summary**

45 Ancient DNA analysis of three individuals dated to ~3000 years before present (BP) from Vanuatu
46 and one ~2600 BP individual from Tonga has revealed that the first inhabitants of Remote Oceania
47 (“First Remote Oceanians”) were almost entirely of East Asian ancestry, and thus their ancestors
48 passed New Guinea, the Bismarck Archipelago, and the Solomon Islands with minimal admixture
49 with the Papuan groups they encountered [1]. However, all present-day populations in Near and
50 Remote Oceania harbor 25-100% Papuan ancestry, implying that there must have been at least one
51 later stream of migration eastward from Near Oceania. We generated genome-wide data for 14
52 ancient individuals from Efate and Epi Islands in Vanuatu ranging from 3,000-150 BP, along with
53 185 present-day Vanuatu individuals from 18 islands. We show that people of almost entirely
54 Papuan ancestry had arrived in Vanuatu by 2400 BP, an event that coincided with the end of the
55 Lapita cultural period, changes in skeletal morphology, and the cessation of long-distance trade
56 between Near and Remote Oceania [2]. First Remote Oceanian ancestry subsequently increased via
57 admixture but remains at 10-20% in most islands. Through a fine-grained comparison of ancestry
58 profiles in Vanuatu and Polynesia with diverse groups in Near Oceania, we find that Papuan
59 ancestry in Vanuatu is consistent with deriving from the Bismarck Archipelago instead of the
60 geographically closer Solomon Islands. Papuan ancestry in Polynesia also shows connections to the
61 ancestry profiles present in the Bismarck Archipelago but is more similar to Tolai from New Britain
62 and Tutuba from Vanuatu than to the ancient Vanuatu individuals and the great majority of present-
63 day Vanuatu populations. This suggests a third eastward stream of migration from Near to Remote
64 Oceania bringing a different type of Papuan ancestry.

65

66 **Keywords:** Near Oceania, Remote Oceania, Pacific Islanders, Lapita, Migration, Ancient DNA

67

68 **Results and Discussion**

69

70 We generated genome-wide data for 14 ancient individuals from Central Vanuatu (**Table 1; Table**
71 **S1**). Of these, 11 individuals are newly reported, and 3 individuals that were previously published
72 are represented here by higher quality data [1]. We identified and selected cochlear bone sections of
73 petrous bones and processed them into powder in dedicated clean rooms at University College

74 Dublin [3]. We then shipped the powder to Harvard Medical School, where in a second set of clean
75 rooms we extracted DNA [4, 5] and created individually barcoded Illumina sequencing libraries,
76 some of which we treated with the enzyme Uracil-DNA Glycosylase (UDG) to greatly reduce the
77 characteristic errors associated with degraded ancient DNA [6, 7]. We screened these libraries for
78 evidence of authentic ancient DNA by enriching for DNA overlapping the mitochondrial genome
79 [8], sequencing on an Illumina NextSeq500 instrument, and assessing the data based on rates of
80 cytosine-to-thymine damage in the terminal nucleotide and consistency with the consensus
81 mitochondrial genome (STAR Methods) [9]. For libraries that were promising after screening, we
82 enriched for regions targeting approximately 1.24 million single nucleotide polymorphisms (SNPs)
83 in the human genome and sequenced the enriched products to greater depth (STAR Methods). We
84 determined sex by examining the ratio of sequences overlapping the Y chromosome and X
85 chromosome, and for males, we additionally estimated contamination based on the rate of
86 polymorphism on the haploid X chromosome (STAR Methods; **Table S1**). The data for the 14
87 individuals passing quality control were derived from a total of 46 Illumina libraries (1-8 per
88 individual; **Table S2**). We also generated genome-wide SNP genotype data on the Human Origins
89 array for 185 present-day individuals from Vanuatu who gave informed consent for studies of
90 genetic variation, with approval from both the University of Oxford and the Vanuatu Cultural
91 Centre (STAR Methods; **Table S3**).

92 93 *Clustering analyses*

94 We performed automated clustering analysis with the ADMIXTURE software [10], using a data set
95 consisting of the ancient and present-day Vanuatu samples together with other Oceanian, East
96 Asian, and worldwide populations genotyped on the Human Origins array [1] (**Figure 1; Figure**
97 **S1**). At $K = 8$ clusters, four ancestry components were inferred to be widespread in Oceania. Three
98 correlate (predominantly) to Papuan ancestry, and are maximized in New Guinea (purple in the
99 ADMIXTURE plot), Mamusi and Baining from New Britain (blue), and Nasioi from Bougainville
100 in the Solomon Islands (red). The fourth component (green) correlates to First Remote Oceanian
101 ancestry, and is maximized in the ancient Lapita individuals from Vanuatu and Tonga. Other
102 Oceanian populations display variable combinations of these components, forming gradients of
103 ancestry between New Guinea, New Britain and New Ireland in the Bismarck Archipelago, and the
104 Solomon Islands. The great majority of present-day as well as ancient groups from Vanuatu show
105 highly similar ratios of the three Papuan ancestry components (although their First Remote

106 Oceanian proportions vary), suggesting that they largely derived their Papuan ancestry from the
107 same source. Among populations in Near Oceania, the most similar to Vanuatu in terms of the
108 Papuan ancestry component ratio (purple-to-blue-to-red) are groups from New Britain in the
109 Bismarck Archipelago with a majority of the blue component and smaller contributions of purple
110 and red, pointing to an origin from the Bismarck Archipelago (rather than the geographically closer
111 Solomon Islands) for the Papuan ancestry in Vanuatu. A similar pattern was previously inferred for
112 the origin of the Papuan ancestry in Santa Cruz to the north of Vanuatu [11] (a result we replicate
113 here), implying similar sources for both island chains.

114
115 We also carried out a principal component analysis focusing on the geographic variation in Papuan
116 ancestry (**Figure S2**). The results confirm those from ADMIXTURE, with the primary feature being
117 a U-shaped cline from top left to top right—encompassing Nakanai (western New Britain), Sulka
118 and Megen (eastern New Britain), most of Vanuatu, Tolai, Tutuba, New Ireland, and finally
119 Bougainville—corresponding closely to a trend of increasing red and decreasing blue components
120 in ADMIXTURE. The position of the Vanuatu samples in the PCA also supports the hypothesis that
121 the inhabitants of the region after the initial Lapita settlement derived ancestry ultimately not from
122 the closer Solomon Islands but from the area of New Britain in the Bismarck Archipelago.

123
124 *Papuan and First Remote Oceanian ancestry proportions*

125 It has been shown that the strongest driver of genetic variation in Oceania today is the widespread
126 but highly variable admixture between Papuan and First Remote Oceanian ancestry sources, the
127 former representing original inhabitants of Near Oceania and the latter descendants of the
128 Austronesian expansion from East and Southeast Asia [1]. From our clustering results, a dramatic
129 turnover is apparent in Vanuatu between around 3000 and 2400 years ago, with First Remote
130 Oceanian populations being joined or possibly completely replaced by individuals of (almost)
131 entirely Papuan ancestry. To provide precise estimates of mixture proportions, we used f_4 -ratio
132 statistics [12], assuming a topology of (Atayal, (Kankanaey, First Remote Oceanian)) for East
133 Asian-derived ancestry and (Australian, (New Guinea, Papuan)) for Papuan ancestry (**Figure 1**;
134 **Table S4**). Taking advantage of our increased coverage compared to the first study of Lapita
135 samples, we find that the ~3000 BP Lapita individuals likely had a small amount of Papuan-related
136 ancestry ($2.4 \pm 0.9\%$), although it remains striking that the initial First Remote Oceanian migrants
137 were only minimally admixed. Given the small proportion, we did not have sufficient statistical

138 power to determine whether this Papuan-related ancestry is derived from the region surrounding
139 New Guinea or could perhaps have been acquired elsewhere, such as in the Philippines or eastern
140 Indonesia. Notably, the first post-Lapita sample (2400 BP from Mele-Taplins, Efate) had almost
141 entirely Papuan ancestry but with a small amount from First Remote Oceanians ($4.2 \pm 1.1\%$). The
142 more recent ancient individuals are similar in their proportions to present-day populations: 8-12%
143 First Remote Oceanian ancestry for 1400-200 BP and 20% for 150 BP (Efate), as compared to a
144 range of 9-38% today (mostly 12-20%; maximized in the Polynesian outlier population of Futuna).
145 For time points with multiple samples, the individuals' mixture proportions are statistically
146 indistinguishable, except at 150 BP ($\sim 14\%$, 21% , and 26% First Remote Oceanian).

147

148 *Dates of admixture*

149 We estimated dates of admixture based on weighted admixture linkage disequilibrium (LD) [13]
150 using ALDER [14], with Ami and New Guinea as references (**Figure 2; Table S4**). We obtain
151 significant evidence for admixture LD in almost all present-day populations and three ancient
152 population groupings (noting that power is highly sample size-dependent). The date estimates are
153 mostly 40-100 generations ago, or 1,100-2,800 years ago assuming 28 years per generation [15],
154 consistent with initial admixture soon after the early settlement of Vanuatu and further mixture
155 continuing through time (in cases of multiple pulse of admixture, ALDER produces a single average
156 date). We observe a modest but significant negative correlation between admixture date and First
157 Remote Oceanian ancestry proportion ($R^2 = 0.32$ for populations in **Figure 2**, nominal $p < 0.01$), as
158 would be expected if a subset of populations (e.g., Efate, Emae, Futuna, Makura) received more
159 recent pulses of gene flow from groups with high proportions of First Remote Oceanian ancestry (a
160 plausible scenario in light of Polynesian cultural influence [16]). We also obtain a direct admixture
161 date of 18 ± 6 generations in the past (500 ± 160 years) for a pair of ancient samples from Vanuatu
162 radiocarbon dated to $\sim 1,400$ years ago, consistent with the ALDER dates in the majority of present-
163 day groups. There has been debate about the timing of admixture between people of East Asian and
164 Papuan ancestry in Remote Oceania, with methods based on wavelet transformations suggesting
165 mixing $>3,000$ BP, prior to the Lapita expansion to Remote Oceania [11, 17], and methods based on
166 admixture LD suggesting more recent dates, implying that mixture must have occurred following
167 later streams of gene flow [18]. It was recently argued that the differences may reflect systematic
168 biases of the methods for dates more than a couple of thousand years old [11], and thus our finding

169 of a definitively post-Lapita date in samples that are within a thousand years of the estimated
170 admixture date strengthens the evidence for more recent mixture.

171

172 *Phylogeny of First Remote Oceanian ancestry*

173 To test whether the First Remote Oceanian ancestry in ancient and present-day groups is more
174 closely related to Lapita samples from Tonga or Vanuatu, we used a block jackknife to evaluate the
175 difference between the statistics $f_4(\text{Test}, \text{Han}; \text{Atayal}, \text{Tonga}_{2600\text{BP}})$ and $f_4(\text{Test}, \text{Han}; \text{Atayal},$
176 $\text{Vanuatu}_{3000\text{BP}})$ for Oceanian populations as *Test* (STAR Methods). We found a trend toward
177 greater allele-sharing with Tonga, with significant results in Polynesian and to a lesser degree
178 Polynesian outlier populations (**Table S5**). These results show that the First Remote Oceanian
179 ancestry in Polynesians today is derived from a source that was closer to the sampled Lapita-period
180 population from Tonga than to the Vanuatu Lapita population. We do not observe significant
181 differences for present-day populations from Vanuatu, but our statistical power is limited due to the
182 small proportions of First Remote Oceanian ancestry.

183

184 *Phylogeny of Papuan ancestry*

185 We built admixture graphs to explore in more detail the different streams of Papuan ancestry
186 present in Oceania. We used as reference populations Australia, Kankanaey, Atayal, and Mixe,
187 together with representatives of major poles of Papuan genetic variation inferred from the
188 ADMIXTURE analysis: Vanuatu_Tanna, Mamusi (New Britain), Nasioi (Solomon Islands), New
189 Guinea, and Tolai (New Britain/New Ireland). To avoid overfitting, we adopted a restricted
190 framework in which the ancestry in each population was modeled as a combination of the same set
191 of source lineages, with the exception of the unadmixed New Guinea population. We found that
192 three Papuan source lineages were necessary in order to obtain a good fit for the model—one
193 maximized in Mamusi, one maximized in Nasioi, and one closest to New Guinea—showing that the
194 implied ancestry components from ADMIXTURE (**Figure S1**) are all well-supported in formal
195 models based on allele-sharing statistics (**Figure S3**). The admixture graph analysis suggests that
196 the blue (Bismarck Archipelago-majority) and red (Solomon Islands-majority) ADMIXTURE
197 components represent admixed ancestry: both include First Remote Oceanian ancestry (~20% for
198 red and ~5% for blue), and the two are additionally admixed with each other, as we could not fit a
199 Solomon Islands population (e.g., Nasioi) and a Bismarck Archipelago population (e.g., Mamusi or
200 Baining) simultaneously without admixture from one to the other. In our models, we included

201 Solomon Islands-type ancestry in Mamusi (approximately one-third of its total Papuan ancestry),
202 although we were unable to distinguish the direction(s) of gene flow. Vanuatu was confidently
203 inferred to have ancestry from all three Papuan sources ($Z > 8$ for omitting any source).

204

205 We next asked if we could add Polynesians (Tongan) as a mixture of a component related to one of
206 the other Oceanian populations along with additional First Remote Oceanian ancestry. Such a
207 model was successful only in one configuration, with Tongan as a mixture of Tolai-related and First
208 Remote Oceanian ancestry (all f_4 -statistics fit to within 2.0 standard errors of their observed values
209 except for one residual, $f_4(\text{Kankanaey, Tongan; Australian, Vanuatu_Tanna})$, at $Z = 2.7$; **Figure 3**
210 **and Figure S3**). Our choice to include Tolai in the model was guided by the ADMIXTURE
211 analysis, in which the Papuan ancestry profile in Polynesians appears to match that in Tolai (and
212 Tutuba, from near Espiritu Santo Island in Vanuatu) more closely than other populations. We note
213 that the Tolai are known to be descended from relatively recent mixture between groups from New
214 Ireland and New Britain (resulting from displacement caused by the eruption of the Rabaul caldera
215 ~1400 BP [19]), so their ancestors cannot represent the true source population of the Papuan
216 ancestry in Polynesians. However, the similarity of Tolai Papuan ancestry to Polynesians suggests
217 that the Papuan component in Polynesians could similarly be from a mixture of multiple Near
218 Oceanian sources. Given that Tolai are intermediate between populations from New Britain and
219 New Ireland (the latter with high Solomon Islands-related ancestry), Polynesians could plausibly
220 have acquired New Britain-related ancestry from Vanuatu or Santa Cruz, along with ancestry more
221 closely related to that in New Ireland or the Solomon Islands via a distinct stream of migration.

222

223 As suggested by their similar mixtures of components in ADMIXTURE, the ancient Vanuatu
224 individuals are broadly consistent with descent from the same common ancestral population as
225 present-day groups from Vanuatu. In the admixture graphs, we could fit the ancient sample groups
226 from 2400-200 BP as sister populations to Vanuatu_Tanna, albeit with different proportions of First
227 Remote Oceanian ancestry. The one exception was the 150 BP grouping of individuals from Efate
228 (with ~20% First Remote Oceanian ancestry), which showed significant un-modeled allele sharing
229 with Tongan (max residual $Z = 3.5$, after accounting for excess First Remote Oceanian ancestry).
230 Some present-day Vanuatu populations, such as Efate and Makura, show a similar pattern when
231 added to the model, likely reflecting migration of Polynesians to Vanuatu in the last thousand years
232 or less.

233

234 *Conclusion*

235 By analyzing a time transect of Vanuatu from initial settlement through the present, combined with
236 dense geographical sampling of surrounding present-day populations, we document a series of
237 dramatic genetic shifts associated with consistently high human mobility through a total of at least
238 four distinct streams of migration and admixture. First, the initial human migration to Vanuatu
239 involved First Remote Oceanians associated with the Lapita culture. Second, by 2400 BP, these
240 groups were almost completely displaced in Vanuatu by Papuan-ancestry populations originally
241 from the Bismarck Archipelago, who remain the source for most of the ancestry of people in
242 Vanuatu today. Third, in Polynesia, we find evidence for a different Papuan ancestry type that
243 reflects a distinct migration. And fourth, finally, these streams of ancestry reconnected in parts of
244 the Vanuatu archipelago, influenced by back-migration from Polynesia. These results highlight the
245 importance of multiple episodes of migration and mixture in shaping the human diversity of
246 Oceania.

247

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268

269 **Author Contributions**

270 R.P. and D.R. supervised the study. M.S., F.V., S.B., R.S., H.B., I.P., G.W., and R.P. provided
271 ancient samples and assembled archaeological and anthropological information. N.R., N.B., O.C.,
272 M.F., M.M., J.O., K.Si., and K.St. performed ancient DNA laboratory work. T.K.H. and D.J.K.
273 carried out and analyzed radiocarbon dating data. K.A., A.H., K.M., S.J.O., T.P., K.R., T.N.W., and
274 A.J.M. provided data from present-day populations. M.L., P.S., S.M., and D.R. analyzed genetic
275 data. M.L., P.S., M.S., and D.R. wrote the manuscript.

276

277 **Declaration of Interests:** The authors declare no competing interests.

278 **Table 1. Details of Ancient Vanuatu Samples Analyzed in this Study**

Sample	Code	Date	Population label	Location	Country	Sex	mtDNA	Y	SNPs
I1370	B17.P3	1160-830 calBCE (3083±26 BP, Wk-21026, corrected for Marine Reservoir Effect)	Vanuatu_3000BP	Teouma, Efate	Vanuatu	F	<u>B4a1a1</u>		237405
I1369	B10B.P3	1050-800 calBCE (3045±30 BP, Poz-81126, corrected for Marine Reservoir Effect)	Vanuatu_3000BP	Teouma, Efate	Vanuatu	F	<u>B4a1a</u>		271048
I1368	B30A.P3	1040-790 calBCE (2983±32 BP, Wk-22657, corrected for Marine Reservoir Effect)	Vanuatu_3000BP	Teouma, Efate	Vanuatu	F	<u>B4a1a</u>		185282
I5951	TeoQE	1258-1088 calBCE (2955±20, PSUAMS-2411)	Vanuatu_3000BP	Teouma Quarry Edge	Vanuatu	M	<u>B4a1a1</u>		23107
I4451	TAP1	516-369 calBCE (2348±32 BP, Wk-20390)	Vanuatu_2400BP	Mele-Taplins, Efate	Vanuatu	M	M28a	K2b1	340152
I4425	EF3_2_E	1652-1950 calCE (200±20 BP, UCIAMS-188795)	Vanuatu_150BP	Ifira, Efate	Vanuatu	F	P2		700783
I4450	SEPU1	1520-1645 calCE (305±15 BP, UCIAMS-188793)	Vanuatu_350BP	Pangpang, Efate	Vanuatu	F	P1d2		735460
I4096	BURU5B	429-595 calCE (1430±20 BP, PSUAMS-1841)	Vanuatu_1400BP	Burumba, Epi Island	Vanuatu	M	<u>B4a1a1k</u>	K2b1	888003
I3921	BURU5D	400-700 CE [429-595 calCE (1430±20 BP, PSUAMS-1841) from burial 5skull B; 551-650 CE (1464±30 BP, Wk-25769)]	Vanuatu_1400BP	Burumba, Epi Island	Vanuatu	M	P1d1	K2b1	855305
I5259	Mang1	1307-1430 calCE (559±30 BP, Wk-20030)	Vanuatu_600BP	Mangaliliu	Vanuatu	F	P1f		799098
I4419	BB1	1678-1940 calCE (135±15 BP, UCIAMS-188792)	Vanuatu_150BP	Banana Bay, Efate	Vanuatu	M	<u>B4a1</u>	K2b1	763556
I4424	EF_Pango1	1661-1950 calCE (190±15 BP, UCIAMS-188794)	Vanuatu_150BP	Pango Village, Efate	Vanuatu	M	<i>R</i>	M1b	780469
I4105	WAMB1	1529-1798 calCE (255±20 BP, PSUAMS-1922)	Vanuatu_200BP	Wambi Bay, Epi Island	Vanuatu	M	M28a	<u>O1a2</u>	1012081
I4106	WAMB2	1645-1950 calCE (225±20 BP, PSUAMS-1923)	Vanuatu_200BP	Wambi Bay, Epi Island	Vanuatu	M	<u>B4a1a1a11</u>	<u>O1a2</u>	1020436

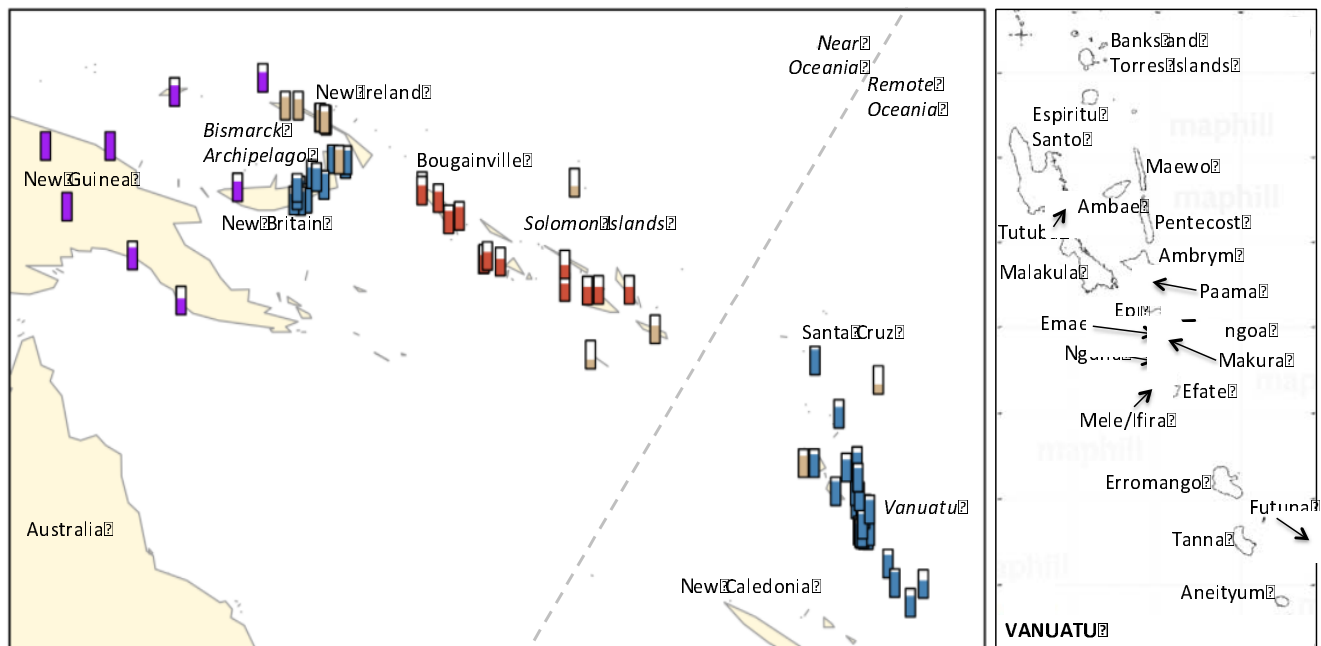
279 Note: Underlining indicates typical East Asian (First Remote Oceanian) haplogroups, while lack of underlining indicates typical
 280 Australo-Papuan haplogroups (the italicized mtDNA haplogroup *R* is unclassified). The first three samples listed are previously
 281 published individuals [1] but with new libraries now added to increase coverage; the other 11 are newly published individuals.

282 **Figure 1. Locations and broad-scale genetic structure of analyzed populations.** (A) Bars
283 represent proportions of Papuan and First Remote Oceanian (white) ancestry. Purple, red, and blue
284 and colors match those in **Figure S1** but here correspond to clusters assigned based on the
285 proximity of populations in the ADMIXTURE results (i.e., overall ratios of Papuan ancestry
286 components) rather than individual ADMIXTURE components: purple, similar to the ratio
287 maximized in New Guinea; blue, similar to New Britain; red, similar to Solomon Islands; brown,
288 mixed between New Britain and Solomon Islands clusters (primarily New Ireland). (B) Map of
289 Vanuatu with islands labeled from which ancient or present-day data are reported in this study. Map
290 data are from freely available sources: (A) was plotted in R using the ‘maps’ package with data
291 from <http://www.naturalearthdata.com/>, and (B) was made with a blank map downloaded from
292 <http://www.maphill.com/vanuatu/simple-maps/blank-map/no-labels/>.

293

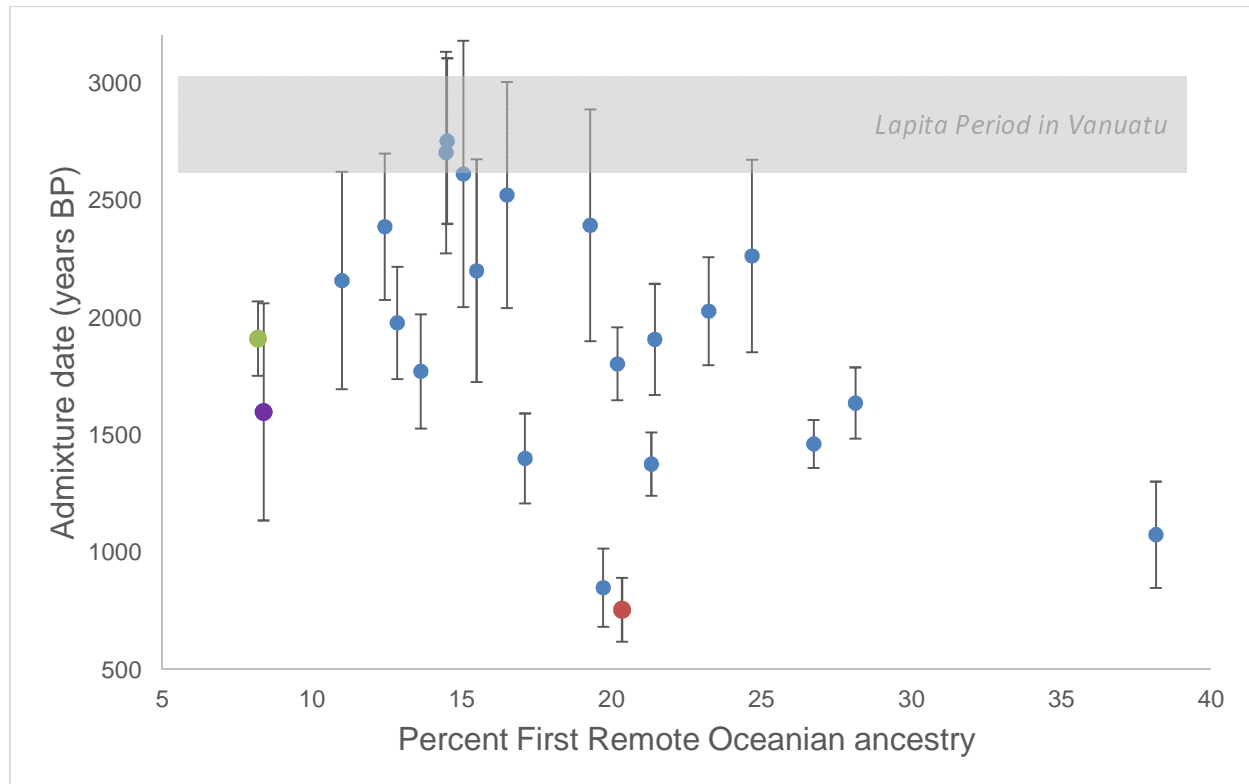
294 **A**

B



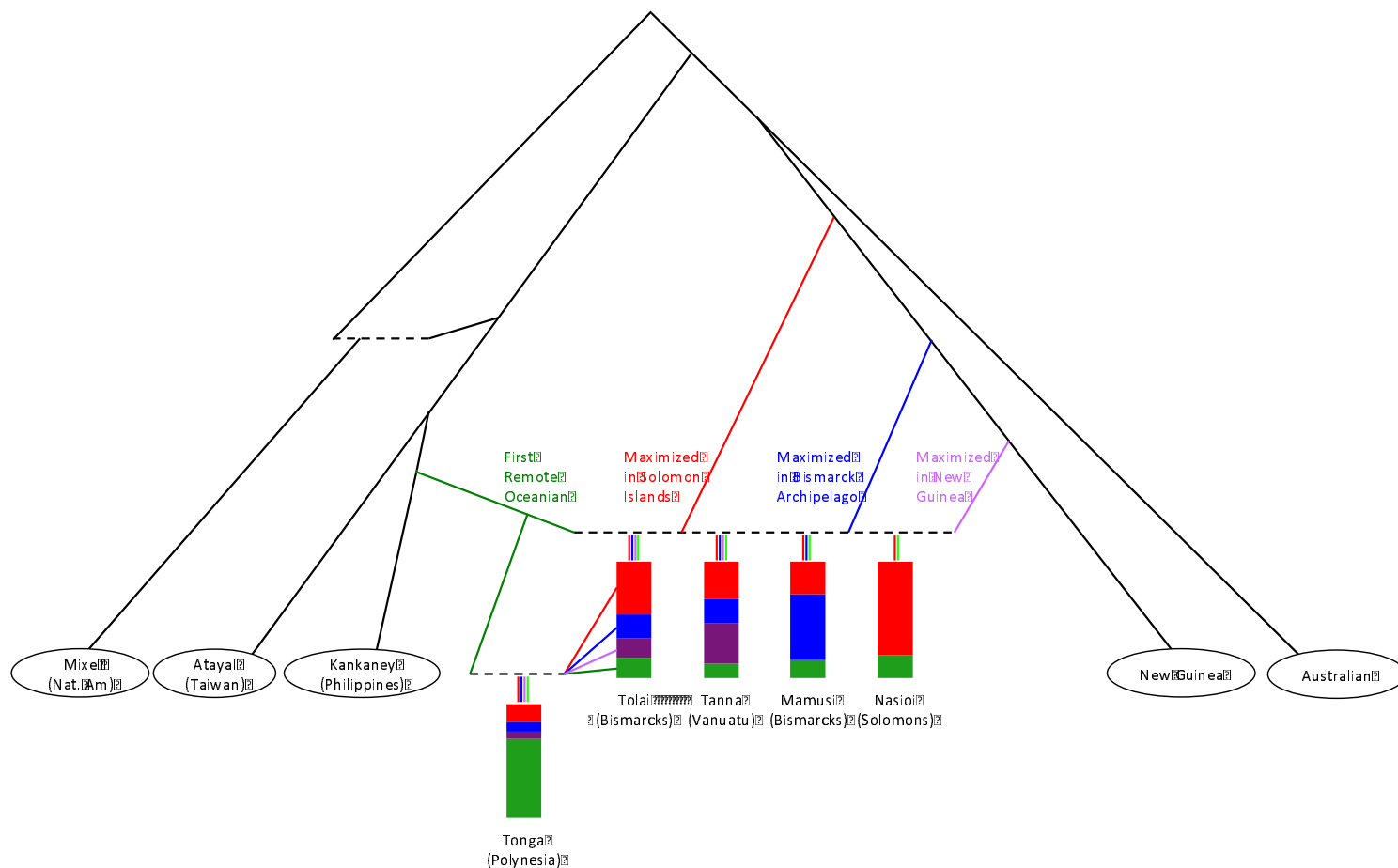
295
296

297 **Figure 2. Ancestry proportions and dates of admixture in Vanuatu.** Blue points represent the 20
298 present-day populations with the most confident admixture date estimates (as measured by Z-score
299 for difference from zero). Colored points represent the ancient population groupings for which we
300 could obtain dates of admixture (adjusted for sample date by assuming 28 years per generation):
301 light green, 1400 BP; purple, 200 BP; red, 150 BP. Bars show one standard error in each direction.
302 See **Table S4** for full results.
303



304

305 **Figure 3. Working admixture graph model with diverse present-day Oceanian populations.** Dotted lines denote admixture events.
 306 For five populations, the proportions of four fitted ancestry sources maximized in First Remote Oceanians (green), Solomon Islands
 307 (red), Bismarck Archipelago (blue) and New Guinea (purple) are shown. Papuan ancestry is inferred to be highly similar in the Tolai and
 308 in Tonga, allowing Tonga to be fit as a mixture of a Tolai-related group and additional ancestry from First Remote Oceanians. We note
 309 that the colors are chosen to be correlated to the components inferred from ADMIXTURE (**Figure S1**), but the ADMIXTURE
 310 components represent combinations of the admixture graph sources given here, and hence the ratios differ between the two methods. Full
 311 model parameters can be found in **Figure S3**.



313 **STAR Methods**

314

315

316 **CONTACT FOR REAGENT AND RESOURCE SHARING**

317

318 Further information and requests for resources and reagents should be direct to and will be fulfilled
319 by the Lead Contact, David Reich (reich@genetics.med.harvard.edu)

320

321

322 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

323

324 **Archaeological Context on Ancient Individuals with New Genome-Wide Data.** We newly
325 report data from 14 ancient skeletons. For 3 of these skeletons we are reporting new ancient DNA
326 data increasing the quality of the dataset beyond what was reported on the samples in a previous
327 study [1]. For the 11 remaining samples the data are entirely new:

328

329 Teouma, Efate Island (~3000 BP) – Lapita Culture (n=4 samples)

330 The Teouma Lapita culture cemetery and settlement site is discussed in detail in the Supplementary
331 Information to Skoglund et al. 2016 and references [1]. The additional sample I5951 was displaced
332 during quarrying activities before controlled archaeological excavations began at the site in 2004.
333 Given its age it was highly likely to have been from a disturbed burial context of Lapita age and can
334 be legitimately considered with the other Lapita-age skeletons from the site.

335

336 • I5951 (TeoQE), Vanuatu_3000BP

337 Newly reported sample

338 Genetic Sex: Male

339 Radiocarbon Date: 1258-1088 calBCE (2955±20, PSUAMS-2411)

340

341 • I1370_all (B17.P3), Vanuatu_3000BP

342 Previously reported in [1]; here we report higher coverage data

343 Genetic Sex: Female

344 Radiocarbon Date: 1160-830 calBCE (3083±26 BP, Wk-21026, corrected for Marine
345 Reservoir Effect [1])

346

347 • I1369_all (B10B.P3), Vanuatu_3000BP

348 Previously reported in [1]; here we report higher coverage data

349 Genetic Sex: Female

350 Radiocarbon Date: 1050-800 calBCE (3045±30 BP, Poz-81126, corrected for Marine
351 Reservoir Effect [1])

352

353 • I1368_all (TB30A.P3), Vanuatu_3000BP

354 Previously reported in [1]; here we report higher coverage data

355 Genetic Sex: Female

356 Radiocarbon Date: 1040-790 calBCE (2983±32 BP, Wk-22657, corrected for Marine
357 Reservoir Effect [1])

358

359 Mele-Taplins, Efate Island (~2400 BP) (n=1 sample)

360 The Mele-Taplins site is described by Valentin and colleagues [20]. The skeleton comes from a
361 subsurface grave in a rockshelter (Taplins 1) at the base of a cliff, excavated by Graeme Ward of
362 The Australian National University in 1973-4 and curated at Otago University, Dunedin, New
363 Zealand. Other burials from the Taplins 2 shelter were of broadly similar age.

364

365 • I4451_all (TAP1), Vanuatu_2400BP

366 Newly reported sample

367 Genetic Sex: Male

368 Radiocarbon Date: 516-369 calBCE (2348±32 BP, Wk-20390)

369

370 Burumba, Epi Island (~1400 BP) (n=2 samples)

371 The Burumba site is described by Valentin and colleagues [20] and excavated in 2006 by
372 Frederique Valentin and Jacques Bole. The graves of nine adults were excavated from an open site
373 at Kalala Plantation 200m from the current beach, dug into sterile sand. Burial 5 was an assemblage
374 of cranial remains of five individuals placed on a pile of coral slabs and blocks.

375

376 • I3921_all (BURU5D), Vanuatu_1400BP

377 Newly reported sample

378 Genetic Sex: Male

379 Radiocarbon Date: 429-663 calCE [429-595 calCE (1530±20 BP, PSUAMS-1841), 619-
380 663 calCE (1395±15, PSUAMS-2428)]

381

382 • I4096_all (BURU5B), Vanuatu_1400BP

383 Newly reported sample

384 Genetic Sex: Male

385 Radiocarbon Date: 545-650 calCE [551-650 calCE (1464±30 BP, Wk-25769), 545-610
386 calCE (1490±15 BP, PSUAMS-2460)]

387

388 Mangaliliu, Efate Island (~600 BP) (n=1 sample)

389 The burial was excavated from a test pit in Mangaliliu village by Richard Shing in 2002 and
390 published in detail by Valentin and colleagues [21]. The originally reported age of the burial was
391 reassessed after direct dating of the skeleton [20].

392

393 • I5259 (burial 1, Mang1), Vanuatu_600BP, Mangaliliu (Efate Island)

394 Newly reported sample

395 Genetic Sex: Female

396 Radiocarbon Date: 1307-1430 calCE (559±30 BP, Wk-20030)

397

398 Pangpang, Efate Island (~350 BP) (n=1 sample)

399 This burial, in a flexed position, was excavated by Richard Shing and Iarawai Philip during
400 archaeological impact assessment related to the Efate Ring Road construction, between the villages
401 of Pangpang and Forari. The body was adorned with ornaments composed of numerous tiny Conus
402 shell and shark vertebrae beads and a large pearl shell pendant. This range of ornaments has been
403 recorded in burial contexts of the last 400 years, prior to and during the initial phases of European
404 contact (unpublished field notes, Vanuatu National Museum).

405

406 • I4450 (SEPU1, Sepulture 1), Vanuatu_350BP

407 Newly reported sample

408 Genetic Sex: Female

409 Radiocarbon Date: 1520-1645 calCE (305±15 BP, UCIAMS-188793)

410

411 Wam Bay, Epi Island (~200 BP) (n=2 samples)

412 The site appears to have been a largely Mission period, late 19th to early 20th century, cemetery of
413 which three burials were exposed and was in proximity to a combustion feature associated with the
414 making of lime-plaster for construction, a European introduced practice. The date of these burials
415 may need to be further calibrated in the light of dietary analysis and could be younger than indicated
416 by current calibration of the bone dates. The site was excavated by Frederique Valentin and
417 Matthew Spriggs in 2006 (unpublished field notes, Vanuatu National Museum).

418

419 • I4105_all (WAMB1), Vanuatu_200BP

420 Newly reported sample

421 Genetic Sex: Male

422 Radiocarbon Date: 1529-1798 calCE (255±20 BP, PSUAMS-1922)

423

424 • I4106_all (WAMB2), Vanuatu_200BP

425 Newly reported sample

426 Genetic Sex: Male

427 Radiocarbon Date: 1645-1950 calCE (225±20 BP, PSUAMS-1923)

428

429 Ifira, Efate Island (Historical Period) (n=1 sample)

430 This tightly flexed burial from a feature containing skeletal remains of two individuals was
431 excavated by Mary Elizabeth and Richard Shutler, Jr, in June 1964 on the small island of Ifira in
432 Vila Harbor, Port Vila, during a test pit survey of the island. It is briefly mentioned in Shutler and
433 Shutler [22]. Unpublished field notes relating to the excavation are held in the files of the Vanuatu
434 National Museum. Ifira is notable as one of the Vanuatu Polynesian Outlier islands and this burial
435 would date to the period of Polynesian cultural influence.

436

437 • I4425 (EF3_2_E, Pit 2; Loc E), Vanuatu_150BP

438 Newly reported sample
439 Genetic Sex: Female
440 Radiocarbon Date: 1652-1950 calCE (200±20 BP, UCIAMS-188795)
441

442 Pango Village, Efate Island (Historical Period) (n=1 sample)

443 This is one of two individuals excavated by Mary Elizabeth and Richard Shutler, Jr, in June 1964 on
444 the Pango Peninsula opposite the small island of Ifira in Vila Harbour, Port Vila. Unpublished field
445 notes relating to the excavation are held in the files of the Vanuatu National Museum, but little
446 detail is available.

447
448 • I4424 (EF_Pango1), Vanuatu_150BP
449 Newly reported sample
450 Genetic Sex: Male
451 Radiocarbon Date: 1661-1950 calCE (190±15 BP, UCIAMS-188794)
452

453 Banana Bay, Efate Island (Historical Period) (n=1 sample)

454 The burial was excavated by Richard Shing and Iarawai Philip during archaeological impact
455 assessment related to the Efate Ring Road construction in the Banana Bay area, southeast Efate. The
456 body, lying on the back, was adorned with ornaments including numerous tiny Conus shell beads
457 and a few European glass beads (unpublished field notes, Vanuatu National Museum).
458

459 • I4419 (BB1, Burial 1), Vanuatu_150BP, Banana Bay (Efate Island)
460 Newly reported sample
461 Genetic Sex: Male
462 Radiocarbon Date: 1678-1940 calCE (135±15 BP, UCIAMS-188792)
463

464 **Data Collection Strategy for Newly Reported Data from Present-Day Vanuatu.** We genotyped
465 185 present-day individuals from 32 populations from Vanuatu spanning 18 islands. All individuals
466 gave informed verbal consent for studies of population history and human health, especially anemia,
467 consistent with the standards prevailing at the time the data were collected. Samples of whole blood
468 were collected as part of a range of research projects undertaken from the late 1970s in
469 collaborations between multiple sites and institutions in Vanuatu and the University of Oxford
470 investigating population differences at the genetic level. In accordance with participant consent,
471 DNA was extracted, anonymized, and stored in batches analyzable only by geographic location of
472 participant origin. Use of the samples for genome-wide analyses including studies of population
473 history was reviewed by the Oxford Tropical Research Ethics Community at the University of
474 Oxford and formally approved in a letter dated July 2 2014 (OXTREC Reference: 537-14). The use
475 of the samples for genetic analysis was also approved by the Vanuatu Cultural Centre in a formal
476 letter dated May 30, 2017.

477
478 **METHOD DETAILS**

479

480 **Ancient DNA laboratory work.** In a dedicated clean room at University College Dublin, we used a
481 dental sandblaster to separate cochlear sections from petrous bones. We milled these samples into
482 fine powder, and shipped them to Harvard Medical School.

483

484 At Harvard Medical School, we extracted DNA following a previously published protocol [4], with
485 two modifications. First, we replaced the combination of a funnel and a MinElute column with
486 Roche columns [5]. Second, we eluted two times in 45µl, obtaining 90µl of extract for each sample.

487

488 We prepared libraries from the extracts using a double-stranded protocol, affixing 7-base-pair
489 sequences to either end to allow multiplexing of the libraries and to prevent contamination from
490 affecting the samples after barcodes were added. We prepared some of the libraries in the presence
491 of the enzyme UDG to remove characteristic damage associated with ancient DNA (**Table S2**) [6].

492

493 We enriched the libraries in solution for sequences overlapping the mitochondrial genome [8] as
494 well as for 3000 nuclear positions, and sequenced on an Illumina NextSeq500 instrument for
495 2x76cycles + 2x7 cycles after adding a pair of unique 7-base-pair indices. For libraries that were
496 promising after screening, we next enriched for sequences overlapping approximately 1.24 million
497 SNPs [9, 23-25]. We added unique 7-base-pair index combinations to each enriched library, and
498 sequenced on a multiplexed pool of samples on a lane of an Illumina NextSeq500 instrument for
499 2x76cycles + 2x7cycles. We iteratively sequenced more sequences from each sample until the
500 number of new SNPs covered per additional sequences generated was less than about 1 in 100.

501

502 For samples for which we wished to obtain more coverage, we prepared additional libraries from
503 existing extract or new extract, up to 8 libraries for some samples. We pooled data from all libraries
504 for further analysis.

505

506 **Bioinformatic processing.** We demultiplexed reads into libraries based on their two indices and
507 two barcodes, allowing no more than one mismatch to the total of four expected 7 base pair
508 sequences. We merged sequences requiring at least 15 base pairs of overlap using *SeqPrep*
509 (github.com/jstjohn/SeqPrep).

510

511 We aligned merged sequences to the mitochondrial RSRS genome [26] (for mitochondrial DNA
512 analyses) and to the hg19 reference (for whole genome analyses). For alignment we used the single-
513 ended aligner “samse” from BWA with default parameters (version 0.6.1) [27]. For samples which
514 are non-UDG treated (and therefore may have higher mismatch rates compared to the reference
515 genome), we used more relaxed alignment parameters, “-n 0.01 -o 2 -l 16500”. This setting disables
516 seeding, allowing for less conservative alignments, helping to align damaged reads.

517

518 **Haplogroup calling strategy on mitochondrial DNA data.** We determined haplogroups using
519 Haplogrep2, which provides a reliability score for assigned haplogroups [28]. We ran Haplogrep2 in
520 three configurations and picked the best rank score to represent the haplogroup for that individual.

521 (a) We restricted sequences to those with characteristic patterns of ancient DNA damage in their
522 terminal nucleotides, which removes contamination. To do this, we used the PMDtools software
523 [29] requiring a minimum score of $\text{pmdscore}=3$. We trimmed the sequences obtained in this way by
524 5 base pairs on either side to remove nucleotides likely to be deaminated prior to running
525 Haplogrep2. (b) As a second approach, we trimmed sequences by 5 base pairs on either side to
526 eliminate characteristic ancient DNA damage and fed these sequences to Haplogrep2 without
527 damage restriction. (c) Finally, we applied no trimming and made a haplogroup call. We manually
528 made two exceptions to the rule of always picking the best ranking call. For S4106.E1.L1, (a) and
529 (c) gave similar ranking scores and we selected B4a1a1a11 from method (a) based on consistency
530 with calls from two other libraries from the same sample. For S4096.E1.L2, we selected B4a1a1k
531 manually from method (a) despite a marginally lower rank score than method (c).

532
533

534 **QUANTIFICATION AND STATISTICAL ANALYSIS**

535

536 **Population genetic analyses**

537

538 All analyses were based on the set of 593,124 autosomal Human Origins SNPs, except for
539 ADMIXTURE, which was performed with all 597,573 Human Origins SNPs. Principal component
540 analysis was carried out using the “lsqproject” and “autoshrink” options in smartpca [30, 31].
541 ADMIXTURE [10] clustering analysis was performed using default parameters, with the cluster
542 components (K) ranging from $K=2$ to $K=8$. f -statistics were computed in ADMIXTOOLS [32],
543 using the qp4diff program for differences between Lapita f_4 -statistics (“allsnps” mode), with
544 standard errors obtained by block jackknife.

545

546 **Admixture graph fitting**

547

548 We constructed admixture graphs using the qpGraph utility in ADMIXTOOLS [32]. Mixe’s
549 position as an outgroup relative to the other populations (in an unrooted sense) means that its
550 eastern and western Eurasian ancestry components can be collapsed into a single lineage with no
551 change in the model. Similarly, we can omit explicit inclusion of Denisovan admixture because of
552 the symmetry of such ancestry in the right-hand clade of the model (as displayed in **Figure S3**).

553

554

555 **DATASET AND SOFTWARE AVAILABILITY**

556

557 Raw sequences from the 14 individuals are available from the European Nucleotide Archive at
558 accession number PRJEB24938. Genotypes are available at <https://reich.hms.harvard.edu/datasets>.
559 To access data for the newly genotyped present-day individuals from Vanuatu, researchers should
560 send a signed letter to D.R. containing the following text: “(a) I will not distribute the data outside
561 my collaboration; (b) I will not post the data publicly; (c) I will make no attempt to connect the

562 genetic data to personal identifiers for the samples; (d) I will use the data only for studies of
 563 population history; (e) I will not use the data for any selection studies; (f) I will not use the data for
 564 medical or disease-related analyses; (g) I will not use the data for commercial purposes.”

565

566 **KEY RESOURCES TABLE**

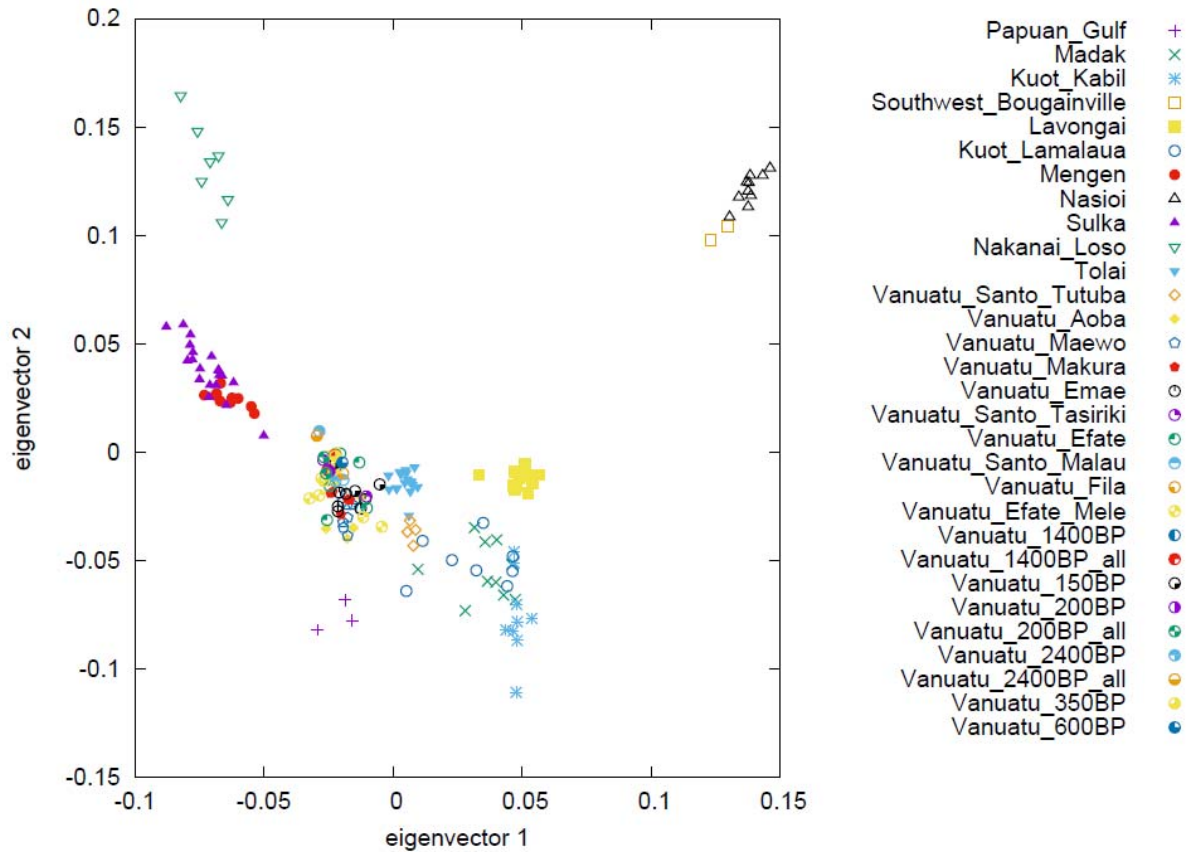
567

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
Pfu Turbo Cx Hotstart DNA Polymerase	Agilent Technologies	600412
Herculase II Fusion DNA Polymerase	Agilent Technologies	600679
2x HI-RPM hybridization buffer	Agilent Technologies	5190-0403
0.5 M EDTA pH 8.0	BioExpress	E177
Sera-Mag™ Magnetic Speed-beads™ Carboxylate-Modified (1µm, 3EDAC/PA5)	GE LifeScience	65152105050250
USER enzyme	New England Biolabs	M5505
UGI	New England Biolabs	M0281
Bst DNA Polymerase2.0, large frag.	New England Biolabs	M0537
PE buffer concentrate	Qiagen	19065
Proteinase K	Sigma Aldrich	P6556
Guanidine hydrochloride	Sigma Aldrich	G3272
3M Sodium Acetate (pH 5.2)	Sigma Aldrich	S7899
Water	Sigma Aldrich	W4502
Tween-20	Sigma Aldrich	P9416
Isopropanol	Sigma Aldrich	650447
Ethanol	Sigma Aldrich	E7023
5M NaCl	Sigma Aldrich	S5150
1M NaOH	Sigma Aldrich	71463
20% SDS	Sigma Aldrich	05030
PEG-8000	Sigma Aldrich	89510
1 M Tris-HCl pH 8.0	Sigma Aldrich	AM9856
dNTP Mix	Thermo Fisher Scientific	R1121
ATP	Thermo Fisher Scientific	R0441
10x Buffer Tango	Thermo Fisher Scientific	BY5
T4 Polynucleotide Kinase	Thermo Fisher Scientific	EK0032
T4 DNA Polymerase	Thermo Fisher Scientific	EP0062
T4 DNA Ligase	Thermo Fisher Scientific	EL0011
Maxima SYBR Green kit	Thermo Fisher Scientific	K0251
50x Denhardt's solution	Thermo Fisher Scientific	750018
SSC Buffer (20x)	Thermo Fisher Scientific	AM9770
GeneAmp 10x PCR Gold Buffer	Thermo Fisher Scientific	4379874
Dynabeads MyOne Streptavidin T1	Thermo Fisher Scientific	65602
Salmon sperm DNA	Thermo Fisher Scientific	15632-011
Human Cot-I DNA	Thermo Fisher Scientific	15279011
Critical Commercial Assays		
High Pure Extender from Viral Nucleic Acid Large Volume Kit	Roche	05114403001
MinElute PCR Purification Kit	Qiagen	28006
NextSeq® 500/550 High Output Kit v2 (150 cycles)	Illumina	FC-404-2002

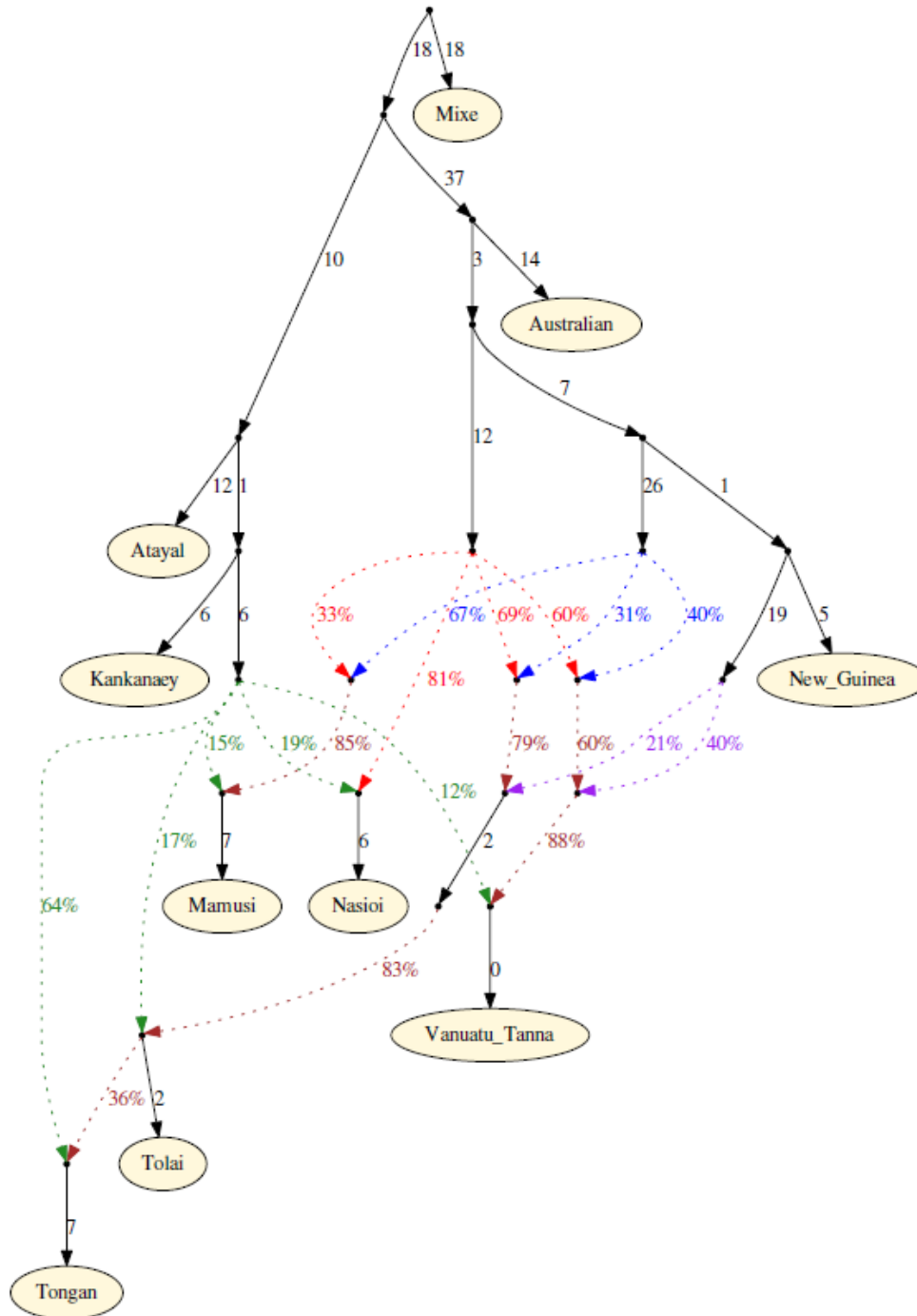
Deposited Data		
Raw and analyzed data	This paper	ENA: PRJEB24938
Software and Algorithms		
Samtools	Li et al., 2009	http://samtools.sourceforge.net/
BWA	Li & Durbin 2008	
ADMIXTOOLS	Patterson et al. 2012	https://github.com/DReichLab/AdmixTools
SeqPrep		https://github.com/jstjohn/SeqPrep
bamrmdup		https://github.com/udo-stenzel/biohazard
smartpca	Patterson et al. 2006	https://www.hsph.harvard.edu/alkes-price/software/
ADMIXTURE	Alexander et al. 2009	https://www.genetics.ucla.edu/software/admixture/download.html
PMDtools	Skoglund et al. 2014	https://github.com/pontusssk/PMDtools
Haplogrep 2	Weissensteiner et al. 2016	http://haplogrep.uibk.ac.at/
Yfitter	Jostins et al. 2016	https://sourceforge.net/projects/yfitter/
ALDER	Loh et al. 2013	http://cb.csail.mit.edu/cb/alder/

568
569

570 **Figure S2. Principal component analysis of Oceanian populations.** We computed axes using
571 present-day populations with 17-25% First Remote Oceanian ancestry and projected ancient
572 samples. For samples with a combination of partial-UDG-treated and non-UDG libraries, the
573 combined data (“_all”) are very similar to the UDG-only data, which enhances our confidence in
574 the results.



577 **Figure S3. Admixture graph model with inferred parameters.** The model shown is the same as
578 in **Figure 3** but with an alternative visualization. Branch lengths are given in units of f_2 genetic drift
579 distance times 1000, and admixture proportions are indicated along corresponding dotted lines. Red,
580 Solomon Islands majority source; blue, Bismarck Archipelago majority source; purple, New
581 Guinea-related source; green, First Remote Oceanian; brown, mixed ancestry. The order of
582 admixture events specified is arbitrary.



583
584
585

586 References

587

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