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ORIGINAL ARTICLE

Genetic structure among Fijian island populations

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We examined nine Y chromosome short tandem repeats (Y-STRs) and the mitochondrial DNA (mtDNA) hypervariable segment 1 region in the Fijian island populations of Viti Levu, Vanua Levu, Kadavu, the Lau islands and Rotuma. We found significant genetic structure among these populations for the Y-STRs, both with and without the Rotumans, but not for the mtDNA. We also found that all five populations exhibited the sex-biased admixture associated with areas settled by Austronesian-speaking people, with paternal lineages more strongly associated with Melanesian populations and maternal lineages more strongly associated with Polynesian populations. We also found that the Rotumans in the north and the Lau Islanders in the east were genetically more similar to Polynesian populations than were the other Fijians, but only for the mtDNA. For the Y-STRs, the Rotumans and the Lau Islanders were genetically as similar to Melanesian populations as were the other three populations. Of the five populations, the Rotumans were the most different in almost every regard. Although past genetic studies treated the Fijians as being genetically homogenous despite known geographic, phenotypic, cultural and linguistic variation, our findings show significant genetic variation and a need for a closer examination of individual island populations within Fiji, particularly the Rotumans, in order to better understand the process of the peopling of Fiji and of the surrounding regions.
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INTRODUCTION

The Pacific Ocean comprises three commonly recognized regions: Melanesia, Micronesia and Polynesia, as seen in Figure 1. Much of Melanesia was first settled ~ 40 000 years ago.[1] Parts of Melanesia and all of Micronesia and Polynesia were settled by Austronesian-speaking Southeast Asians who likely originated on or near the island of Taiwan and reached Melanesia ~ 3500 years ago.[1] In the area of the Bismarck Archipelago, the Lapita culture developed. By ~ 3200 years ago, the Lapitans were expanding eastward toward Polynesia, and by no later than ~ 725 years ago their descendants had reached all of the islands of Polynesia and became the Polynesians we know today.[2]

Several models have attempted to characterize the nature of settlement of Polynesia. At one extreme, the 'Express Train' model[3] asserts that the Polynesians' ancestors moved relatively rapidly from Southeast Asia through Melanesia without significantly admixing with the people already present. At the other extreme, the 'Entangled bank' model[4] asserts that the Polynesian people resulted from a long and complex history of human interaction and admixture starting with the first occupation of Melanesia during the Pleistocene. However, the intermediate 'Slow Boat' model,[5] which asserts that the Polynesians' ancestors originated in Southeast Asia relatively recently and significantly admixed with people already present in Melanesia on their way to Polynesia, is the most strongly supported and widely accepted.[6–8] One version of the Slow Boat model suggests that the initial movements of Southeast Asians into Melanesia were likely small-scale exploratory and/or trading voyages, and that these earlier voyages

at least resulted in the knowledge of and gene flow into the region into which later larger-scale movements occurred and may have resulted in the establishment of relationships or even settlements that facilitated them.[9]

Fiji has been characterized as the 'Gateway to Polynesia', and yet the process of the peopling of Fiji and Western Polynesia remains unclear. Fiji was originally settled by the Lapitans ~ 3100 years ago,[10] but today the Fijians are a complex blend of Melanesian and Polynesian characteristics, possibly due in part to one or more subsequent Melanesian migrations into Fiji.[1,11] As a group, Fijians have traditionally been classified as Melanesian based on cultural practices and some morphological features,[12] but they share much in common linguistically,[13] phenotypically[14] and genetically[6] with Polynesian populations. However, most Fijians reside on the larger western islands of Viti Levu, Vanua Levu and Kadavu, and are culturally and morphologically more influenced by Melanesia,[12] whereas a significant minority of Fijians reside on the northern island of Rotuma and the eastern Lau Islands, and are culturally,[1] phenotypically[14] and linguistically[15] more influenced by Polynesia. In fact, there is some archeological support for moving the boundary between Melanesia and Polynesia to within the Fijian group, with Rotuma and the Lau Islands being grouped with Polynesia.[16] Others consider all of Fiji to be a part of Polynesia.[6,8]

Approximately 65% of Fijian autosomal DNA is Asian and ~ 35% is Melanesian, and this admixture appears to have occurred substantially after the initial settlement of the region,[11] that is, there was a

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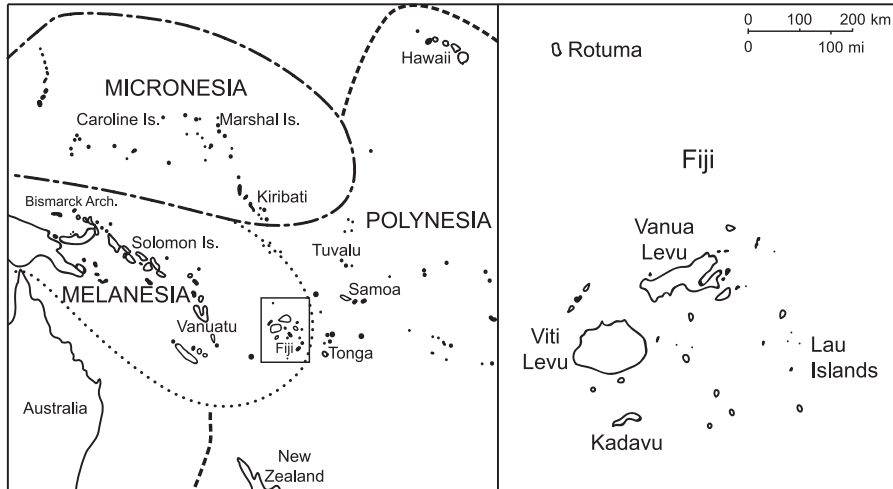
Figure 1 The larger mid-Pacific region (left), and the Fijian island group (right).

substantial subsequent contact between Fijians and Melanesians that did not extend to Polynesia. Approximately 80% of Fijian mitochondrial DNA (mtDNA) is Asian and ~79% of Fijian Y chromosome DNA is Melanesian,[6] which are consistent with the sex-biased admixture found among populations descended from Austronesian-speaking settlers,[17] but substantially different proportions than are found in Polynesians. In fact, Y chromosome DNA and mtDNA data support a closer relationship between Fijians and Melanesians than between Polynesians and Melanesians.[6] For example, the frequencies of Melanesian mtDNA haplogroups in Fijians is ~20%, while in Polynesians it is ~6%.[6] On the one hand, Fijians have a higher diversity and frequency of Y chromosome DNA haplogroups of Melanesian origin than any Polynesian population except, perhaps, the Cook Islanders, and have a higher diversity and frequency of mtDNA haplogroups of Melanesian origin than any Polynesian population.[6] On the other hand, Fijians and Polynesians share haplogroups (for example, the O-M122/DYS385 triplet) that are not found in Melanesia.[6] This complexity of genetic, phenotypic, cultural and linguistic variation found in Fiji likely reflects (1) the Fiji's role as the 'Gateway to Polynesia' for the Austronesian-speaking people, who ultimately settled in Polynesia, and (2) the subsequent contact between Fijians and Melanesian people. However, much of this treats the Fijians as a homogenous whole and ignores the potentially very different experiences of the individual island populations.

Geographically, Fiji includes 332 islands spread over ~18 300 square kilometers (7100 square miles). In general, genetic studies have treated Fijians as a unit,[6,8,11] and despite their importance in understanding the origins of the Polynesians, little systematic work has been done to determine and understand the genetic history of the geographically and culturally diverse island populations of the group. We therefore sought to determine whether the Fijians are genetically homogenous, whether they exhibit the sex-biased admixture commonly associated with areas settled by Austronesian-speaking people, and whether the Rotumans in the north and the Lau Islanders in the east are genetically more similar to Polynesians than is the rest of Fiji.

Throughout this paper we use the Melanesia/Polynesia distinction for the Pacific rather than the Near/Remote Oceania distinction because the former is more relevant to our questions and to our findings. In particular, Fiji is located at the boundary between Melanesia and Polynesia, and its genetic structure strongly reflects and is most meaningful with regard to this liminality.

MATERIALS AND METHODS

Our samples were obtained in 2008 from individuals at the University of the South Pacific's main campus in the capital city of Suva, on Vitu Levu. In particular, the team collected buccal cell samples from which DNA was later extracted using the phenol–chloroform method.[18] Most participants had ancestral ties to the Fijian islands of Viti Levu, Vanua Levu, Kadavu, the Lau Islands and Rotuma, and some participants had ties to Samoa, Tonga and Vanuatu. With regard to the samples used for Y chromosome short tandem repeat (Y-STR) analysis, all participants were able to identify their own and their father's (and 78% were able to identify their father's father's) islands of birth. With regard to the samples used for mtDNA analysis, all participants were able to identify their own and their mother's (and 68% were able to identify their mother's mother's) islands of birth. All participants gave informed consent, and all samples were obtained and handled in accordance with the human subjects committees at the University of Kansas and the University of the South Pacific in Fiji.

With regard to the Y chromosome, 102 male samples were haplotyped using an AmpFlSTR YFILER PCR amplification kit (Applied Biosystems, Waltham, MA, USA) to determine the alleles for 17 Y-STR loci (DYS19, DYS385a/b, DYS389I, DYS389II-I, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and YGATAH4). The kit protocol called for a total reaction volume per sample of 25 μl, including 10 μl of DNA at a concentration of 0.1 ng μl^{-1} (that is, a total of 1 ng), which allowed for haplotyping 100 samples. For most of the samples, however, we successfully used a total reaction volume per sample of 6.25 μl, including 0.75 μl of DNA at 2.5 ng μl^{-1} (thats is, a total of 1.875 ng), which allowed for haplotyping up to 400 samples. A small subset of the samples did not return complete data at this DNA concentration, and were rerun with 1.25 μl of DNA. We opted to use our own positive control sample with a DNA concentration of 2.5 ng μl^{-1} and alleles of known length. The PCR profile specified by the kit was used. All samples were diluted 1:30 with H$_2$O after purification. Length determination of the 17 Y-STR loci was performed by the University of Kansas DNA Sequencing Laboratory, reported by GENEMAPPER (Applied Biosystems), and checked in PEAK SCANNER (Applied Biosystems); both GPS and AJR examined all Y-STR data. Our Y-STR alleles are provided in Supplementary Table S1.

Additional Y-STR data were taken from the literature,[19] and additional Y-STR data for the Polynesian islands of Samoa, Tonga and Tahiti were provided by AJR. The latter data were collected and sequenced in a manner similar to that described above, and are also provided in Supplementary Table S1. The data for Polynesia were limited to alleles for nine loci (DYS19, DYS385a/b, DYS389I, DYS389II-I, DYS390, DYS391, DYS392 and DYS393), so the data for Fiji and Melanesia were correspondingly reduced. An analysis of molecular variance for the five Fijian populations was performed and Slatkin's linearized F_{ST} (fixation index) genetic diversity distances[20] between the various

Table 1 Y-STR genetic diversity and Slatkin's linearized F$_{ST}$ (fixation index) genetic distance values for certain Fijian island populations, as well as F$_{ST}$ values for the centroids of Polynesia (in italics and represented by Samoa, Tonga and Tahiti) and Melanesia (in italics and represented by Vanuatu and 10 non-Polynesian Outlier islands within the Solomon Islands)

| | | | | YDNA | | | | | | |
| | | | | | Slatkin's linearized F$_{ST}$ genetic distances values | | | | | |
Population	N	Number of haplotypes	Gene diversity	Mean no. of pairwise differences	Viti Levu	Vanua Levu	Kadavu	Lau	Rotuma	Polynesia	Melanesia
Viti Levu	47	35	0.98 ± 0.01	5.79 ± 2.82							
Vanua Levu	17	16	0.99 ± 0.02	6.59 ± 3.27	0.10						
Kadavu	10	10	1.00 ± 0.05	6.53 ± 3.38	0.00	0.01					
Lau	18	16	0.98 ± 0.03	6.18 ± 3.08	0.01	0.09	0.00				
Rotuma	10	8	0.93 ± 0.08	5.38 ± 2.83	0.16	0.14	0.00	0.05			
Polynesia	*58*	*37*	*0.96 ± 0.01*	*5.13 ± 2.52*	*0.14*	*0.32*	*0.22*	*0.17*	*0.55*		
Melanesia	*501*	*319*	*1.00 ± 0.00*	*6.06 ± 2.89*	*0.18*	*0.15*	*0.01*	*0.12*	*0.06*	*0.25*	

Abbreviations: YDNA, Y chromosome DNA; Y-STR, Y chromosome short tandem repeat.

Melanesian, Fijian and Polynesian populations were determined using ARLE-QUIN 3.11 (University of Bern, Bern, Switzerland),[21] and a multi-dimensional scaling plot based on those distances were created using NTSYSPC 2.02 (Exeter Software, East Setauket, NY, USA). In particular, separate analyses were performed in which the Polynesian and Melanesian island populations were treated separately, and in which they were collapsed into single populations. Additionally, average genetic distances within Fiji were calculated by averaging the genetic distances between each island population and the other island populations.

With regard to the mtDNA, 107 samples were haplotyped using light-chain (L) primer L-15996 (5′-ACTCCACCATTAGCACCCAAAGC-3′) and heavy-chain (H) primer H-16401 (5′-CACCATCCTCCGTGAAATCA-3′) to determine the sequence for a 405 bp fragment from the mtDNA hypervariable segment 1 (HVS1) region. The PCR profile involved initial denaturation at 94 °C for 3 min; then 30 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s and elongation at 72 °C for 45 s; and a final extension at 72 °C for 3 min. PCR products were cleaned using a QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) according to the normal protocol.

Sequencing of the HVS1 fragments was performed by the University of Kansas DNA Sequencing Laboratory. The forward and reverse fragments were visualized using SEQUENCHER 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA), and aligned to the revised Cambridge Reference Sequence (CRS). Sections of low quality sequence were removed. Each substitution within the sequence was inspected on the chromatogram to ensure proper sequence calling. After the sequence was constructed by merging the two fragments, the ends were trimmed to fit the revised CRS. The final mtDNA fragment length was 362 bp. The final fragments were aligned to the revised CRS, and variable substitution sites were identified using MEGA4.[22] MtDNA haplogroups were assigned based on substitutions identified in the literature,[23,24] and each haplogroup was characterized as either Melanesian or Asian based on the origin of the lineage rather than the location where the particular haplogroup may have arisen. For example, we characterized haplogroup B4a1a1 as Asian because the B4 lineage originated in Asia, even though B4a1a1 may have arisen in Melanesia among Asian-descended people.[9]

These assignments were tested by the construction of a phylogenetic tree (not shown) in which the samples clustered in accordance with their assigned haplogroups. A total of 41 unique, previously unpublished haplotypes were observed. In most cases, these haplotypes possessed at least one additional substitution outside of the defining substitutions for the haplogroup. Our mtDNA HVS1 sequences are available in GenBank (accession numbers KJ842340–KJ842483).

Additional mtDNA sequences for various Melanesian islands reported in the literature[19] were obtained from GenBank (accession numbers JN017205–JN017907). These sequences were 340 bp long, so the Fijian sequences were

correspondingly trimmed. The mtDNA data were analyzed in the same manner as the Y-STR data.

RESULTS

In Table 1, Y-STR data for the five individual Fijian island populations were compared with Polynesians as a group, to Melanesians as a group, and to each other. Slatkin's linearized F$_{ST}$ genetic distance values indicated that the Kadavuans were genetically the most like Melanesian populations (or the most 'Melanesian-like'), and the Viti Levuans were the most like Polynesian populations (or the most 'Polynesian-like'). In fact, all but the Viti Levuans were more Melanesian- than Polynesian-like, but the Rotumans were by far the least Polynesian-like and had the largest difference (Rotuman-Polynesian F$_{ST}$ = 0.55, Rotuman-Melanesian F$_{ST}$ = 0.06). As among the Fijian populations, the Viti Levuans were most different from the Rotumans and least different from the Kadavuans, and had an average difference within Fiji of F$_{ST}$ = 0.07. Additionally, the Viti Levuans had the largest sample size and the highest number of different haplotypes, but they did not have either the highest gene diversity or the highest mean number of pairwise differences. The Vanua Levuans were most different from the Rotumans and least different from the Kadavuans, with an average difference within Fiji of F$_{ST}$ = 0.09. The Kadavuans were genetically identical to the Viti Levuans, the Lau Islanders and the Rotumans, and only slightly different from the Vanua Levuans, which resulted in an average difference within Fiji of F$_{ST}$ = 0.00, which placed it at the Fijian centroid. The Lau Islanders were most different from the Vanua Levuans and least different from the Kadavuans, and had an average difference within Fiji of F$_{ST}$ = 0.04. The Rotumans were the most different from the Viti Levuans and least different from the Kadavuans, and had an average difference within Fiji of F$_{ST}$ = 0.09. Additionally, the Rotumans had the smallest sample size, the lowest number of different haplotypes, the lowest gene diversity, and the lowest mean number of pairwise differences.

In Figure 2, Y-STR genetic distance data were plotted for the five Fijian island populations; the Polynesian island populations of Samoa, Tonga and Tahiti; and the Melanesian island group population of Vanuatu, 10 non-Polynesian Outlier island populations in the Solomon Islands, and four Polynesian Outlier island populations in the Solomon Islands. Four of the five Fijian populations grouped relatively intermediate between the Polynesian and Melanesian clusters, with the Kadavuans and Rotumans being closer to the
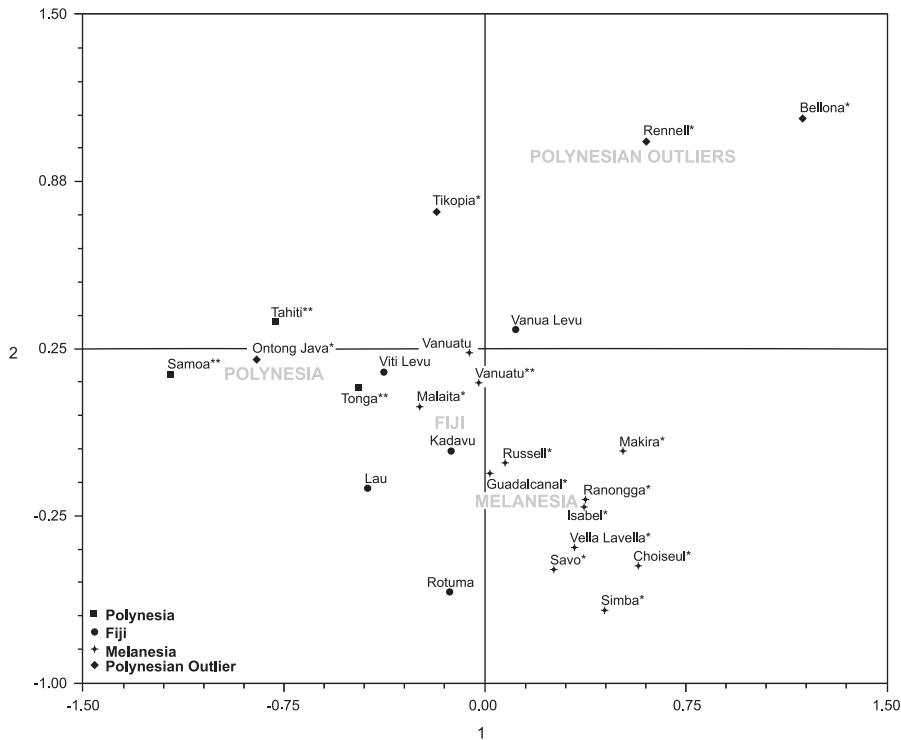
Figure 2 Two-dimensional monotonic multi-dimensional scaling plot of Slatkin's linearized F_{ST} genetic distance values based on nine Y chromosome short tandem repeats (Y-STRs): 19, 385a, 385b, 389I, 389II, 390, 391, 392, and 393; *denotes Y-STR data from the literature;[19] **denotes Y-STR data provided by AJR; final stress = 0.14.

Table 2 Mitochondrial DNA haplogroup frequencies for certain Fijian island populations and for Fiji as a whole (in italics)

Population	N	Asian haplogroups			Melanesian haplogroups										
		B4b1	B4a1a1	B4a1a1a	P1	P1e	Q1	Q1a2	Q2	M27a	M27b	M28	M28a	M28b
Fiji	*107*	*0.009*	*0.093*	*0.710*		*0.019*	*0.028*	*0.009*	*0.056*			*0.009*	*0.065*	
Viti Levu	22		0.136	0.636				0.045	0.136				0.045	
Vanua Levu	21			0.762		0.095	0.095		0.048					
Kadavu	21		0.095	0.619									0.286	
Lau	22		0.136	0.682			0.045		0.091			0.045		
Rotuma	21	0.048	0.095	0.857										

Melanesian populations. However, the four central Fijian populations were neither as clearly intermediate between the Polynesian and Melanesian clusters nor as tightly clustered themselves for the Y-STRs as they were for the mtDNA HVS1 region. The Rotumans did not associate with the Polynesian Outlier populations.

In Table 2, we found that, as a group, Fijian mtDNA haplogroups were 81.2% Asian and 18.7% Melanesian. In comparison, one prior study found them to be 79.6% and 20.5%,[7] and another prior study found them to be 66.7 and 22.1%.[19] The B4a1a1a mtDNA haplogroup, which has a very high frequency in Polynesia,[25] had the highest frequency in all of the Fijian populations. The Viti Levuans and Vanua Levuans had intermediate frequencies of Asian and Melanesian mtDNA haplogroups. The Kadavuans had the lowest frequency of Asian haplogroups, and had a very high frequency of the Melanesian M28a mtDNA haplogroup. The Lau Islanders had the second highest frequency of Asian mtDNA haplogroups (behind the Rotumans), which may be explained by the Lau Islanders' relatively close geographical proximity to Polynesia. The proportions of Asian

mtDNA haplogroups for the four central island populations were lower than that for the Polynesians in general, which supports the possibility of an earlier presence[6] and/or later influx[16] of Melanesians. However, the Rotumans had exclusively (100%) Asian mtDNA haplogroups, which suggests that they had a substantially different experience.

In Table 3, mtDNA data for the five individual Fijian island populations were compared with the Polynesians as a group, to the Melanesians as a group, and to each other. Genetic distance values indicated that the Vanua Levuans were the most Melanesian-like, and the Rotumans were the most Polynesian-like, though all five island populations were more Polynesian- than Melanesian-like. In contrast to the Y-STR data, the Rotumans were by far the least Melanesian-like and had the largest difference (Rotuman–Polynesian $F_{ST} = 0.00$, Rotuman–Melanesian $F_{ST} = 0.45$). Among the Fijian populations, the Viti Levuans were most different from the Rotumans and least different from the Vanua Levuans and Lau Islanders, and had an average difference within Fiji of $F_{ST} = 0.04$. The Vanua Levuans were

Table 3 MtDNA HVS1 genetic diversity and Slatkin's linearized F_{ST} genetic distance values for certain Fijian islands populations, as well as F_{ST} values for the centroids of Polynesia (in italics and represented by Samoa and Tonga) and Melanesia (in italics and represented by Vanuatu and 14 non-Polynesian Outlier islands within the Solomon Islands)

				MtDNA						
				Slatkin's linearized F_{ST} genetic distance values						
Population	N	Number of haplogroups	Gene diversity	Mean no. of pairwise differences	Viti Levu	Vanua Levu	Kadavu	Lau	Rotuma	Polynesia	Melanesia
Viti Levu	22	10	0.76±0.10	5.91±2.93							
Vanua Levu	21	11	0.74±0.11	6.31±3.12	0.00						
Kadavu	21	9	0.85±0.06	6.64±3.26	0.01	0.03					
Lau Islands	22	10	0.75±0.10	5.12±2.58	0.00	0.00	0.02				
Rotuma	21	3	0.35±0.13	0.73±0.57	0.13	0.10	0.24	0.08			
Polynesia	*24*	—	—	*1.78±1.07*	*0.08*	*0.07*	*0.19*	*0.04*	*0.00*		
Melanesia	*519*	—	—	*5.26±2.55*	*0.24*	*0.23*	*0.25*	*0.25*	*0.45*	*0.43*	

Abbreviations: mtDNA, mitochondrial DNA; Y-STR, Y chromosome short tandem repeat.
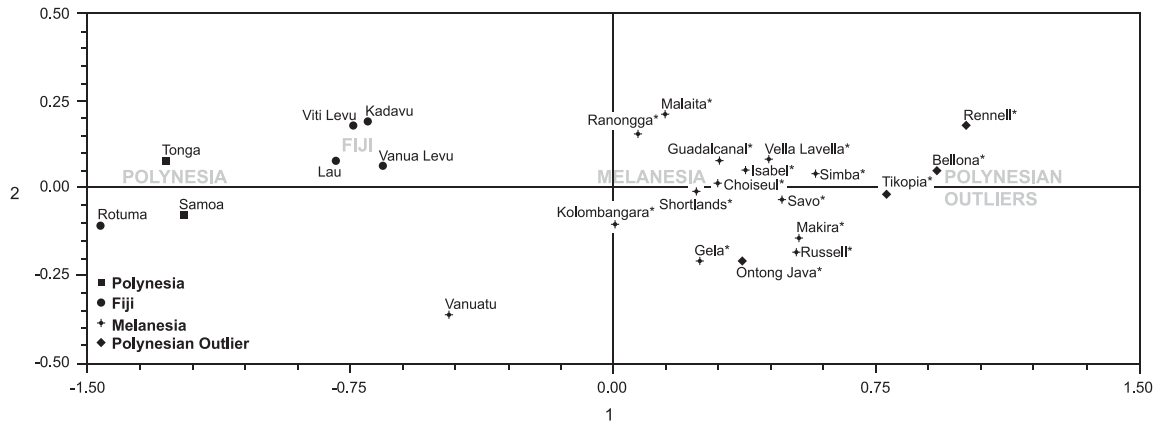
Figure 3 Two-dimensional monotonic multi-dimensional scaling plot of Slatkin's linearized F_{ST} genetic distances based on the mtDNA HVS1 region; *denotes mtDNA data from the literature;[19] final stress=0.11.

Table 4 Average Slatkin's linearized F_{ST} genetic distance values among Fiji island populations and F_{ST} associations outside of Fiji, and mtDNA dominant haplogroup frequencies for certain Fijian island populations

	YDNA		*MtDNA*		
Population	Average F_{ST} distance to other Fijian populations	Regional affinity based on F_{ST} distance	Average F_{ST} distance to other Fijian populations	Regional affinity based on F_{ST} distance	Asian haplogroup frequency
Viti Levu	0.07	Slightly more Polynesian	0.04	Polynesian	Asian 77.2%
Vanua Levu	0.09	Melanesian	0.03	Polynesian	Asian 76.2%
Kadavu	0.00	Much more Melanesian	0.08	Slightly more Polynesian	Asian 71.4%
Lau	0.04	Slightly more Melanesian	0.03	Polynesian	Asian 81.4%
Rotuma	0.09	Melanesian; much less Polynesian	0.14	Much more Polynesian; much less Melanesian	Asian 100.0%

most different from the Rotumans and least different from the Viti Levuans and Lau Islanders, with an average difference within Fiji of $F_{ST}=0.03$. The Kadavuans were most different from the Rotumans and least different from the Viti Levuans, with an average difference within Fiji of $F_{ST}=0.08$. Additionally, the Kadavuans had the highest gene diversity and the highest mean number of pairwise differences. The Lau Islanders were most different from the Rotumans and least

different from the Viti Levuans and Vanua Levuans, and had an average difference within Fiji of $F_{ST}=0.03$. The Rotumans were most different from the Kadavuans and least different from the Lau Islanders, and had an average difference within Fiji of $F_{ST}=0.14$. As with the Y-STRs, but to a much greater extreme, the Rotumans had the lowest number of different haplotypes, the lowest gene diversity and the lowest mean number of pairwise differences.

In Figure 3, mtDNA HVS1 genetic distance data were plotted for the five Fijian island populations; the Polynesian island populations of Samoa and Tonga; and the Melanesian island group population of Vanuatu, fourteen non-Polynesian Outlier island population in the Solomon Islands, and four Polynesian Outlier island populations in the Solomon Islands. Four of the five Fijian populations grouped clearly intermediate between the Polynesian and Melanesian clusters, with the Rotumans grouping much more closely with the Polynesians. In particular, the four central Fijian populations were much more clearly intermediate between the Polynesian and Melanesian populations and much more tightly clustered for the mtDNA HVS1 region than they were for the Y-STRs. As with the Y-STRs, the Rotumans did not associate with the Polynesian Outlier populations.

In Figures 1 and 2, both data sets for the Vanuatuans clustered with the Fijian populations intermediate between the Melanesia and Polynesia clusters, and though our mtDNA data for the Vanuatuans did not cluster with the Fijian populations, it was still intermediate between the Melanesian and Polynesian clusters and was slightly closer to the Fijian centroid than the Melanesian centroid. Similarly, the Y-STR data for the Malaitans and the Y-STR data for the Tongans clustered with the Fijian populations.

Table 4 summarizes our findings with regard to genetic structure among the five Fijian island populations.

DISCUSSION

We found that the Fijian island populations are not genetically homogenous. There is a statistically significant genetic structure among these island populations for the Y-STRs, both with and without the Rotumans ($P = 0.01$ and 0.04, respectively), but not for the mtDNA HVS1 region ($P = 0.07$ and 0.45, respectively). Each of the Fijian populations exhibit the sex-biased admixture commonly associated with areas settled by Austronesian-speaking people, with the paternal lineages being genetically more similar to Melanesian populations and the maternal lineages being genetically more similar to Polynesian populations. The Kadavuans exhibited an unusually high frequency of the Melanesian M28a mtDNA haplogroup, the highest levels of genetic diversity, the highest Y-STR similarity to Melanesian populations and the lowest mtDNA HVS1 region similarity to Polynesian populations, which may indicate that Kadavu was an entry point of a Melanesian migration into Fiji. The Rotumans in the north and the Lau Islanders in the east are genetically more similar to the Polynesian populations than are the other Fijian island populations, but only for the mtDNA HVS1 region. For the Y-STRs, the Rotumans and the Lau Islanders were approximately as similar to the Melanesian populations as were the other three populations, though Rotuma was by far the least similar to the Polynesian populations. Of the five Fijian populations, the Rotumans were the most different in almost every regard. Additionally, the fact that the Rotumans did not associate with the four Polynesian Outlier populations, either for the Y-STRs or for the mtDNA HVS1 region, suggests that Rotuma was neither settled by the same source population nor were the Rotumans involved in settling those particular Polynesian Outliers. Finally, there are potentially interesting relationships between the Fijians and the Vanuatuans, Malaitans and Tongans that may be worthy of further investigation.

Our study has several limitations. First, our sample sizes were relatively small. For the Y-STR analysis in particular, $N < 20$ for four of the five island populations, and $N = 10$ for the Kadavuans and the Rotumans. Importantly, however, the Rotuman participants and their mothers, mothers' mothers, fathers and fathers' fathers represented at least six of the seven Rotuman districts (only the northern Malhaha district may be unrepresented), so the sample, though small, was

diverse. Second, our samples were collected from individuals on the campus of the University of the South Pacific in Suva, and these individuals may not be fully genetically representative of their home island populations. Third, our examination was limited to certain STRs on the Y chromosome and the HVS1 region of the mtDNA genome, so an examination of other areas of the Y chromosome or mtDNA genome or of autosomal DNA might yield different results. In particular, we note that position 16 247 in the HVS1 region, which we used to distinguish between the B4a1a1 and B4a1a1a haplogroups, has been found to back-mutate,[26,27] so some small portion of the participants identified as belonging to B4a1a1 may actually belong to B4a1a1a, and sequencing and analyzing other portions of the mtDNA genome would clarify these haplogroup assignments.

The results of our study contribute to understanding the genetic structure among the Fijian island populations. Although prior studies treated the Fijians as genetically homogenous, we found the important genetic differences among the various island populations. Data collection that does not take these differences into account could yield unreliable results. For both paternal and maternal lineages, four of the five island populations were generally intermediate between the Melanesians and Polynesians, but the people of the isolated northern island of Rotuma had paternal haplotypes more strongly associated with Melanesian populations, maternal haplotypes much more strongly associated with Polynesian populations, and exclusively Asian maternal haplogroups. Thus, our findings support the need for a closer examination of individual island populations within Fiji, particularly with regard to the Rotumans, in order to better understand the process of the peopling of Fiji and of the surrounding regions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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