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Three phases of ancient migration shaped the ancestry of human populations in Vanuatu

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Summary

The archipelago of Vanuatu has been at the crossroads of human population movements in the Pacific for the past three millennia. To help address several open questions regarding the history of these movements, we generated genome-wide data for 11 ancient individuals from the island of

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Author Contributions

M.S., R.P., and D.R. supervised the study. M.S., F.V., S.B., R.S., W.Z., H.B., and R.P. provided samples and assembled archaeological and anthropological materials and information. F.P. performed radiocarbon dating analysis. R.M. served as a liaison for the project with other stakeholders. M.L. and D.R. analyzed genetic data. O.C. and N.R. performed and supervised ancient DNA laboratory work. M.L., M.S., and D.R. wrote the manuscript with input from all coauthors.

Declaration of Interests

The authors declare no competing interests.

Supplemental Data (Excel)

Data S1: Supplementary data files, related to Table 1 and Figures 3–4.

Efate dating from its earliest settlement to the recent past, including five associated with the Chief Roi Mata's Domain World Heritage Area, and analyzed them in conjunction with 34 published ancient individuals from Vanuatu and elsewhere in Oceania, as well as present-day populations. Our results outline three distinct periods of population transformations. First, the four earliest individuals, from the Lapita-period site of Teouma, are concordant with eight previously described Lapita-associated individuals from Vanuatu and Tonga in having almost all of their ancestry from a 'First Remote Oceanian' source related to East and Southeast Asians. Second, both the Papuan ancestry predominating in Vanuatu for the past 2500 years and the smaller component of Papuan ancestry found in Polynesians can be modeled as deriving from a single source most likely originating in New Britain, suggesting that the movement of people carrying this ancestry to Remote Oceania closely followed that of the First Remote Oceanians in time and space. Third, the Chief Roi Mata's Domain individuals descend from a mixture of Vanuatu- and Polynesian-derived ancestry and are related to Polynesian-influenced communities today in central, but not southern, Vanuatu, demonstrating Polynesian genetic input in multiple groups with independent histories.

eTOC Blurb:

Lipson et al. report new genetic data and analyses shedding light on three human migrations to Vanuatu. The first involved people with primarily East Asian-related ancestry; the second, shortly afterward, and likely following a similar route from New Britain, primarily Papuan ancestry; and the third, more recently, Polynesian ancestry.

Keywords

Oceania; Vanuatu; Polynesia; Lapita; Roi Mata; Ancient DNA

Introduction

A key distinction within Pacific studies has been between Near Oceania, the part of the Western Pacific (comprising New Guinea; the Bismarck Archipelago, including New Britain and New Ireland; and the main Solomon Islands) settled for approximately 50,000 years by modern humans, and Remote Oceania [1]. Remote Oceania encompasses the whole of Micronesia and Polynesia and the geographically-designated Melanesian island groups of Vanuatu, New Caledonia, and Fiji (as well as the scattered islands of the Reefs and Santa Cruz groups in the southeast Solomons), which were only settled starting around 3000 years before present (BP) [1].

Vanuatu is a key archipelago in the history of Pacific settlement given its status both as the first major island group in southern Remote Oceania to be occupied by humans and as an important regional crossroads during the succeeding three millennia [2, 3]. Our understanding of the genetic history of Vanuatu has been advanced by three studies reporting genome-wide ancient DNA data from individuals who lived in the archipelago over the course of its human settlement [4–6]. The earliest sampled individuals, who belong to the first human migration to Vanuatu (labelled by some commentators as Migration 1 or M1 [7, 8]), are associated with early phases of the Lapita cultural complex and likely with the initial spread of Austronesian languages into Oceania (where Austronesian is now by far the most

widespread language family) [9, 10]. They had almost entirely East Asian-related ancestry, from a source that originated in Taiwan and has been termed ‘First Remote Oceanian’ (FRO) [4]. Later individuals (including present-day people, who identify as ‘Ni-Vanuatu’), by contrast, have largely Papuan ancestry likely originating in New Britain, which reached the Reefs-Santa Cruz [11] and Vanuatu [5, 6] either during latest Lapita or Post-Lapita times after 2800 BP. (We use the term ‘Papuan’ to refer to the deep ancestral lineage that contributes the majority of the ancestry found in present-day populations from Near Oceania.) Previous papers differed in their interpretation of this second migration (M2) as being either a time-constrained event [6] or a slower process of continuing genetic exchange through time [5].

Previous studies [5, 6] also noted but did not address in detail signals of a third distinct migration stream (M3) occurring within the last millennium and associated with the establishment of ‘Polynesian Outlier’ communities in Vanuatu (as in other areas of Melanesia and Micronesia); that is, islands where Polynesian sub-group languages are spoken and where elements of Polynesian material and non-material culture are practiced [12, 13]. Polynesian impacts in Vanuatu also extend to a number of islands neighboring the Outlier communities showing Polynesian influence but without full language replacement. Little is known, however, about the degree of population movement accompanying these Polynesian-derived cultural and linguistic changes (Zinger et al. unpublished data) [14].

One such Polynesian-influenced island is Efate in central Vanuatu, where two Polynesian language-speaking communities exist today, one on the small off-shore island of Ifira and one at Mele on the southwest of the island. Also located on Efate and the adjacent small islands of Eretok and Lelepa is ‘Chief Roi Mata’s Domain,’ which was inscribed on the UNESCO World Heritage Area list in 2008 on the basis of strong links between oral traditions and a spectacular mortuary site excavated in the 1960s [15]. Some versions of the local oral traditions and aspects of the associated material culture have suggested strong Polynesian influence, illustrated by stories about Chief Roi Mata and his political role on Efate and adjacent islands of the Shepherd Group [15, 16]. The burial site at Eretok was thought initially to date to the 13th century CE [15], but subsequent radiocarbon dates from Eretok and from Mangaas (Mangaasi), the village site on Efate said to have been the home of Chief Roi Mata and his closest followers [15], now place the burials at c. 1600 CE [17].

To gain a genetic perspective on the history of Chief Roi Mata’s Domain, and more generally on the history of Polynesian influence in Vanuatu, we sampled three individuals from the Eretok (also known as Retoka or ‘Hat Island’) Island complex where Roi Mata was buried (according to tradition), along with two individuals from sub-floor burials at Mangaas, for ancient DNA analysis. We also report new genome-wide ancient DNA data from six additional individuals from Efate, complementing published data [4, 6]: four from the Teouma Lapita cemetery (~3000–2750 BP, thus doubling the sample size available from that site), one from the Taplins 1 rockshelter, and one from Banana Bay. We combined these 11 individuals with 26 ancient Vanuatu individuals from the literature (who have previously not been analyzed together) [4–6], eight other published ancient Oceanian individuals, and diverse present-day populations, to shed light on the following primary questions pertaining to the population movements referred to above as M1, M2, and M3:

M1. Does the increased sample of Lapita-period burials from Teouma, combined with other sites, reveal a more diverse founding population than was previously documented?

M2. Can we better elucidate the source, timing, and duration of Papuan migration into Vanuatu?

M3. Do the newly reported individuals from Eretok and Mangaas within the Chief Roi Mata's Domain World Heritage Area show particular relatedness to Polynesians as some oral traditions and features of the archaeological record would suggest?

RESULTS

Sample and data preparation

We generated genome-wide ancient DNA data for 11 new individuals (Figure 1; Table 1; STAR Methods; Data S1A) and increased sequencing coverage for one previously reported individual from Teouma [6] (I5951/TeoQE, previously 23,107 sites covered, now 120,830). In dedicated clean rooms, we extracted DNA from either petrous bone samples (Teouma, Mangaas, and two Eretok individuals) or teeth (Taplins, one Eretok, and Banana Bay) and prepared next-generation sequencing libraries, enriching for a set of ~1.2 million single nucleotide polymorphisms (SNPs). Based on a combination of criteria, all yielded authentic ancient DNA (STAR Methods). We created genotype data for analysis by assigning the observed base from one randomly chosen sequencing read covering each targeted SNP. For most analyses, we merged the new data with published data from both ancient and present-day Oceanians [4–6] (Data S1B). We also obtained three new radiocarbon dates to help establish chronology in relation to previously dated samples [18]; notably, the dates from Eretok and Mangaas confirm that the individuals lived within the past several centuries (Table 1; Data S1C).

PCA

We began by performing a principal component analysis (PCA) in which we computed axes using Kankanaey (Philippines), Nasioi (Solomon Islands), and New Guinea Highlanders and projected all other individuals (STAR Methods; Figure 2). Visually, PC1 corresponds to relative proportions of FRO ancestry (lower on the left, higher on the right), while PC2 corresponds to affinity to populations from the Solomon Islands versus New Guinea (up and down, respectively). Present-day groups from New Britain and Vanuatu form a cluster with relatively uniform values along PC2 but a moderate amount of spread along PC1, with Polynesians and Polynesian Outlier populations farther to the right. Ancient individuals mostly overlap present-day groups from the same island chains, but the Lapita-associated individuals from Teouma (Vanuatu) and Talasiu (Tonga), the ancient individuals from Malakula, and some individuals from Eretok and Mangaas fall farther to the right.

The direction of greatest variation within Vanuatu in Figure 2 is approximately left to right (likely reflecting differential FRO/Papuan mixture proportions), which is well aligned with the primary direction of variation linking New Britain, Vanuatu, Polynesia, and the ancient Lapita-associated individuals. This pattern suggests the possibility that many or all of the populations along this extended cline can be modeled in a simple way as having a shared

pair of ancestry components in different proportions: one represented by Papuan ancestry related to that found in some parts of New Britain and Vanuatu at close to 100%, and one represented by FRO ancestry related to that found in the Lapita-associated individuals at close to 100% [4–6].

Explicit admixture modeling

Guided by the PCA results, we tested candidate admixture models using the *qpAdm* software [19, 20]. Previous results [4–6, 11], as well as Figure 2, indicate a high degree of regional population structure in Near Oceania, with largely distinct clusters of Papuan ancestry found in New Guinea, the Solomon Islands (excluding Santa Cruz and Polynesian Outliers), New Britain, and New Ireland, although many populations (e.g., from New Ireland) can be modeled as having mixtures of multiple Papuan ancestry components. In the following analyses, we often use Nasioi (non-Austronesian-speakers from the island of Bougainville) and Baining (non-Austronesian-speakers from New Britain) to represent the Solomon Islands and New Britain clusters, respectively, because they are the populations with both the lowest proportions of FRO ancestry (~20% and ~5%) and the highest proportions of the distinctive local Papuan ancestry from their clusters in our data set [4–6, 11].

For almost all of the ancient Vanuatu individuals, we obtain successful *qpAdm* models (i.e., high *p*-values for model fit) using Baining (Marabu subgroup) and Kankanaey (Austronesian speakers from the Philippines related to the ancestors of FRO) as the two proxy sources, even with Nasioi as an outgroup (STAR Methods; Data S1D). Conversely, if we use Nasioi as a proxy source in place of Baining, almost none of the models are successful. We note that poor fits can result from any un-modeled shared ancestry between the outgroups and either the test population or the proxy sources, for example from small amounts of contamination (for ancient individuals) or if the FRO-related ancestry in Nasioi (as an outgroup) is a better source than the FRO-related ancestry in Kankanaey. For Polynesians and Polynesian Outliers, our power to distinguish between different lineages is limited by their lower proportions of Papuan ancestry, but we observe similar results, with better fits when using Baining rather than Nasioi as a proxy source. As previously reported [5], the fits improve with Malaita (a Solomon Islands population with some New Britain-related ancestry; see Figure 2 and ref. [6]) in place of Nasioi, but they are worse than with Baining and are rejected at $p < 0.05$ for most populations.

The quantitative mixture proportion estimates from *qpAdm* (Figure 3) are also in good agreement with PCA. The lowest proportions of FRO ancestry we observe are 0–3.6% and 0.6–6.6% (truncated 95% CIs) for post-Lapita individuals from Efate and Tanna, respectively, and the highest proportions are 96.4–99.2%, 96.4–100%, and 87.4–100% for Lapita-associated individuals from Teouma, Talasiu (Tonga), and Malakula, respectively. The individuals from Chief Roi Mata's Domain are relatively variable, ranging from a low of 17.3–22.0% total FRO ancestry for I10966 (Mangaas) to a high of 38.3–44.2% FRO ancestry for I10969 (Eretok). We also compared ancestry proportion estimates on the autosomes and X chromosomes to test for possible sex-biased admixture. We observed isolated signals of sex bias, replicating previously reported instances for present-day

Polynesians and ancient Malakula (Data S1D) [4, 5]; additional examples may exist, but our statistical power is limited by sequencing coverage and available sample sizes.

Dates of admixture

Previous work [4–6] has shown that the majority of present-day populations in Vanuatu have average admixture dates centered around ~2000 BP, in line with other Oceanians, although some groups, especially those with potential Polynesian-related ancestry, yield more recent dates (e.g., Futuna, ~1075 ± 225 BP [6]). We estimated dates of admixture for the Eretok and Mangaas individuals using both MALDER [21] and DATES [22] and inferred average dates of roughly 20–30 generations, or 550–850 years, before the individuals lived (i.e., ~1400–700 BP; Table 2). This range extends somewhat earlier than the likely arrival of westward-moving Polynesian groups in Vanuatu, which, based on archaeological evidence, occurred around 1000–750 BP [13, 23]. However, under a scenario of Polynesian influx, the expected average admixture dates would reflect a combination of recent and older events, given that both Polynesians and local groups would have been admixed already. We did not detect significant evidence of multiple waves of admixture from MALDER, but because both proximal sources would have had mixtures of the same (Papuan and FRO) types of ancestry, it is difficult to disentangle the different episodes [21]. Still, the relatively recent dates for Eretok and Mangaas, together with the observed heterogeneity in mixture proportions [24], provide evidence of more recent admixture processes.

Sources of Papuan and FRO ancestry

We explored the cline of Papuan and FRO ancestry in Remote Oceania in more detail through allele-sharing symmetry tests. To allow us to compare different populations along the cline, we plotted f_4-statistics of interest as a function of a separate statistic (f_4(X, New Guinea Highlanders; Kankanaey, Australian)) proportional to FRO ancestry (Figure S1). If all test populations X can be modeled as having mixtures of ancestry related to the same two source populations (in different proportions), then such plots are expected to show a straight line (STAR Methods).

First, we computed the statistic f_4(X, Dai; Nasioi, New Guinea Highlanders), which tests for relative allele-sharing between the test population X and groups from the Solomon Islands and New Guinea (Figure 4a; Data S1E). Two test populations would be expected to yield different values of this statistic (after correcting for proportions of FRO ancestry) if they have different sources for their Papuan ancestry (for example, one from New Britain and the other from New Guinea, New Ireland, or the Solomon Islands). With a few exceptions (Erromango, Z = −3.2; Teouma, Z = −2.5; I10969, Z = 2.3; Tutuba, Z = 4.0; all others within |Z| = 2 of the regression line), present-day and ancient Remote Oceanians give highly uniform results (purple and green points and regression line in Figure 4a), consistent with a common source for their Papuan ancestry. Tutuba, as a copra plantation island, plausibly experienced recent admixture between Ni-Vanuatu and introduced plantation laborers from other parts of Melanesia. Why Erromango is an exception is unclear; it was a much-visited island in the 19th century by groups purchasing and cutting sandalwood and, as a result of such contacts, suffered population collapse through introduced diseases [25]. Among Near Oceanians, as expected, groups from New Guinea are generally below the Remote Oceanian

line, and groups from the Solomon Islands are above. A subset of populations from New Britain, however, closely track the Remote Oceanians, suggesting that they represent good proxies for the source of Papuan ancestry that contributed (predominantly) to Vanuatu and Polynesia. We confirmed this result using *qpWave* [26], where we obtain reasonably good two-component fits (rank 1 $p = 0.18$ without Nasioi as an outgroup, $p = 0.02$ with Nasioi added; STAR Methods) for 10 ancient Vanuatu population groups together with present-day Tongan plus Melamela (Austronesian speakers from New Britain with $|Z| < 2$ deviation from the regression line in Figure 4a). Present-day Vanuatu populations require four ancestry sources (rank 3 $p = 0.17$ without Nasioi as an outgroup, $p = 0.02$ with Nasioi added), plausibly due to small proportions of distinct Papuan (as in Erromango and Tutuba) or other (e.g., East Asian or European) ancestry resulting from recent contacts.

Next, we performed similar tests for possible different sources of FRO ancestry. We first computed f_4(X, New Guinea Highlanders; Teouma, Kankanaey) to test relatedness of FRO ancestry across Oceania to the Teouma individuals versus present-day Kankanaey. All populations yield positive values highly correlated with levels of FRO ancestry (Figure S2a; Data S1F), indicating that the ancestry is more closely related to the Teouma individuals [4]. We then computed f_4(X, New Guinea Highlanders; Teouma, Talasiu) to test whether the FRO ancestry is more closely related to the Lapita-associated individuals from Vanuatu or from Tonga. Although our statistical power is limited by the close relationship between the two Lapita-associated groups, we obtain significantly non-zero values for populations having relatively high FRO ancestry, with the negative slope implying (slightly) greater affinity to Talasiu than to Teouma (Figure S2b; Data S1G). However, we observe only minor deviations from the regression line (max $|Z| = 2.5$). Thus, the FRO ancestry found in sampled ancient and present-day Oceanian populations appears to be relatively uniform in its relationships to the Lapita-associated individuals from Vanuatu and Tonga, and slightly closer to the latter.

Polynesian genetic legacy

Using similar methods, we tested for the presence of specifically Polynesian-related ancestry via the statistic f_4(X, Tolai; Kankanaey, Tongan) (STAR Methods; Figure 4b; Data S1H; Figure S3). As expected, other Polynesians show very strong allele-sharing with Tonga ($|Z| > 9$ for Samoa, Tahiti, and the Polynesian Outliers of Ontong Java, Rennell and Bellona, and Tikopia). Within Vanuatu, most groups are consistent with the baseline level established by Near Oceanians, but some – generally those with higher total proportions of FRO ancestry – display excess allele-sharing with Tonga. These include one ~150 BP Efate (Ifira) individual ($Z < -3$) and present-day Aneityum, Banks, Efate, Emae, Futuna, Makura, Mele (high-FRO subgroup, from the island of Efate), and Tongoa (all $Z < -4$). Among our newly reported ancient individuals, both from Mangaas and two of the three from Eretok have strong signals of Polynesian affinity ($-5.0 \leq Z \leq -3.6$).

We also attempted to determine the source of this Polynesian affinity more precisely using statistics f_4(X, Tolai; Polynesian1, Polynesian2) (Data S1I–L). We did not detect significant differences in allele-sharing relative to Tonga versus Samoa, but for a number of Polynesian-influenced groups in Vanuatu, we observed modest excess allele-sharing with Tonga versus

Polynesian Outliers (max |Z| = 3.6, 2.5, and 2.5 for Ontong Java, Rennell and Bellona, and Tikopia, respectively). One exception was excess relatedness between Namaram (from the island of Pentecost) and Ontong Java (Z = 3.2). However, for the most part, the source of the Polynesian-related ancestry in the Vanuatu groups appears to be slightly more closely related to populations from Polynesia than to other Polynesian Outlier communities in Melanesia (at least in their current genetic makeup).

We then tested for excess allele-sharing between the Eretok and Mangaas individuals and other Vanuatu populations (STAR Methods, Data S1M–Q). We detected several significant signals: (i) between the five ancient individuals and present-day Efate (Z = 1.8–3.2) and especially the high-FRO subgroup of present-day Mele (Z = 4.2–7.5); (ii) between the Eretok individuals I10968 and I10969 and the ~150 BP individual from Ifira (Z = 2.7–3.6); and (iii) among the five Eretok and Mangaas individuals themselves (Z = 1.8, 2.2, 2.5, 2.7, 2.9, 3.6, 6.5, 7.2, 7.4, and 16.4). A separate statistic testing for allele-sharing with present-day Futuna identified a strong relationship with Aneityum (Z > 9) but confirmed no particular relatedness to Eretok or Mangaas (Data S1R). Follow-up analyses also indicated that the Eretok individual I14493 and the Mangaas individual I10967 are close family relatives (probably second-degree; Figure S4), explaining their especially high allele-sharing (Z = 16.4) and confirming oral traditions directly linking both sites in the Roi Mata stories.

Admixture graph analysis

Finally, we built an admixture graph to explore relationships among multiple populations simultaneously, including present-day Tanna and Futuna, a ~600 BP individual (I5259) from Efate [6], Eretok and Mangaas, Polynesians, and diverse Near Oceanians (Figure 5; Figure S5; STAR Methods). The final model predicts all *f*-statistics relating the populations to within 2.7 standard errors of their observed values. We inferred two admixture events [6] among four ancestral Papuan lineages (associated with New Guinea, the Solomon Islands, and two with New Britain), one of which can parsimoniously characterize the Papuan ancestry in Melamela (New Britain), Vanuatu, and Tonga.

Within Vanuatu, the model contains separate two-stage admixture histories in the southern and central parts of the archipelago. Present-day Futuna can be modeled as having 56% ancestry related to individuals from Tanna (who themselves are inferred to have 12% FRO ancestry and 88% Papuan ancestry) and 44% related to Polynesians. For Efate, I5259 (from Mangaliliu, but not necessarily associated with the Chief Roi Mata's Domain sites) is inferred to have 11% FRO ancestry and 89% Papuan ancestry, and the Eretok/Mangaas group can be modeled as having 63% of their ancestry related to I5259 and 37% related to Polynesians (for a total of ~33% FRO ancestry). If we model Eretok/Mangaas and Futuna as having excess FRO (but not specifically Polynesian-related) ancestry, the log-likelihood of the model is more than 30 units lower, with residual poorly predicted *f*-statistics (Z > 5). Tanna and I5259 may not be exact representatives for the true ancestral source groups, so the inferred proportions of Polynesian-related ancestry may be slightly inaccurate, but they are plausible proxies based on both the regional genetic context and the fit quality of the final model.

Discussion

The human genetic history of Vanuatu is complex, featuring interactions between multiple populations with diverse origins. This complexity is not surprising given that the archipelago stretches for more than 1000 km and forms a crucial intervisible link in the southwest Pacific from the Reefs and Santa Cruz (at the eastern edge of the Solomon Islands) to New Caledonia. Furthermore, in light of the great cultural diversity that characterizes Vanuatu today, it would not be surprising if different parts of the archipelago have experienced different demographic dynamics in the past.

The results in this study further our understanding of three population movements (M1-M3) that contributed substantially to the genetic makeup of Vanuatu through time, with new evidence presented pertaining to several open questions.

Four newly reported individuals from Teouma (Efate) join published data to make a total of 12 sampled Lapita-associated individuals (all represented by petrous bones) from Remote Oceania dating to 3000–2500 BP (eight from Teouma, three from Talasiu in Tonga, and one from Malakula), all of whom have nearly entirely FRO-related ancestry [4–6]. Thus, while future sampling could potentially still reveal greater genetic diversity during this period, ancient DNA results to date support the hypothesis that the first people of Remote Oceania, who were responsible for spreading the Lapita cultural complex (M1), were mostly descended from a population with roots in East and Southeast Asia [4].

After about 2500 BP, sampled individuals from post-Lapita contexts testify to an influx of Papuan ancestry (M2), although with different trajectories in different parts of Vanuatu. The three earliest individuals from this period from central and southern Vanuatu (one newly reported here) have the smallest proportions of FRO ancestry in our data set, pointing to a major local genetic shift. The increased FRO ancestry in later populations from the same islands, combined with estimated dates of admixture that postdate the Lapita period, show that mixture subsequently occurred between populations with different proportions of FRO and Papuan ancestry [5, 6]. Previously published late Lapita and Post-Lapita individuals (2500–2000 BP) from Malakula in northern Vanuatu provide direct documentation of such an admixture process, as reflected in widely varying individual-level ancestry proportions along with recent estimated dates of admixture [5] (cf. ref. [24]). Unlike the other ancient individuals, those from Malakula come from a site that was continuously occupied for 1000 years, from the founding Lapita population until around 2000 BP. There are also indications that elements of the Lapita culture persisted for longer in this region than in central and southern Vanuatu [27, 28].

Our reanalysis of ancient and present-day data supports a single source for the main component of Papuan ancestry found in Vanuatu from 2500 BP to the present, with most of the (few) exceptions potentially relating to post-European-contact movements. In particular, although we do not have contemporaneous ancient DNA data available from Near Oceania, the location of this source, based on the strong present-day regional genetic structure, is likely to have been New Britain, and we do not detect more than isolated evidence of gene flow from the (geographically closer) Solomon Islands (in agreement with ref. [11]). This

relative homogeneity (across Vanuatu as well as through time) favors the hypothesis of a short-term migration episode responsible for introducing Papuan ancestry beginning around the late Lapita period. Inferred dates of admixture in Vanuatu (aside from Polynesian-influenced groups) also point to mixture of FRO and Papuan ancestry around this time [5, 6]. *A priori*, the most likely movements and interactions would be expected to be between neighboring archipelagoes rather than distant ones, i.e., from the main Solomons chain to the Reefs and Santa Cruz to Vanuatu. However, this appears not to have be the case either for M1, on archaeological and linguistic grounds [29], or for M2, on the basis of direct genetic links between Vanuatu and New Britain to the exclusion of the Solomons.

In light of results from both genetics and archaeology, a parsimonious explanation could be that M2 was effectively a continuation of M1 in late Lapita times, but involving migrants having mostly different ancestry. Cultural connections between New Britain and Vanuatu include the presence of New Britain obsidian in earliest Lapita deposits in Vanuatu [30], changes in dietary and mortuary behaviors and skeletal morphology subsequent to this earliest Lapita phase [31, 32], and distinctive practices (of unknown time depth), such as head-binding and the production of fully circular pig's tusks, that are exclusive to those locations [5, 33]. We also find that, contrary to the more complex proposals in previous studies [5, 6], we can model the Papuan ancestry found in Polynesians using the same New Britain-related source as for Vanuatu, raising the possibility that both were derived predominantly from the same phase of migration. However, as with the FRO component, future work is necessary to determine whether or not people carrying this ancestry passed through Vanuatu *en route* to Polynesia.

In accordance with archaeological and anthropological evidence of Polynesian cultural influence in Efate over the past several centuries, our analysis of five individuals from the Chief Roi Mata's Domain World Heritage Area demonstrates an influx of Polynesian-related ancestry as well (M3), through signals of higher FRO ancestry proportions, relatively recent dates of admixture, and specifically high allele-sharing with Polynesians. The present-day Polynesian Outlier community of Mele, as well as other present-day and recent-past individuals from Efate and nearby islands (but not more distant groups), also display shared ancestry with the Eretok and Mangaas individuals, while the Polynesian Outlier population of Futuna and the neighboring island of Aneityum in southern Vanuatu likely represent a separate instance of Polynesian influence (we currently lack data for comparison from communities such as those of Lelepa and Mangaliliu in the immediate World Heritage Area vicinity). Thus, while the ancestry of present-day Ni-Vanuatu groups can largely be traced to the early human history of the archipelago, later migrations – in particular of Polynesians – have also contributed to the genetic diversity of Vanuatu today.

STAR Methods

RESOURCE AVAILABILITY

Lead contact—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).

Materials Availability—This study did not generate new unique reagents.

Data and Code Availability—The aligned sequences are available through the European Nucleotide Archive under accession number PRJEB40109. Genotype data files are available at https://reich.hms.harvard.edu/datasets.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The following are brief descriptions of the sites and burials where the ancient individuals included in this study were found.

Teouma.—The Teouma site is located in southern Efate, on the edge of a large sheltered bay. It was once located near the sea but due to uplift it is now some 800 m from the current coast and 8 m above sea level. It comprises a colonizing Lapita settlement and associated cemetery dating from c. 2950 BP. Six field seasons of excavation were carried out at the site [9]. They revealed an extensive cemetery with up to 68 burial features. The burials were placed in solution holes in the ancient uplifted reef or in shallow graves on the old coral beach foreshore. They were directly associated with Lapita pottery and a range of ornaments also typical of Lapita. Manipulation of the bodies and the skeletal remains was standard procedure with all the adult skulls being removed from the initial interments [10, 34]. In a number of rare cases skulls were placed within other graves. Petrous portions of four of them were analyzed here (B10A, B10C, B30B, B30C). Continuing Lapita-period occupation at the site along with subsequent post-Lapita occupation ultimately buried the cemetery. The site appears to have been abandoned by about 2500 BP.

Mangaas.—The Mangaas or Mangaasi site is located on the west coast of Efate opposite Lelepa Island. The site was first excavated by José Garanger in 1967 as part of his wider pioneering archaeological research on central Vanuatu [15]. In oral traditions the site is said to be the location of the village of Roi Mata, a powerful chief who transformed the socio-political organization of the region. Deeply buried deposits were identified that were associated with distinctive pottery, subsequently named Mangaasi. Two burials (represented by petrous bones) recovered in the upper layers of the site are the subject of analysis here while five graves and two groups of disturbed human remains were recorded by Garanger. The same site and a much more extensive area immediately adjacent to the west were subsequently re-investigated from 1996–2003 [35, 36]. It has now been established that the region was first occupied around 2800 BP with continuing settlement in the region up to the present, primarily focused parallel to the coast. The earliest settlement is now some 80 m from the current beach due to continuing uplift, and, over millennia, settlements have continued to shift to maintain their location near the coast. The earlier archaeological deposits are generally deeply buried due to subsequent slopewash and tephra deposits.

Eretok.—Eretok (also known as Retoka or Hat Island) is located just offshore of Efate and Lelepa Islands on the west coast of Efate. It is the location of a cemetery that was associated with the death of chief Roi Mata in c. 1600 CE. Oral traditions tell of the death of this very important chief and how subsequently he was buried as part of a large communal ceremony undertaken on the island. Dozens of people apparently volunteered to be buried with the

chief as part of the ceremony. The site was excavated by José Garanger in 1967 after he was informed of its location by local community members working on the site of Roi Mata's village at Mangaas [15]. More than 50 individuals were identified with many buried as couples and others individually. Three of them, represented by two petrous bones and one tooth, are analyzed here. Roi Mata is identified as being buried in a more deeply excavated zone in front of a series of standing stones, alongside a number of individuals ostentatiously decorated with traditional shell and other ornaments.

Taplins.—Taplins comprises two rockshelters, Taplins 1 and 2, located at the base of a cliff on uplifted terraces behind Mele Bay in the southwestern part of Efate. Five subsurface graves were excavated at these sites by Graeme Ward in 1973 and 1974 [37, 38]. Both the earlier analysed individual and the subject of this study came from Taplins 1. The loose tooth studied here was initially hypothesized to belong to the same individual as the previously published petrous bone sample [6], but the genetic analysis shows that a second individual is represented (different mtDNA and Y chromosome haplogroups, and genome-wide allele-matching rates at the level of unrelated individuals).

Banana Bay.—Four burials were located during drainage works associated with road improvements around Efate Island [39]. The site is located on the southeast coast of the island. Local informants said that there had been a large village located in this area up to European contact. Burial 1, a burial in a supine position some 1.5 m below the current ground surface, was clearly associated with the historic period as the body was adorned with a shell and glass bead necklace. That individual was analyzed in ref. [6]. The tooth studied here is associated with a group of bones representing at least one other individual, found close by burial 1.

METHOD DETAILS

Ancient DNA laboratory procedures.—For the Teouma and Taplins samples, powder was drilled from bones or teeth in a clean room facility at University College Dublin, and DNA was then extracted in dedicated clean rooms at Harvard Medical School following previously published protocols [40–42] (additional sample preparation information can be found in Data S1A). Powder was obtained from four of the Mangaas and Eretok samples via cranial base drilling [43] at the Musée de l'Homme in Paris, while for I14493, the drilling step was omitted, and the tooth was submerged directly in 1.5 ml of extraction buffer for 4 hours. Laboratory work for EFE005 took place at the Max Planck Institute for the Science of Human History in Jena, Germany. The tooth was cut along the enamel/dentin junction and drilled into the pulp chamber, with the extraction then proceeding as above.

Barcoded sequencing libraries (1–5 per individual) were prepared from the extracts, utilizing the enzyme uracil-DNA glycosylase (UDG; partial treatment, for all but EFE005) to reduce the rate of deamination-induced ancient DNA damage artifacts [44–47]. The libraries were enriched for sequence fragments overlapping the mitochondrial genome and ~1.2 million genome-wide SNPs via two rounds of in-solution target capture [19, 48–51], with 7-base-pair indices added for the libraries generated at Harvard Medical School [52]. The libraries

were sequenced on an Illumina HiSeq machine with single-end reads (EFE005) or an Illumina NextSeq 500 machine with 76-base paired-end reads (others).

Bioinformatic processing.—For the ten individuals for whom data were generated at Harvard Medical School, we assigned sequencing reads to their respective libraries based on their barcodes, requiring at most one mismatch per read pair. We merged overlapping reads, trimmed barcodes and adapters, and then mapped to the mitochondrial reference genome RSRS [53] and to the human reference genome (version hg19) using the 'samse' command with default parameters in BWA (version 0.6.1) [54]. After aligning, we removed duplicate molecules and imposed a mapping quality filter of 10. Finally, we trimmed terminal bases (2 for UDG-treated libraries and 5 for untreated) to eliminate most damage-induced errors, and we called pseudo-haploid genotypes for genome-wide analyses by selecting one allele at random per targeted SNP site. Data for EFE005 were processed at the Max Planck Institute in Jena as described elsewhere [5].

Uniparental haplogroups and authentication.—We determined genetic sex of each individual by examining the fractions of sequence fragments mapping to the X and Y chromosomes [55]. We called mitochondrial haplogroups using HaploGrep2 [56] and Y-chromosome haplogroups by comparing SNP genotypes (using all reads) to the International Society of Genetic Genealogy Y-tree (http://www.isogg.org).

We assessed the authenticity of the data through five measures (Data S1A). First, we computed the rate of damage-induced errors in terminal positions of sequenced molecules to confirm the presence of ancient DNA signatures. We then tested for possible contamination by (a) confirming that genetic sex could be determined as male or female, (b) computing the rate of matching of mtDNA sequences to the consensus haplogroup call for each individual [57], and (c) measuring apparent heterozygosity at variable sites on the X chromosome in males [58]. Finally, we noted any signals in the genome-wide ancestry analyses that could suggest possible contamination from present-day human DNA.

For the individuals with lower coverage (fewer than 100,000 SNPs), the metrics are noisier, and the contamination estimates are generally less reliable, so our typical approach was to run our analyses for these individuals but to be cautious in interpreting the results and not to draw fine-grained conclusions. For the higher-coverage individuals, all metrics indicated at most a few percent contamination. One individual (I14493) had lower than expected damage rates (2.4% for mapped nuclear reads) but low contamination estimates (about 2–5% from both mtDNA and X chromosome). As an empirical test, we fit an admixture model for I14493 in *qpAdm* using damage-restricted data, and the results were extremely similar to those for all data ($p = 0.61$, $82.7 \pm 4.4\%$ Baining-related ancestry, and $17.3 \pm 4.4\%$ Kankanaey-related ancestry, versus $p = 0.84$, $79.8 \pm 1.4\%$, and $20.2 \pm 1.4\%$; Data S1D). Thus, we continued to use the data in our analyses.

Radiocarbon Dates.—We report new direct AMS radiocarbon dates for three individuals (EFE005, I10967, and I14493), which we combined with previously published dates for I5267 and I5268 [18]. Dates were calibrated using OxCal [59] version 4.3 with a mixture of the Marine13 and Intcal13 curves [60] as determined by linear interpolation between dietary

terrestrial and marine $\delta^{13}C$ isotopic endpoints (−21‰/−12‰) with an uncertainty of ± 10% on the percent marine carbon result, following the methodology outlined in ref. [61] to assess the proportion of marine, reef, and terrestrial food contribution to the bone protein. A location-specific reservoir correction (ΔR) of 40 ± 44 ^{14}C years was also applied to the marine curve to adjust for regional oceanic variation in ^{14}C around Vanuatu [62].

QUANTIFICATION AND STATISTICAL ANALYSIS

Data set construction.—We merged our newly generated data with published ancient and present-day data [4–6, 63, 64]. Unless otherwise specified, we used a set of ~398,000 autosomal SNPs from the Human Origins array, excluding (a) C-to-T transition SNPs at CpG dinucleotides, and (b) a set of SNPs with high rates of missing data in present-day genotype data. For *f*-statistic-based analyses (as reflected in the sample sizes in Data S1B), we excluded 20 present-day individuals who were outliers relative to their ethnolinguistic groups: UV128 (Tolai); UV219 (Mengen); UV220 (Sulka); UV726 (Kuot Kabil); UV516 and UV519 (Kuot Lamalaua); UV533 (Nailik); UV1166 (Melamela); Jk2663, Jk2665, and Jk2669 (Samoan); and nine individuals from Vanuatu who were identified as outliers in previously published data curations [6].

PCA.—We performed PCA with smartpca [65], using the ‘lsqproject’ and ‘shrinkmode’ options. We used three populations (Kankanaey, Nasioi, and New Guinea Highlanders) to define axes and projected all other individuals. Projecting ancient individuals prevents bias due to missing data; we chose to project present-day populations as well in order to create a two-dimensional plot with equivalent procedures for all individuals (aside from the three axis populations) and with minimal effects of population-specific drift. We note that the ancient individuals with lower coverage have more uncertainty associated with their positions.

Formal modeling of admixture.—We tested admixture models using the *qpAdm* software [19, 20]. Our basic outgroup list consisted of New Guinea, Australian, French, Dai, Onge, and Mixe, a set of populations with largely phylogenetically distinct positions relative to the mixing populations in our applications: Papuan, deeply Papuan-related, western Eurasian, East Asian, deep eastern Eurasian, and Native American, respectively. If a given model fits poorly (i.e., low *p*-value), that implies that it is poorly specified, in the sense that not all of the ancestry in the test population is more closely related to the proxy sources than to the outgroups. In other words, either the test population shares some ancestry with one or more of the outgroups more closely than with the specified proxy sources, or one of the proxy sources shares ancestry with one or more of the outgroups more closely than with the test population. To search for possible sex-biased admixture, we compared mixture proportion estimates on the autosomes and the X chromosome, computing a quasi-Z-score by dividing the difference by the standard error for the X estimate (which is much larger than the autosomal standard error). To maximize coverage on the X chromosome, we did not apply the two SNP exclusion criteria described in the ‘Data set construction’ subsection above (which should have a negligible effect on mixture proportion estimates in the units of ratios of *f*-statistics). When computing FRO and Papuan ancestry proportions, we used

Baining as the proxy for Papuan-related ancestry and corrected the estimates for the fact that Baining themselves have ~5% FRO ancestry.

We also tested the compatibility of multiple populations with having common sources of admixture without a formal model, using *qpWave* [26]. Our test set for ancient Vanuatu plus other Oceanian populations consisted of present-day Tongan (6 individuals) and Melamela (9), plus the following ancient Vanuatu groupings: Efate 150–400 BP (5), Efate ~600 BP (1), Efate ~2400 BP (2), Epi ~150 BP (2), Epi ~1300 BP (2), Futuna ~1100 BP (4), Malakula 2000–2500 BP (6), Tanna ~150 and ~2500 BP (2), Mangaas (2), and Eretok (3). For present-day Vanuatu, we used all population groups in our data set. In both analyses, our outgroup set was the same as for *qpAdm*, either with or without Nasioi. As in *qpAdm*, a higher *p*-value indicates a better fit for the proposed model, where a rank of k implies $k+1$ distinct ancestry sources combining to form the test set of populations.

Dates of admixture.—We estimated dates of admixture using MALDER [21] and DATES [22]. MALDER extends the linkage disequilibrium (LD)-based model of ALDER [66] by integrating information from multiple reference populations and searching for evidence of multiple waves of admixture. We used all ~590k autosomal Human Origins SNPs, and our reference set consisted of New Guinea Highlanders, Papuan, Australian, Baining (both subgroups), Teouma, Talasiu, Kankanaey, Ami, and CDX (1000 Genomes Dai).

DATES implements a regression-based ancestry covariance estimate that can be applied to single individuals. We used all ~1.15 million autosomal SNPs from our capture set, and our reference pair was Papuan [64] and CDX [63]. For both methods, we assumed an average generation interval of 28 years when converting results to years in the past and estimated standard errors by block jackknife.

f_4 regression analysis.—We used a linear regression-based method to test for asymmetrical allele-sharing in cases where the f_4-statistics of interest are confounded by differential ancestry proportions across the test population set. Instead of searching directly for non-zero values, we plotted pairs of f_4-statistics in which the dependent variable is the statistic of interest and the independent variable is a statistic (f_4(X, New Guinea Highlanders; Kankanaey, Australian)) measuring levels of Papuan ancestry. This approach is based on the linearity of f_4-statistics for a collection of test populations ('X') with mixtures of ancestry related to the same two source populations but in different proportions (see below for derivation). If some test populations violate the proposed two-way model, they will tend to deviate from the expected linear relationship between the dependent and independent variables. We computed the f_4-statistics in ADMIXTOOLS [67], with standard errors estimated by block jackknife. This approach is in some ways similar to the f_4-biplots introduced in ref. [19], but the scenarios of interest and the interpretations of the results are different.

Taking the example of Figure 4B, suppose we had a collection of populations, each of whose ancestry is a mixture from the same two sources, P and F, but in different proportions. Let A = f_4(P, New Guinea Highlanders; Kankanaey, Australian), B = f_4(F, New Guinea

Highlanders; Kankanaey, Australian), C = f_4(P, Tolai; Kankanaey, Tongan), and D = f_4(F, Tolai; Kankanaey, Tongan). If one of our test populations, X, has a proportion α of P-related ancestry and (1-α) of F-related ancestry, then (in expectation) f_4(X, New Guinea Highlanders; Kankanaey, Australian) = α*A + (1-α)*B = α*(A-B) + B, and f_4(X, Tolai; Kankanaey, Tongan) = α*C + (1-α)*D = α*(C-D) + D = [constant1]* f_4(X, New Guinea Highlanders; Kankanaey, Australian) + [constant2] (where the first constant is (C-D)/(A-B) and the second is also a rational function of A, B, C, and D). Thus a pair of f_4-statistics are expected to have a linear relationship under the assumption that the set of populations in the first position (with the other three positions fixed) have mixtures of ancestry from the same two sources.

We performed linear regression via inverse-variance-weighted least-squares. Given the resulting best-fit equation f_4(2) = m*f_4(1) + b, we evaluated the deviation of each population by calculating its empirical value of f_4(2) - m*f_4(1) - b, assessing the statistical significance by a Z-test (estimating the standard error on the value directly with a block jackknife). In most cases, we used one data point for each population group, except in cases of ancient populations with substantial heterogeneity (the distinction being accommodated naturally because of the weighting scheme).

To maximize power given the relatively low-coverage data for the Lapita-period individuals, we computed the statistic f_4(X, New Guinea Highlanders; Teouma, Talasiu) indirectly, via f_4(X, New Guinea Highlanders; CDX, Talasiu) - f_4(X, New Guinea Highlanders; CDX, Teouma) (including non-overlapping SNPs), with a block jackknife to estimate the standard error. When computing deviations from the regression line for this statistic, we then used the raw standard error rather than the full residual described above; empirically, this likely results in slight underestimates of the standard error (although this is conservative, in the sense that we observe only minor deviations for this test).

We note that the choice of comparison population in the second position (e.g., New Guinea in the previous paragraph) only serves to shift all statistic values up or down a constant amount, because f_4(X, Pop1; Y, Z) - f_4(X, Pop2; Y, Z) = f_4(Pop2, Pop1; Y, Z), which is a constant for all X. For Polynesian-related ancestry tests, to improve power, we used Tolai in the second position because (a) it is the Oceanian population with the largest sample size in our data set, (b) it has an intermediate proportion of FRO ancestry, and (c) we were not specifically interested in the history of Tolai as a test population in these analyses.

When testing for specific relatedness to the Eretok and Mangaas individuals, we used the statistics f_4(X, Tolai; Eretok/Mangaas individual, Futuna ~1100 BP) for each individual in turn. We used the set of four ancient individuals from Futuna [5] in the fourth position rather than a present-day group in order to prevent artificial signals of allele-sharing when X is ancient.

Admixture graph fitting.—We built our admixture graph using the *qpGraph* software in ADMIXTOOLS [67], with 13 populations included: Mixe (from Mexico) and Australian as outgroups; Atayal and Kankanaey (FRO-related); New Guinea Highlanders; Nasioi (Solomon Islands); Baining (Marabu subgroup) and Melamela (New Britain); Tongan; and

four groups or individuals from Vanuatu – Eretok and Mangaas, ~600 BP Efate (I5259) [6], present-day Futuna, and present-day Tanna. We specified the options 'outpop: NULL', 'lambdascale: 1', and 'diag: 0.0001.' For a model of this size, the space of possible topologies is extremely large, so we cannot conclude that our final graph is the unique one that provides a good fit to the data. Instead, we use it in conjunction with our other analyses to investigate which results are supported when modeling the relationships among many populations simultaneously and to discover any additional admixture events necessary to obtain a good fit.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights:

- New ancient DNA supports a shift in ancestry during early migrations to Vanuatu.
- A single spread from New Britain can explain most of the ancestry of later groups.
- More recent Polynesian migrations contributed both cultural and genetic legacies.

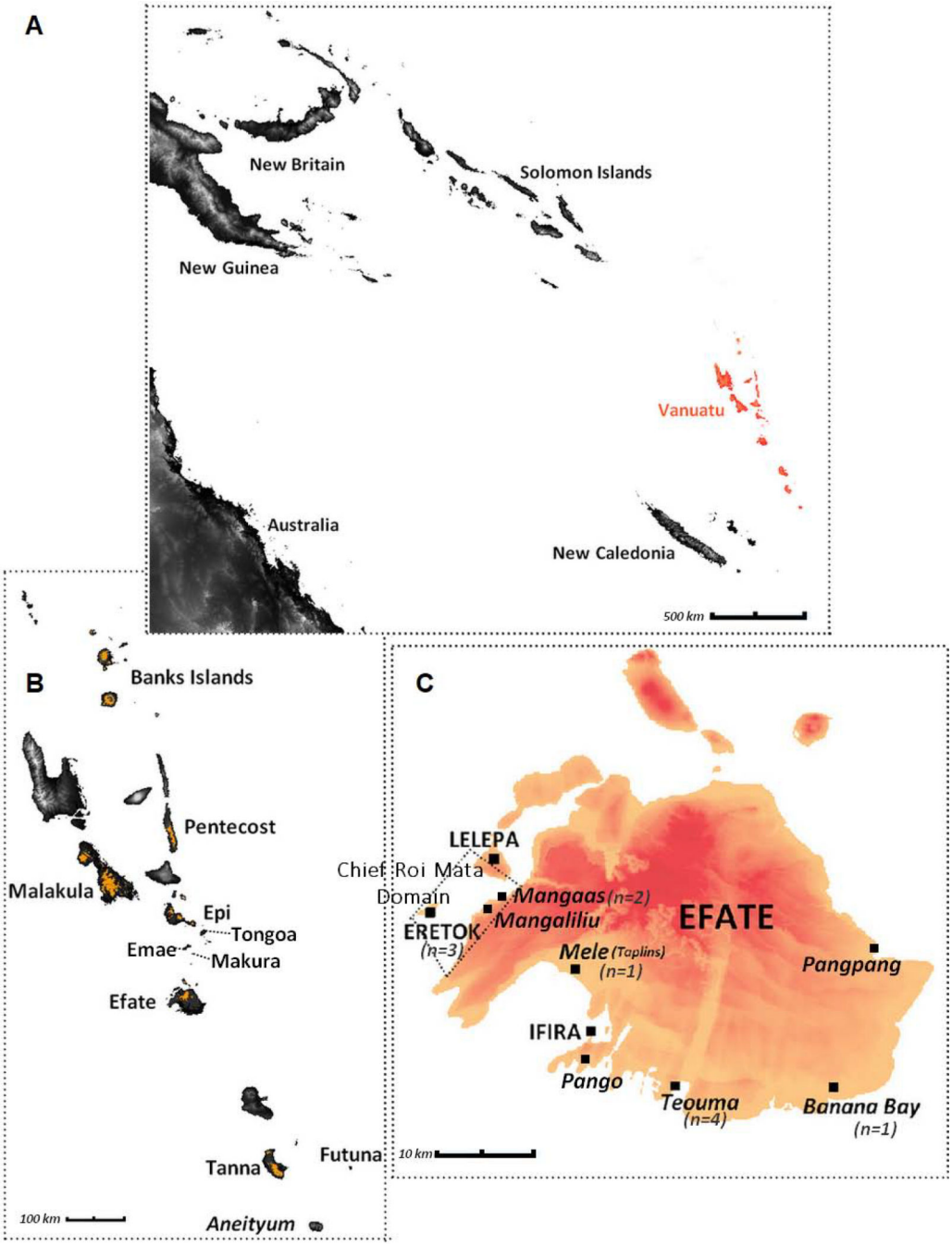

Figure 1. Geographic context.

(A) Regional map. (B) Map of Vanuatu. (C) Map of Efate with sample sizes for newly reported individuals from each site.

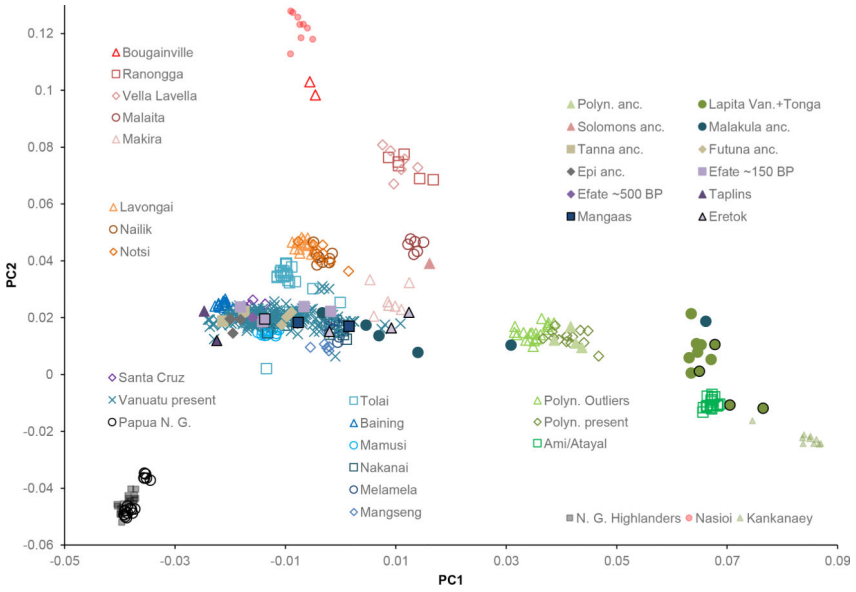

Figure 2. PCA results.

Axes were computed using three present-day populations (bottom right legend), and other present-day (no fill) and ancient (large filled symbols; newly reported with black outline) individuals were projected and plotted using the first two PCs. Colors correspond to genetic clusters centered around the Solomon Islands (red), New Ireland (orange), New Britain (blue), New Guinea (black), and Polynesia and Taiwan (green). N. G., New Guinea; Polyn., Polynesian; Van., Vanuatu; anc., ancient.

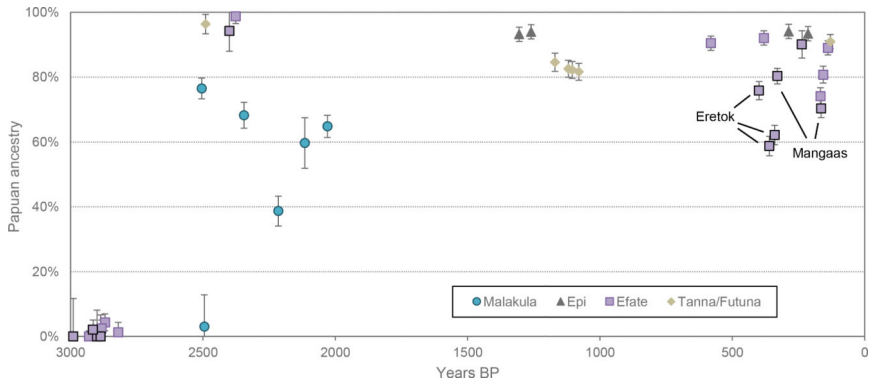

Figure 3. Ancestry proportions for ancient Vanuatu individuals.

Results are from two-component *qpAdm* models estimating total proportions of Papuan and FRO ancestry, truncated at 0% for four individuals with negative point estimates. Newly reported individuals are represented by points with black outlines. Some points are shifted slightly left and right for legibility. Bars show two standard errors in both directions (for truncated individuals, upper limit of point estimate plus two standard errors). See Table 1 for full date intervals and Data S1D for full *qpAdm* results.

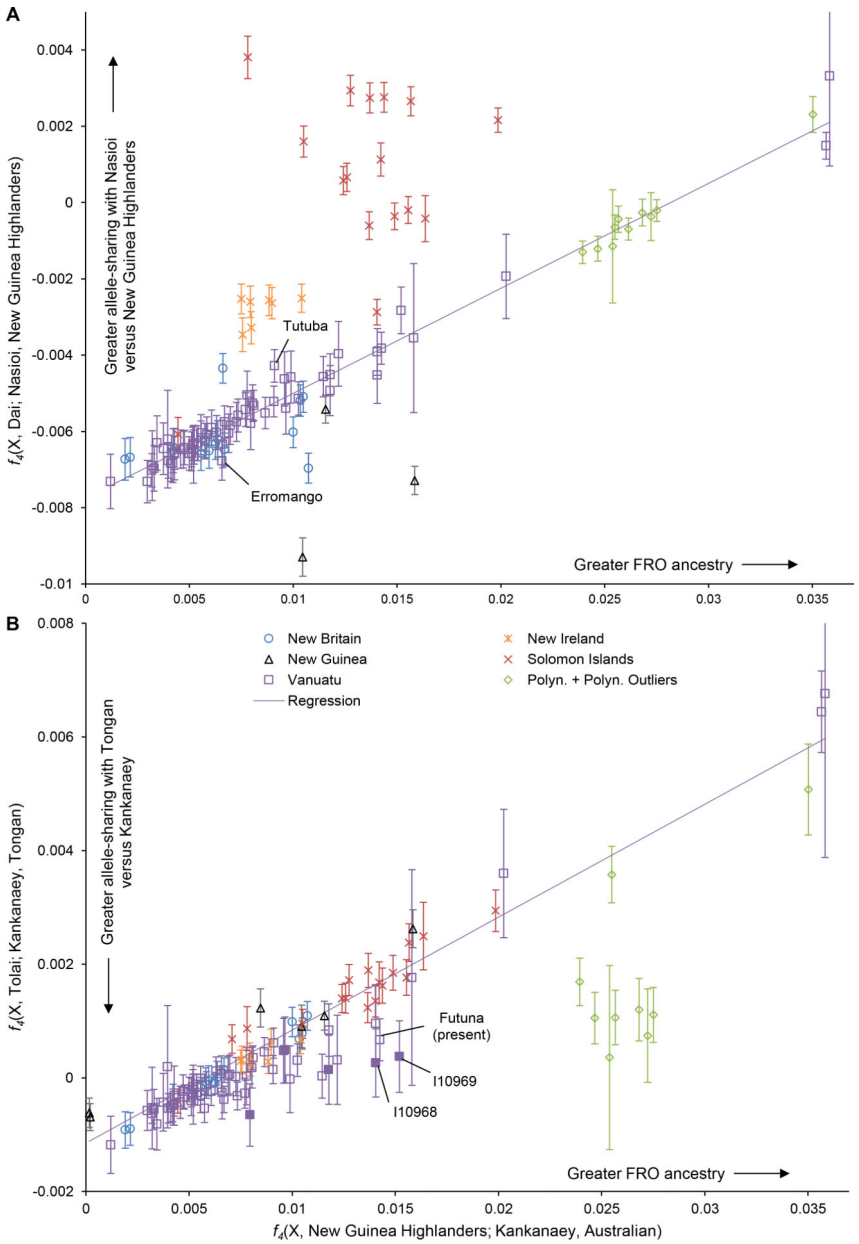

Figure 4. Allele-sharing regression tests.

(A) Test for differential Papuan ancestry. The regression line was computed using groups from Vanuatu and Polynesia, except for the Lapita-associated individuals (rightmost three points). (B) Test for Polynesian influence. The regression line was computed using Near Oceanian populations. Filled points represent the Eretok/Mangaas individuals. The legend is the same for both panels (the 'New Guinea' label includes some closely related populations from nearby islands; some in the far lower left in (A) are omitted for scale), and bars show two standard errors in each direction. Polyn., Polynesian. See also Figures S1–S4 and Data S1E–R.

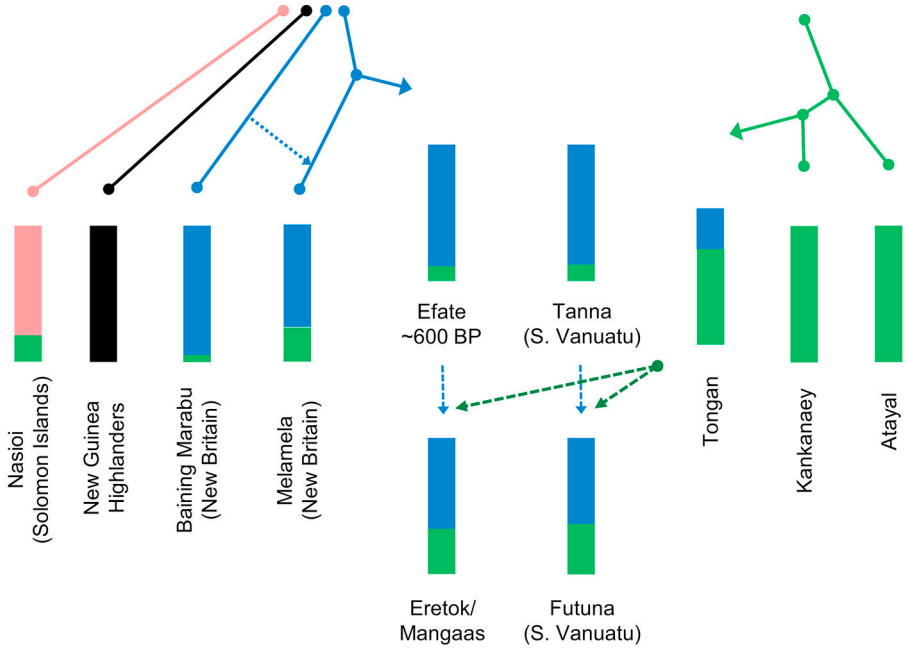

Figure 5. Schematic of admixture graph results.

Inferred phylogeny is shown for FRO-related ancestry (light green) and four Papuan lineages (pink, black, and blue, shown separately because they are not related by a simple tree). Arrows denote Papuan ancestry found in Vanuatu and Polynesia (solid blue), admixed FRO ancestry (green), local Ni-Vanuatu ancestry (dashed blue), Polynesian-related ancestry (dashed dark green), and intra-New Britain admixture (dotted blue). Colored bars give inferred total ancestry proportions (excluding outgroups Australian and Mixe). See Figure S5 for full results. S., southern.

Table 1.
Information for newly reported individuals.

Date, calibrated radiocarbon date (95.4% CI) or burial context estimate (brackets); Mt/Y hap, mitochondrial DNA/Y chromosome haplogroup; SNPs, unique autosomal target sites covered at least once / sites covered in primary analysis data set. See also Data S1A–C.

Lab ID	Skeletal code (element)	Date	Location	Sex	Mt hap	Y hap	SNPs
I5265	Teo_B10A (petrous)	[3000–2750 BP]	Efate, Teouma	M	No call	O	13,594/4,469
I5266	Teo_B10C (petrous)	[3000–2750 BP]	Efate, Teouma	M	B4a1a1	O	136,137/45,599
I5267	Teo_B30B (petrous)	3170–2810 calBP (3050±49 BP, Wk-22658)	Efate, Teouma	M	No call	No call	8,612/2,802
I5268	Teo_B30C (petrous)	3010–2760 calBP (2995±21 BP, Wk-22659)	Efate, Teouma	M	No call	No call	4,165/1,396
I6188	TAP_E149 (tooth)	[2600–2200 BP]	Efate, MeleTaplins	M	Q1b	C1b2a	23,812/8,088
I10966	Musée de l'Homme 25788 (petrous)	[500–200 BP]	Efate, Mangaas	F	Q1	n/a	648,879/230,929
I10967	Musée de l’Homme 25787 (petrous)	290–0 calBP (180±20 BP, PSUAMS-5494)	Efate, Mangaas	F	Q2a3	n/a	469,594/167,469
I10968	Musée de l’Homme 25793 (petrous)	[500–200 BP]	Eretok	M	B4a1a1	C1b2a	848,415/295,552
I10969	Musée de l’Homme 25791 (petrous)	[500–200 BP]	Eretok	F	P2	n/a	749,208/267,632
I14493	Musée de l’Homme 25797 (tooth)	490–310 calBP (350±20 BP, PSUAMS-6698)	Eretok	M	P2	C1b2a	506,596/179,141
EFE005	EFE005 (tooth)	310–0 calBP (234±19 BP, MAMS-29695)	Efate, Banana Bay	M	P1d2	C1b2a	74,434/25,228

Table 2:

Inferred average dates of admixture.

Gen/yr, generations/years before the individuals lived (mean ± 1 SE).

Test group or individual	MALDER result (gen / yr)	DATES result (gen / yr)
Eretok (triple)	16.4 ± 4.5 / 459 ± 126	24.1 ± 5.0 / 675 ± 140
Mangaas (pair)	36.5 ± 15.4 / 1023 ± 432	22.6 ± 6.9 / 633 ± 193
I10968 (Eretok)		18.4 ± 4.6 / 514 ± 128
I10969 (Eretok)		29.6 ± 9.1 / 828 ± 256
I14493 (Eretok)		28.3 ± 9.3 / 793 ± 261
I10966 (Mangaas)		31.7 ± 13.5 / 888 ± 378
I10967 (Mangaas)		18.5 ± 7.4 / 517 ± 207

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Ancient human skeletal elements	This study	See Table 1
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
Silica magnetic beads	G-Biosciences	786–915
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	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
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

REAGENT or RESOURCE	SOURCE	IDENTIFIER
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
NextSeq® 500/550 High Output Kit v2 (150 cycles)	Illumina	FC-404–2002
Deposited Data		
Raw and analyzed data	This paper	ENA: PRJEB40109
Software and Algorithms		
In-house bioinformatics tools	https://github.com/DReichLab/ADNA-Tools	https://github.com/DReichLab/ADNA-Tools
In-house data workflow	https://github.com/DReichLab/adna-workflow	https://github.com/DReichLab/adna-workflow
Samtools	[68]	http://samtools.sourceforge.net/
BWA	[54]	http://bio-bwa.sourceforge.net/
Picard	https://broadinstitute.github.io/picard/	https://broadinstitute.github.io/picard/
ADMIXTOOLS	[67]	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	[65]	https://www.hsph.harvard.edu/alkes-price/software/
PMDtools	[69]	https://github.com/pontussk/PMDtools
Haplogrep 2	[56]	http://haplogrep.uibk.ac.at/
htsbox	https://github.com/lh3/htsbox	https://github.com/lh3/htsbox
contamMix	[57]	contact Philip Johnson plfj@umd.edu
ANGSD	[58]	https://github.com/ANGSD/angsd
OxCal	[59]	https://c14.arch.ox.ac.uk/oxcal.html
MALDER	[21]	https://github.com/joepickrell/malder/tree/master/MALDER
DATES	[22]	https://github.com/priyamoorjani/DATES