STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Ancient skeletal element	This study	S1368 / TB30A.P3
Ancient skeletal element	This study	S1369 / B10B.P3
Ancient skeletal element	This study	S1370 / B17.P3
Ancient skeletal element	This study	S3921 / BURU5D
Ancient skeletal element	This study	S4096 / BURU5B
Ancient skeletal element	This study	S4105 / WAMB1
Ancient skeletal element	This study	S4106 / WAMB2
Ancient skeletal element	This study	S4419 / BB1
Ancient skeletal element	This study	S4424 / Pango1
Ancient skeletal element	This study	S4425 / Pit 2, Loc E
Ancient skeletal element	This study	S4450 / Sepulture 1
Ancient skeletal element	This study	S4451 / TAP1
Ancient skeletal element	This study	S5259 / Mang1
Ancient skeletal element	This study	S5951 / TeoQE
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 (1 μm, 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	5030
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

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
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
DyNAmo HS SYBR Green qPCR Kit	Thermo Fisher Scientific	F410L
Methanol, certified ACS	VWR	EM-MX0485-3
Acetone, certified ACS	VWR	BDH1101-4LP
Dichloromethane, certified ACS	VWR	EMD-DX0835-3
Hydrochloric acid, 6N, 0.5N & 0.01N	VWR	EMD-HX0603-3
Critical Commercial Assays		
High Pure Extender from Viral Nucleic Acid Large Volume Kit	Roche	5114403001
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	[21]	http://samtools.sourceforge.net/
BWA	[22]	http://bio-bwa.sourceforge.net/
ADMIXTOOLS	[23]	https://github.com/DReichLab/AdmixTools
SeqPrep	https://github.com/jstjohn/SeqPrep	https://github.com/jstjohn/SeqPrep
bamrmdup	https://bitbucket.org/ustenzel/biohazard	https://bitbucket.org/ustenzel/biohazard
smartpca	[24]	https://www.hsph.harvard.edu/alkes-price/software/
ADMIXTURE	[11]	https://www.genetics.ucla.edu/software/admixture/download.html
PMDtools	[25]	https://github.com/pontussk/PMDtools
Haplogrep 2	[26]	http://haplogrep.uibk.ac.at/
Yfitter	[27]	https://sourceforge.net/projects/yfitter/
ALDER	[16]	http://cb.csail.mit.edu/cb/alder/

CONTACT FOR REAGENT AND RESOURCE SHARING

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

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Archaeological Context on Ancient Individuals with New Genome-Wide Data

We newly report data from 14 ancient skeletons. For 3 of these skeletons we are reporting new ancient DNA data increasing the quality of the dataset beyond the data reported from the same samples in a previous study [1]. For 11 samples the data are entirely new:

Teouma, Efate Island (~2900 BP) – Lapita Culture (n = 4 samples)

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

I5951 (TeoQE), Vanuatu_2900BP
Newly reported sample
Genetic Sex: Male

Radiocarbon Date: 2920–2720 calBP (2955 ± 20 BP, PSUAMS-2411, marine corrected)
I1370 (B17.P3), Vanuatu_2900BP
Previously reported in [1]; here we report higher coverage data
Genetic Sex: Female
Radiocarbon Date: 3080–2780 calBP (3083 ± 26 BP, Wk-21026, marine corrected)
I1369 (B10B.P3), Vanuatu_2900BP
Previously reported in [1]; here we report higher coverage data
Genetic Sex: Female
Radiocarbon Date: 3020–2750 calBP (3045 ± 30 BP, Poz-81126, marine corrected)
I1368 (TB30A.P3), Vanuatu_2900BP
Previously reported in [1]; here we report higher coverage data
Genetic Sex: Female
Radiocarbon Date: 2990–2750 calBP (2983 ± 32 BP, Wk-22657, marine corrected)

Mele-Taplins, Efate Island (~2300 BP) (n = 1 sample)

The Mele-Taplins site is described by Valentin and colleagues [28]. The skeleton comes from a subsurface grave in a rockshelter (Taplins 1) at the base of a large boulder, excavated by Graeme Ward of The Australian National University in 1973-4 and curated at Otago University, Dunedin, New Zealand. Other burials from the Taplins 2 shelter were of broadly similar age.

I4451 (TAP1), Vanuatu_2300BP
Newly reported sample
Genetic Sex: Male
Radiocarbon Date: 2360–2160 calBP (2348 ± 32 BP, Wk-20390, marine corrected)

Burumba, Epi Island (~1300 BP) (n = 2 samples)

The Burumba site is described by Valentin and colleagues [28] and excavated in 2006 by Frederique Valentin and Jacques Bole. The graves of nine adults were excavated from an open site at Kalala Plantation 200 m from the current beach, dug into sterile sand. Burial 5 was an assemblage of cranial remains of five individuals placed on a pile of coral slabs and blocks.

I3921 (BURU5D), Vanuatu_1300BP
Newly reported sample
Genetic Sex: Male
Radiocarbon Date: 1340–1180 calBP [1410–1280 calBP (1530 ± 20 BP, PSUAMS-1841), 1300–1170 calBP (1395 ± 15 BP, PSUAMS-2428), marine corrected]
I4096 (BURU5B), Vanuatu_1300BP
Newly reported sample
Genetic Sex: Male
Radiocarbon Date: 1380–1240 calBP [1380–1270 calBP (1490 ± 15 BP, PSUAMS-2460), 1350–1180 calBP (1464 ± 30 BP, Wk-25769), marine corrected]

Mangaliliu, Efate Island (~500 BP) (n = 1 sample)

The burial was excavated from a test pit in Mangaliliu village by Richard Shing in 2002 and described in detail by Valentin and colleagues [29]. The originally reported age of the burial was reassessed after direct dating of the skeleton [28].

I5259 (burial 1, Mang1), Vanuatu_500BP, Mangaliliu (Efate Island)
Newly reported sample
Genetic Sex: Female
Radiocarbon Date: 630–330 calBP (559 ± 30 BP, Wk-20030, marine corrected)

Pangpang, Efate Island (~150 BP) (n = 1 sample)

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

I4450 (SEPU1, Sepulture 1), Vanuatu_Efate_150BP
Newly reported sample
Genetic Sex: Female
Radiocarbon Date: 430–0 calBP (305 ± 15 BP, UCIAMS-188793, marine corrected)

Wam Bay, Epi Island (~150 BP) (n = 2 samples)

The site appears to have been a largely Mission period cemetery of the late 19th to early 20th centuries. Three burials were exposed in proximity to a combustion feature associated with the making of lime-plaster for construction, a European introduced practice. The site was excavated by Frederique Valentin and Matthew Spriggs in 2006 (M.S. and F.V., unpublished data; field notes at Vanuatu National Museum).

I4105 (WAMB1), Vanuatu_Epi_150BP
Newly reported sample
Genetic Sex: Male
Radiocarbon Date: 300–0 calBP (255 ± 20 BP, PSUAMS-1922, marine corrected)
I4106 (WAMB2), Vanuatu_Epi_150BP
Newly reported sample
Genetic Sex: Male
Radiocarbon Date: 280–0 calBP (225 ± 20 BP, PSUAMS-1923, marine corrected)

Ifira, Efate Island (~150 BP) (n = 1 sample)

This tightly flexed burial from a feature containing skeletal remains of two individuals was excavated by Mary Elizabeth and Richard Shutler, Jr, in June 1964 on the small island of Ifira in Vila Harbor, Port Vila, during a test pit survey of the island. It is briefly mentioned in Shutler and Shutler [30]. Unpublished field notes relating to the excavation are held in the files of the Vanuatu National Museum. Ifira is notable as one of the Vanuatu Polynesian Outlier islands and this burial would date to the period of Polynesian cultural influence.

I4425 (EF3_2_E, Pit 2; Loc E), Vanuatu_Efate_150BP
Newly reported sample
Genetic Sex: Female
Radiocarbon Date: 270–0 calBP (200 ± 20 BP, UCIAMS-188795, marine corrected)

Pango Village, Efate Island (~150 BP) (n = 1 sample)

This is one of two individuals excavated by Mary Elizabeth and Richard Shutler, Jr. on the Pango Peninsula opposite the small island of Ifira in Vila Harbour, Port Vila. Unpublished field notes relating to the burial are held in the files of the Vanuatu National Museum, but the notes provide limited detail.

I4424 (EF_Pango1), Vanuatu_Efate_150BP
Newly reported sample
Genetic Sex: Male
Radiocarbon Date: 280–0 calBP (190 ± 15 BP, UCIAMS-188794, marine corrected)

Banana Bay, Efate Island (~150 BP) (n = 1 sample)

The burial was excavated by Richard Shing and Iarawai Phillip during archaeological impact assessment related to the Efate Ring Road construction in the Banana Bay area, southeast Efate. The body, lying on the back, was adorned with ornaments including numerous tiny Conus shell beads and a few European glass beads (R.S. and I.P., unpublished data; field notes at Vanuatu National Museum).

I4419 (BB1, Burial 1), Vanuatu_Efate_150BP, Banana Bay (Efate Island)
Newly reported sample
Genetic Sex: Male
Radiocarbon Date: 260–20 calBP (135 ± 15 BP, UCIAMS-188792, marine corrected)

Genotyping Data from Present-Day Vanuatu

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

METHOD DETAILS

Ancient DNA laboratory work

In dedicated clean rooms at University College Dublin, we used a dental sandblaster to separate cochlear sections from petrous bones. We milled these samples into fine powder, and shipped them to Harvard Medical School.

In dedicated clean rooms at Harvard Medical School, we extracted DNA following a previously published protocol [5], with two modifications. First, we replaced the combination of a funnel and a MinElute column with Roche columns [6]. Second, we eluted two times in 45 μl, obtaining 90 μL of extract for each sample (Data S1).

We prepared libraries from the extracts using a double-stranded protocol, affixing 7-base-pair sequences to either end to allow multiplexing of the libraries and to prevent contamination from affecting the samples after barcodes were added. We prepared some of the libraries in the presence of the enzyme UDG to remove characteristic damage associated with ancient DNA (Data S1) [7].

We enriched the libraries in solution for sequences overlapping the mitochondrial genome [9] as well as for 3000 nuclear positions, and sequenced on an Illumina NextSeq500 instrument for 2x76cycles + 2x7 cycles after adding a pair of unique 7-base-pair indices. For libraries that were promising after screening, we enriched for sequences overlapping approximately 1.24 million SNPs on the nuclear genome [10, 31–33]. We added two unique 7-base-pair indices to each enriched library and sequenced a multiplexed pool of samples with an Illumina NextSeq500 instrument for 2x76cycles + 2x7cycles. We iteratively sequenced more from each sample until the number of new SNPs covered per additional sequences generated was less than about 1 in 100.

For samples for which we wished to obtain more coverage, we prepared additional libraries from existing extract or new extract, leading to a total of up to 8 libraries for some samples. We pooled data from all libraries for further analysis. We also prepared versions of the sample data using only UDG-treated libraries. We use the suffix "_all" to refer to the versions of each sample with all libraries in Data S1 and Figure S2. We use the "_all" versions for our primary analyses, but also perform some analyses on the entirely UDG-treated versions to assess if there is evidence that any results are influenced by ancient DNA artifacts (all appear to be robust).

Bioinformatic processing

We demultiplexed reads from the NextSeq500 lanes into individual libraries based on the sequences of their two indices and two barcodes. To assign a read pair to a library we required no more than one mismatch to the total of four expected 7 base pair sequences. We merged sequences using *SeqPrep* (https://github.com/jstjohn/SeqPrep), requiring at least 15 base pairs of overlap. At positions of overlap, we used the allele and quality score of the read of higher quality.

We aligned merged sequences to the mitochondrial RSRS genome [34] (for mitochondrial DNA analyses) and to the hg19 reference (for whole genome analyses) using the command "samse" from BWA with default parameters (version 0.6.1) [22]. For non-UDG treated libraries, which are expected to have higher mismatch rates compared to the reference genome, we used more relaxed alignment parameters, "-n 0.01 -o 2 -l 16500." This setting disables seeding, allowing for less conservative alignments and helping to align damaged sequences. We chose one allele at random per site ("pseudo-haploid" genotypes) from aligned sequences to use in analyses.

Mitochondrial DNA haplogroup determination

We determined mitochondrial DNA haplogroups for each library separately as well as for pools of libraries for each sample using Haplogrep2, which provides a ranking score measuring the reliability of the haplogroup assignments [26] (Data S1). The procedure used here is designed to extract the maximally informative data from the sample during haplogroup assignment by building a mitochondrial consensus sequence in multiple ways and then using the rank score to select the most confident call. First, we restricted to sequences with characteristic ancient DNA damage in their terminal nucleotides (a procedure that removes potentially contaminating sequences at the cost of greatly reduced coverage). To restrict to damaged sequences, we used the PMDtools software [25], requiring a minimum score of pmdscore = 3, and then trimmed the sequences by 5 base pairs on either side to remove nucleotides likely to be deaminated before calling a haplogroup with Haplogrep2. As a second approach, we trimmed sequences by 0-7 base pairs on either side to eliminate characteristic ancient DNA damage and fed these sequences to Haplogrep2 without damage restriction (hence retaining more data), calling a haplogroup at each trim level.

Direct Accelerator Mass Spectrometry (AMS) Radiocarbon Dates

We prepared 10 new bone samples for AMS radiocarbon dating at the Human Paleoecology and Isotope Geochemistry Laboratory at the Pennsylvania State University. After preparation, we AMS dated the samples either at the W.M. Keck Carbon Cycle Accelerator Mass Spectrometry Laboratory at the University of California, Irvine (lab code: UCIAMS) or at the Accelerator Mass Spectrometer Laboratory at the Pennsylvania State University (lab code: PSUAMS). We co-analyzed our 10 newly generated dates with 6 previously published dates generated by other laboratories: five by the Radiocarbon Dating Laboratory at the University of Waikato (lab code: Wk) and one by the Poznan Radiocarbon Laboratory (lab code: Poz) [1, 28, 35].

Bone samples for the newly and previously reported direct AMS ^{14}C dates were manually cleaned and demineralized in weak HCl and soaked in an alkali bath (NaOH) at room temperature to remove contaminating soil humates. Samples were then rinsed to neutrality in Nanopure H_2O and gelatinized in HCL [36]. The resulting gelatin was lyophilized and weighed to determine percent yield as a measure of collagen preservation (% crude gelatin yield). Collagen was either directly AMS ^{14}C dated (Wk-20390, Wk-20030) or further purified using ultrafiltration prior to analysis. At PSUAMS we hydrolyzed two bone samples with low collagen yields and purified the resulting amino acids using XAD chromotography [37].

We use stable carbon and nitrogen isotopic analysis of bone collagen (or amino acids) as an additional quality control measure. For all samples, we examined the %C, %N and C:N ratios. C:N ratios for well-preserved samples fall between 2.9 and 3.6 [38], and all our samples met this criterion. We also used stable carbon and nitrogen isotope measurements to determine the marine

reservoir correction for each individual as described below. The detailed bone preparation and quality control methods we used for the newly reported dates from PSUAMS and UCIAMS are reported elsewhere [37, 39].

To calibrate the dates, we began with an adjustment for the marine reservoir effect, applying a correction (ΔR) of 40 ± 44 BP based on marine shell measurements to adjust for local oceanic variation in ^{14}C levels around Vanuatu as previously described by Petchey and colleagues [35]. We then corrected for mass-dependent fractionation with measured ^{13}C values [40] and calibrated in OxCal 4.3 [41] via a mixture of the IntCal13 Southern Hemisphere and Marine13 calibration curves [42] using the Mix_Curves function in OxCal according to the marine component of each individual's diet (estimated by $\delta^{13}C$ values). This method uses $\delta^{13}C$ endpoints of -21 and $-12‰$, the former representing a highly terrestrial diet and the latter indicating a diet rich in marine foods, with the percentage of marine dietary contribution estimated via linear interpolation [35]. In two cases, individuals were sampled twice for radiocarbon dating and stable isotope analysis: I3921 (PSUAMS-1841, PSUAMS-2428) and I4096 (PSUAMS-2460, Wk-25769). In these instances, we combined radiocarbon dates using the R Combine function in OxCal 4.3 and estimated the marine contribution according to the mean of two $\delta^{13}C$ measurements. For calBP dates (calibrated years before present), we use the standard definition of 1950 CE as 0 calBP.

QUANTIFICATION AND STATISTICAL ANALYSIS

Analysis dataset

All analyses are based on 593,124 SNPs on chromosomes 1-22 genotyped on the Affymetrix Human Origins array [23], with the newly reported data from ancient and present-day Vanuatu individuals merged with published Human Origins data [1]. Based on the ADMIXTURE results, we excluded 26 present-day individuals from analyses as outliers: 14 from Vanuatu (2 Efate, 3 Emae, 2 Malekula, 1 Ambrym, 1 Nguna, 1 Erromango, 3 Aneityum, and 1 Banks) with evidence of European admixture, 4 from Samoa also with evidence of European admixture, and 7 Tolai and 1 Tutuba with evidence of recent admixture or otherwise non-representative ancestry profiles.

Clustering analyses

We performed ADMIXTURE [11] clustering analysis using default parameters, with the cluster components (K) ranging from K = 2 to K = 8. We carried out principal component analysis (PCA) using the "lsqproject" and "autoshrink" options in smartpca [24, 43], computing axes using the present-day populations and projecting ancient samples. For PCA, we restricted to populations within a narrow range of Papuan ancestry proportions in order to minimize the variance due to Papuan versus First Remote Oceanian ancestry and capture components related to variation in Papuan ancestry sources. The specific range (~80% Papuan ancestry) was chosen as the highest possible that included groups from all of the major Oceanian island chains and genetic clusters. We did not project the Vanuatu_2900BP individuals because of their near-zero Papuan ancestry.

For the Papuan ancestry clusters defined in Figure 1B, we manually assigned populations based on their majority Papuan component in ADMIXTURE (out of red, blue, and black). For borderline populations with large red and blue components and small black components, we created a mixed cluster containing Kuot_Kabil, Kuot_Lamalaua, Lavongai, Madak, Nailik, Notsi, and Tigak (New Ireland); Ontong Java, Rennell and Bellona, and Tikopia (Polynesian Outliers); Makira (Solomon Islands); Tolai (New Britain); and Tutuba (Vanuatu). We assigned two borderline populations with large black components to the black cluster (Kove and Mussau).

Allele-sharing statistics

We computed allele-sharing statistics (*f*-statistics) in ADMIXTOOLS [23], with standard errors obtained by block jackknife. To replicate the findings from ADMIXTURE and PCA and test for differential allele sharing of populations from Vanuatu with groups from the Solomon Islands versus the Bismarck Archipelago, we computed the statistic f_4(Australian, *Vanuatu*; *Solomon Islands*, *Bismarck Archipelago*). We used the same populations as in the PCA (10 from Vanuatu, 8 from the Bismarck Archipelago, and 2 from the Solomon Islands; Figure S2) to avoid confounding from differential proportions of First Remote Oceanian ancestry.

We computed Papuan ancestry proportions using the f_4-ratio statistic f_4(Atayal, Australian; Kankanaey, *Test*)/f_4(Atayal, Australian; Kankanaey, New_Guinea_Highlander). The form of this statistic assumes a topology of (Atayal, (Kankanaey, First Remote Oceanian)) for the First Remote Oceanian ancestry in the *Test* population.

We measured differential affinity to Lapita samples from Vanuatu and Tonga by computing the difference between the statistics f_4(*Test*, Han; Atayal, Tonga_2600BP) and f_4(*Test*, Han; Atayal, Vanuatu_2900BP), using the qp4diff program ("allsnps" mode). In expectation, this difference is equal to the single statistic f_4(*Test*, Han; Vanuatu_2900BP, Tonga_2600BP), but our formulation allows us to increase power by utilizing the union rather than the intersection of the SNPs covered by the relatively low-coverage Vanuatu_2900BP and Tonga_2600BP samples.

Dates of admixture

We estimated dates of admixture using ALDER [14]. As reference populations we used published Human Origins data for Ami (aboriginal Taiwanese; n = 10 individuals) and New Guinea Highlanders (n = 19). In computing the correlation between ancestry proportions and dates of admixture, we performed a weighted linear regression of present-day Vanuatu population groups with ALDER date estimates (treated independently; either all 31 such groups or only the 20 with most confident estimates, as shown in Figure 2), weighting by the Z-score for the difference of the date estimate from zero [44].

Admixture graph fitting

We constructed admixture graphs using the qpGraph utility in ADMIXTOOLS [43]. The position of Mixe (a Native American population from present-day Mexico) as an outgroup relative to the other populations (in an unrooted sense) means that its eastern and western Eurasian ancestry components can be collapsed into a single lineage with no change in the model. Similarly, we can omit explicit inclusion of Denisovan admixture because of the symmetry of such ancestry in the right-hand clade of the model (as displayed in Figure S3).

DATA AND SOFTWARE AVAILABILITY

Raw sequences from the 14 individuals are available from the European Nucleotide Archive at accession number PRJEB24938. Genotype files are available at https://reich.hms.harvard.edu/datasets. To access data for the newly genotyped present-day individuals from Vanuatu, researchers should send a signed letter to D.R. containing the following text: "(a) I will not distribute the data outside my collaboration; (b) I will not post the data publicly; (c) I will make no attempt to connect the genetic data to personal identifiers for the samples; (d) I will use the data only for studies of population history; (e) I will not use the data for any selection studies; (f) I will not use the data for medical or disease-related analyses; (g) I will not use the data for commercial purposes."