Genetic Evidence for Modifying Oceanic Boundaries Relative to Fiji

Gerhard P. Shipley,[1] Diana A. Taylor,[1] Antoine D. R. N'Yeurt,[2] Anand Tyagi,[3] Geetanjali Tiwari,[4] and Alan J. Redd[1]

ABSTRACT

We present the most comprehensive genetic characterization to date of five Fijian island populations: Viti Levu, Vanua Levu, Kadavu, the Lau Islands, and Rotuma, including nonrecombinant Y (NRY) chromosome and mitochondrial DNA (mtDNA) haplotypes and haplogroups. As a whole, Fijians are genetically intermediate between Melanesians and Polynesians, but the individual Fijian island populations exhibit significant genetic structure reflecting different settlement experiences in which the Rotumans and the Lau Islanders were more influenced by Polynesians, and the other Fijian island populations were more influenced by Melanesians. In particular, Rotuman and Lau Islander NRY chromosomal and mtDNA haplogroup frequencies and Rotuman mtDNA hypervariable segment 1 region haplotypes more closely resemble those of Polynesians, while genetic markers of the other populations more closely resemble those of the Near Oceanic Melanesians. Our findings provide genetic evidence supportive of modifying regional boundaries relative to Fiji, as has been suggested by others based on a variety of nongenetic evidence. Specifically, for the traditional Melanesia/Polynesia/Micronesia scheme, our findings support moving the Melanesia-Polynesia boundary to include Rotuma and the Lau Islands in Polynesia. For the newer Near/Remote Oceania scheme, our findings support keeping Rotuma and the Lau Islands in Remote Oceania and locating the other Fijian island populations in an intermediate or "Central Oceania" region to better reflect the great diversity of Oceania.

In prior work (Shipley et al. 2015), we examined genetic markers in five Fijian island populations (Viti Levu, Vanua Levu, Kadavu, Rotuma, and the Lau Islands) and found that Fiji is not genetically homogeneous but, rather, exhibits significant genetic structure among these populations. In particular, we found significant genetic structure for nonrecombinant Y (NRY) chromosomal short tandem repeat (STR) haplotypes, both with and without the Rotumans, and that Rotuman mitochondrial DNA (mtDNA) haplogroup frequencies and hypervariable segment 1 (HVS1) region haplotypes are much more similar to those of Polynesian populations than to those of the other Fijian populations. However, that study was limited by the number and types of genetic markers and the relatively small Rotuman sample size. In the present study, we examined NRY chromosomal single nucleotide polymorphisms (SNPs) to determine Y chromosomal haplogroup

[1] Department of Anthropology, University of Kansas, Lawrence, Kansas, USA.
[2] Pacific Centre for Environment and Sustainable Development (PaCE-SD), University of the South Pacific, Suva, Fiji.
[3] Faculty of Science (CEST), Fiji National University, Lautoka, Fiji.
[4] Department of Religious Studies, University of Kansas, Lawrence, Kansas, USA.

*Correspondence to: Gerhard P. Shipley, Department of Anthropology, University of Kansas, 622 Fraser Hall, 1415 Jayhawk Blvd., Lawrence, Kansas 66045-7556 USA. E-mail: Fiji_Genetics@outlook.com.

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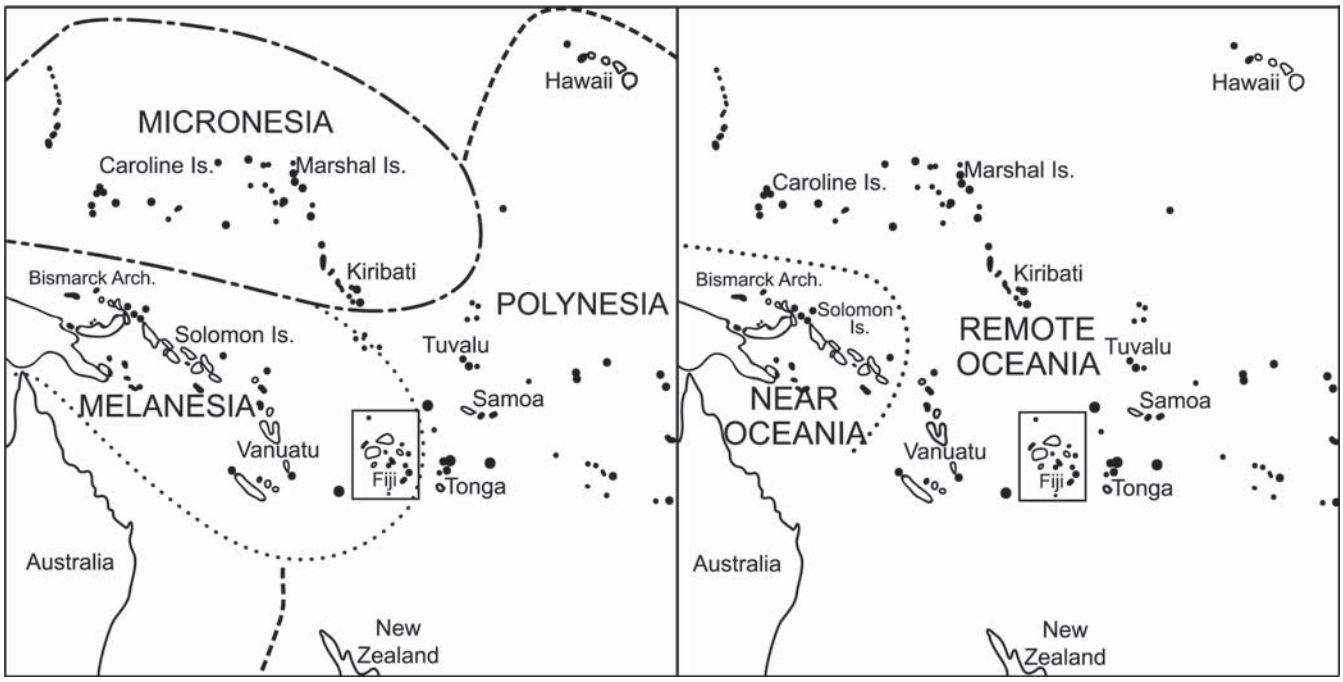

FIGURE 1. Current Melanesia-Polynesia-Micronesia scheme (left) and current Near-Remote Oceania scheme (right), with Fiji boxed.

frequencies, substantially increased the number of Rotuman samples, and applied our findings to the ongoing debate over Oceanic boundaries relative to Fiji, both with regard to the traditional Melanesia/Polynesia/Micronesia (MPM) scheme and the newer Near/Remote Oceania (NRO) scheme.

The origins of and arguments for and against the MPM and NRO schemes are complex, and a full treatment is beyond the scope of this article, but a short introduction is necessary to better understand the significance of our findings and conclusions. The MPM scheme (Figure 1, left) resulted from Jules Dumont d'Urville's (1832) initial division of Oceania into three regions (actually four, including Malaysia; Tcherkezoff 2003). "As geographic referents, the terms Melanesia, Polynesia, and Micronesia have generally neutral connotations" (Clark 2003: 157) but, like many such concepts of the period, also carry racial implications. Dumont d'Urville located the lighter-skinned Polynesians (and Malaysians) higher, the Micronesians intermediate, and the darker-skinned Melanesians lower on a socio-evolutionary scale (Clark 2003, citing Dumont d'Urville 1832; Tcherkezoff 2003). Fijians were ranked highest among Melanesian populations because they had been "improved" by contact with Polynesians (Clark 2003, citing Dumont d'Urville 1832). While recognizing the need to divide Oceania into manageable and meaningful regions, some, such as Thomas et al. (1989), have expressed dislike for the MPM scheme because of its tainted beginnings and have argued for a different scheme that is supported by scientific evidence. Others, such as Sahlins (1989), have argued that assertions that the continued use of the terms *Melanesia* and *Polynesia* perpetuates racism and bigotry can only be sustained if these distinctions have no value whatsoever and are instead nothing more than ideological survivals (Thomas et al. 1989). This second group has noted that work done in all areas of anthropology is "sufficient to explain the continuing anthropological disposition to distinguish Polynesia and Melanesia—*despite rather than because* of the original basis of the contrast, long ago disavowed"—that is, the MPM scheme *is* supported by scientific evidence (Thomas et al. 1989: 37). A small third group, which includes Guiart (1982) and Spriggs (1984), has argued against what they see as a racial bias against Melanesia and in favor of Polynesia and ultimately asserted that there is no legitimate basis for distinguishing between Melanesians and Polynesians or for deriving Polynesians from any place but Melanesia. However, that assertion contradicts strong scientific evidence. In particular, Guiart (1982: 143) seems primarily concerned with pan-Pacific nationalism and the "unity of

Oceanic peoples," and Spriggs (1984: 222) seems primarily concerned with the political utility of "a Melanesian origin for the Polynesians."

The NRO scheme (Figure 1, right) was introduced by Pawley and Green (1973) and subsequently refined by Green (e.g., Green 1991) as an alternative to the MPM scheme. Into Near Oceania they placed New Guinea, the Bismarck Archipelago, and the Solomon Islands east to San Cristobal, almost all of which were settled by Papuan-speaking peoples no later than ~40,000 years ago (Kirch 2000). Into Remote Oceania they placed all of the islands east and north of the Santa Cruz Islands, almost all of which were settled by Austronesian-speaking peoples beginning ~3,200 years ago (Kirch 2000). Pawley and Green (1973) based their bipartite division on such factors as settlement date, material culture, language, island density, and floral and faunal diversity differences but, importantly, not on genetic or other biological evidence. Perhaps realizing that reducing the already overly inclusive three regions to two regions did an even greater disservice to the great diversity of Oceania, Pawley and Green (1973) further defined an Eastern Pacific division within Remote Oceania, including all of the islands east of Samoa and Niue plus New Zealand and the Chatham Islands, based on material culture differences. Similarly, Finney (1994), though favoring the NRO scheme but perhaps also sensing the unwieldiness of Remote Oceania as a single category, divided Remote Oceania into West Polynesia, in which he included Fiji, Tonga, and Samoa, and East Polynesia, in which he included the remainder of the original Remote Oceania region. The NRO scheme more accurately reflects the clearly distinct initial settlement dates of the two regions, with parts of Near Oceania having been settled as much as 55,000 years earlier than the remotest islands of Remote Oceania. However, this treats settlement as a single event rather than a process, as though the moment the first Lapitan set foot on previously uninhabited eastern Melanesian lands the act of settling those lands was complete. Just as the process of settling the Americas likely involved multiple waves of settlers over an extended period of time (e.g., Reich et al. 2012), the process of settling eastern Melanesia (or western Remote Oceania), especially a boundary area like Fiji, also spanned a period of time during which admixing occurred with one or more waves of eastwardly migrating Melanesians and westwardly migrating Polynesians.

Although many have argued either explicitly against the long-standing MPM scheme (e.g., Thomas et al. 1989; Finney 1996), ambiguously against it (e.g., Kirch [2010], who acknowledged the value of "Polynesia" and allowed that "Micronesia" was an exception within Remote Oceania but rejected the usefulness of "Melanesia"), or for it (e.g., Sahlins 1989; Stephenson 1989), many continue to use it rather than or alongside the NRO scheme. "There is still little evidence that Dumont d'Urville's tripartite division of the Pacific is in any danger of being replaced" (Clark 2003: 157). Perhaps this is because the tripartite scheme has evolved beyond its original basis and is now supported by meaningful anthropological evidence, or perhaps it is because the bipartite scheme is less reflective of the great diversity of Oceania, especially its genetic diversity. However, both schemes can be improved through boundary adjustments as new information comes to light. In particular, as Sahlins (1989) noted, the most disputed boundary between Melanesian Fiji and Polynesian Tonga is likely outmoded and in need of closer examination (Thomas et al. 1989).

Fiji is treated very differently by the two schemes. Under the MPM scheme, Fiji is located at the boundary of Melanesia and Polynesia, which accurately reflects its liminal nature and transitionary characteristics as the "Gateway to Polynesia." Fijians have traditionally been classified as Melanesian based on their cultural practices and some morphological features (Spriggs 1997), but they share much in common linguistically (Geraghty 1983), phenotypically (Howells and Moss 1933), and genetically (Kayser et al. 2006; Shipley et al. 2015) with Polynesian populations. Tellingly, as many as 35% of Samoan legends connect it with Fiji, including Samoa's creation story, which tells of the simultaneous creation of Samoa, Tonga, and Fiji (Barnes and Hunt 2005). Thus, although originally settled by the Lapitans ~3,100 years ago (Rutherford et al. 2012), modern Fijians are a complex blend of Melanesian and Polynesian characteristics, due at least in part to forward and backward migrations into Fiji during the settlement process (e.g., Kirch 2000; Clark 2003; Barnes and Hunt 2005; Addison and Matisoo-Smith 2010; Wollstein et al. 2010; Sheppard 2011; Duggan and Stoneking 2014; Shipley

et al. 2015). For example, based on an analysis of ~1 million SNPs, Wollstein et al. (2010) found that Fijians were of 65% Polynesian and 35% Near Oceanic ancestry and have approximately twice as much Near Oceanic ancestry than do Polynesians, "thereby suggesting substantial contact between Fiji and Near Oceania that did not extend to Polynesia."

The majority of Fijians reside on the larger western islands of Viti Levu, Vanua Levu, and Kadavu and are culturally, phenotypically, and genetically more influenced by Melanesia (Spriggs 1997), while a significant minority of Fijians reside on the northern island of Rotuma and the eastern Lau Islands and are culturally (Kirch 2000), phenotypically (Howells and Moss 1933), and linguistically (Geraghty 1986) more influenced by Polynesia. In that light, some have suggested dividing Fiji between Melanesia and Polynesia. For example, in his Outline Map of the South Pacific, Linton (1926) depicted the Melanesia-Polynesia boundary bisecting Fiji but gave no indication as to which Fijian islands belonged in which region. In his isolation plot of Polynesian islands, Irwin (1990) placed Fiji in a voyaging sphere with western Polynesia (i.e., Samoa and Tonga) but showed Rotuma as being distinct from the rest of Fiji. Burley (2013) identified archaeological support for moving the boundary between Melanesia and Polynesia to within the Fijian group, with Rotuma and the Lau Islands being grouped with Polynesia. Others consider all of Fiji to be within Polynesia (Kayser et al. 2006; Mirabal et al. 2012), while still others have characterized western Polynesia as consisting of Tonga, Samoa, and Tuvalu and have left Fiji in Melanesia (Whyte et al. 2005). As Kirch (2000: 156) noted, "Anthropologists have never quite known how to deal with Fiji. It is a sort of 'between' archipelago, situated geographically closer to Western Polynesia . . . yet usually classified as a 'Melanesian' culture. . . . Fiji thus shares an identical foundation culture as Western Polynesia . . . [but] continued in later millennia to receive both genetic and cultural influences from the west (i.e., from 'Melanesia')." Despite this, Fiji has no special significance whatsoever under the NRO scheme, being located approximately 1,250 km east of the boundary between Near and Remote Oceania. Even under Pawley and Green's (1973) and Finney's (1994) attempts to further subdivide Remote Oceania, Fiji's nonliminal location within the scheme is incommensurate with its liminal reality.

Among Fijian island populations, Rotuma probably received its first settlers ~3,000 years ago along with the region generally, and the earliest physical evidence for human occupation was found at Itu'muta and carbon-dated to ~2,000 years ago (Howard and Rensel 2007). After perhaps several hundred years of insignificant contact with other peoples, a backward flow of Polynesians, particularly Samoans and Tongans, from east to west reached Rotuma (Howard and Rensel 2007). Rotumans' own oral history supports the influence of Samoa, Tonga, and other-than-Fijian influence on their language and culture, as Rotuma was visited by voyagers from Niuafo'ou, Tonga, Futuna, Tuvalu, Tarawa, and Polynesian outliers and, in turn, early Rotuman voyagers traveled as far as Tikopia and Anuta to the west and Bora Bora to the east (Howard and Rensel 2007).

HMS Pandora made the first recorded European sighting of Rotuma in 1791, while searching for the mutineers of *HMS Bounty*. Like many Pacific islands, Rotuma received a number of European and non-European castaways and ship-jumpers, suffered tragic depopulation due to the introduction of foreign diseases such as measles, and experienced sex-biased out-migration as young men left on European vessels (Howard and Rensel 2007). As a result, there are several potential influences on Rotuman genetics, including (a) an initial founder effect, (b) genetic drift due to small population size, (c) precontact gene flow with other Oceanic peoples, (d) postcontact gene flow with Europeans and other non-Oceanic peoples, and (e) one or more potential bottleneck effects due to, for example, disease or sex-biased migration. Phenotypically, most modern Rotumans are Polynesian in appearance, with light skin, black wavy hair, and Polynesian facial features (Howard and Rensel 2007). Linguistically, Rotuman shares a substantial portion of its vocabulary with Samoan and Tongan (Howard and Rensel 2007). Genetically, Rotumans exhibit the sex-biased admixture, which is so distinctive of Polynesia (Shipley et al. 2015).

Similarly, the Lau Islands are located closest to Polynesia and served as a bridge between greater Fiji and Tonga (Kirch 2000). In fact, Tongans

established colonies in Lau, and Tongan canoe builders worked in Lau to access the larger trees, resulting in "strongly Polynesianized Lauan" society (Thompson 1938: 193). The largest island, Lakeba, contains the largest Lapita site found in Fiji and western Polynesia (Best 1984), and historically served as a "Crossroads of the Sea." Obsidian flakes found on Lakeba and dated to 2,500 years ago have been identified as products of Tonga (Best 1984; confirmed by Reepmeyer and Clark 2010). Linguistically, the Lauan language has been heavily influenced by Tongan and contains a large number of Polynesian loan words (Geraghty 1983).

Materials and Methods

The majority of buccal cell samples were obtained in 2008 from individuals at the University of the South Pacific's main campus in the capital city of Suva, on Viti Levu. Additional Rotuman buccal cell samples were obtained in 2014 from Suva and the island of Rotuma. DNA was extracted from these samples using the phenol-chloroform method (Sambrook and Russell 2001). Participants whose samples were used for Y chromosome analysis were able to identify their own and at least their father's island of birth, and those whose samples were used for mtDNA analysis were able to identify their own and at least their mother's island of birth. All participants gave informed consent, and all samples were obtained and handled in accordance with the human subject research requirements of the University of Kansas and the University of the South Pacific in Fiji.

With regard to the Y chromosome, in addition to the 102 male samples previously reported (Shipley et al. 2015), 16 new male Rotuman samples were similarly processed using an AmpFlSTR Yfiler PCR Amplification Kit (Applied Biosystems) to determine the alleles for 17 NRY-STR loci (DYS19, DYS385a/b, DYS389I, DYS389II-I, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, and YGATAH4). Fragment analyses of the new samples were performed by the University of Arizona Genetics Core (UAGC), and fragment lengths were determined using Peak Scanner software (Applied Biosystems). Additional NRY-STR data were taken from the literature (Delfin et al. 2012), and additional NRY-STR data for the Polynesian islands of Samoa, Tonga, and Tahiti were provided by one of us (A.J.R.). For the Y chromosomal analysis, recognizing the genetically intermediate nature of Eastern Melanesia (or Central Oceania), no Remote Oceanic populations (i.e., the Santa Cruz Islanders and the Vanuatuans) or Polynesian Outlier populations were included in our genetic characterization of Melanesia. The data for Polynesia were limited to alleles for nine loci (DYS19, DYS385a/b, DYS389I, DYS389II-I, DYS390, DYS391, DYS392, DYS393), so the data for all populations were correspondingly reduced to facilitate analysis. An analysis of the molecular variance (AMOVA) for the five Fijian populations was performed, and Slatkin's linearized F_{ST} genetic diversity distances (Slatkin 1995) between the various Melanesian, Fijian, and Polynesian populations were determined using Arlequin (version 3.11; Excoffier and Schneider 2005). Separate analyses were performed in which Polynesian and Melanesian island populations were treated as distinct island populations and in which they were collapsed into two respective regional populations.

Additionally, NRY-SNP haplogroups were determined for 100 of the male Fijian samples using the primers and PCR profiles shown in Table 1. Sequencing was performed by the UAGC, and haplogroup-defining SNP positions were examined in Sequencher (version 4.8; Gene Codes Corp.). NRY-SNP haplogroups were characterized as Asian, Asian-descended, Polynesian (i.e., C2a1-P33, which is Melanesian-descended but arose in Polynesia and is characterized as strongly Remote Oceanic by, e.g., Cox et al. 2007; Delfin et al. 2012), or Melanesian based on characterizations in the literature (Kayser et al. 2006; Delfin et al. 2012). To facilitate comparison, the NRY-SNP haplogroup frequencies for Melanesia and Polynesia (from Delfin et al. 2012) shown in Table 2 were limited to the same individual haplogroups and haplogroup families determined for the five Fijian island populations as shown in Table 3.

With regard to the mtDNA, in addition to the 107 male and female samples previously reported (Shipley et al. 2015), 19 new male Rotuman samples were similarly processed using light-chain primer L-15996 (5′-ACTCCACCATTAGCACCCAAAGC-3′) and heavy-chain primer H-16401 (5′-CACCATCCTCCGTGAAATCA-′) to determine the sequence for a

Table 1. Primers and PCR Profiles for NRY-SNPs

SNP ID	Haplogroup	Primers[a]	SNP	Profile[b]
RPS4Y	C family	F 5′-CTGTACTTACTTTTATCTCCTC-3′ R 5′-CAGCAACAGTAAGTCGAATG-3′	C→T	Standard, X = 54°C
M38	C2 family	F 5′-CAGTTTTTAGAGAATAATGTCCT-3′ R 5′-TTAAAGAAAAGAAAAGCAGATG-3′	T→ G	Standard, X = 60°C
M208	C2a family	F 5′-ATAAATACAAAATCACCTGATGGAT-3′ R 5′-TTAAACAGCGAAATTACTAACAAAA-3′	C→T	Standard, X = 60°C
P33	C2a1	F 5′-GTGCAAGATAATGACTCTTAT-3′ R 5′-GTGCTAGGTCCAAATATG-3′	TT→TC	P33
M9	K, NO, P, S families	F 5′-GCAGCATATAAAACTTTCAGG-3′ R 5′-GAAATGCATAATGAAGTAAGCG-3′	G→C	Standard, X = 54°C
P79	K3	F 5′-TCTTTGCATAAGTTGTGTCCAAT-3′ R 5′-AAATGAGGCTAATCAATGGAACA-3′	T→C	Standard, X = 57°C
P256	M family	F 5′-TCTTGGTTTTCCCATTGACC-3′ R 5′-CATCTCCCAACTTGTCTGTGC-3′	G→A	Standard, X = 54°C
M4	M1 family	F 5′-TCCTAGGTTATGATTACAGAGCG-3′ R 5′-TAAAACACTTCTGTGGATGGCA-3′	T→C	Standard, X = 60°C
M353	M2 family	F 5′-GAATGGCTCATGGCTGAACT-3′ R 5′-TACTATCAGGGCCCACCAAG-3′	G→A	Standard, X = 60°C
P117	M3	F 5′-CTGATTATTCTTTTCTACCTTG-3′ R 5′-CTTAATCTGATGTGTCACTGA-3′	C→A	Standard, X = 53°C
M175	O family	F 5′-CCCAAATCAACTCAACTCCAG-3′ R 5′-TTCTACTGATACCTTTGTTTCTGTTCA-3′	TTCTC→A	M175
M119	O1a family	F 5′-GAATGCTTATGAATTTCCCAGA-3′ R 5′-TCCACACAATATACAAGATGTATTCTT-3′	A→C	Standard, X = 60°C
M268	O2 family	F 5′-CATGCCTAGCCTCATTCCTC-3′ R 5′-CTGGATGGTCACGATCTCCT-3′	A→G	Standard, X = 56°C
M122	O3 family	F 5′-GTTGCCTTTTGGAAATGAATAAATC-3′ R 5′-CACTTGCTCTGTGTTAGAAAAGATAGC-3′	T→C	Standard, X = 58°C

[a]F, forward; R, reverse.

[b]*Standard profile:* 2.5 ng/ml DNA; 95°C for 11 min; forty cycles of 94°C for 30 sec, X°C for 30 sec, 72°C for 45 sec; then 72°C for 10 min. *P33 profile:* 5 ng/ml DNA; 94°C for 3 min; 40 cycles of 94°C for 45 sec, 62°C 45 sec ramp down to 52°C in 0.5°C increments over first 20 cycles and then hold at 52°C, 72°C for 45 sec; then 72°C for 45 min. *M175 profile:* 25 ng/ml DNA; 95°C for 10 min; 40 cycles of 94°C for 15 sec, 60°C for 45 sec; then 60°C for 5 min.

Table 2. Summary of Genetic Marker Characterizations for Five Fijian Island Populations, Melanesia, and Polynesia

Population	NRY				mtDNA			
	SNP Haplogroup Frequency		STR F_{ST} Distance		Haplogroup Frequency		F_{ST} Distance	
	Asian, Asian-Descended, and Polynesian	Near Oceanic Melanesian	From Polynesian Centroid	From Melanesian Centroid	Asian and Asian Descended	Melanesian	From Polynesian Centroid	From Melanesian Centroid
Melanesia	0.155	0.845	—	—	0.606	0.394	—	—
Viti Levu	0.070	0.930	0.108	0.207	0.773	0.227	0.080	0.257
Vanua Levu	0.250	0.750	0.270	0.177	0.762	0.238	0.069	0.230
Kadavu	0.250	0.750	0.118	0.017	0.714	0.286	0.185	0.261
Lau Islands	0.500	0.500	0.119	0.131	0.818	0.182	0.043	0.261
Rotuma	0.810	0.190	0.261	0.088	0.949	0.051	0.000	0.456
Polynesia	0.770	0.230	—	—	0.964	0.036	—	—

Data for Melanesia and Polynesia are from Delfin et al. (2012).

Table 3. NRY-SNP Haplogroup Frequencies for Five Fijian Island Populations

Haplogroup	Population				
	Viti Levu (*n* = 43)	Vanua Levu (*n* = 12)	Kadavu (*n* = 8)	Lau Islands (*n* = 16)	Rotuma (*n* = 21)
Asian and Asian-descended haplogroups					
O1a family-M119			0.250	0.063	
O3 family-M122	0.047	0.083		0.250	0.762
Melanesian-descended Polynesian haplogroup					
C2a1-P33	0.023	0.167		0.188	0.048
Near Oceanic Melanesian haplogroups					
K, NO, S family, or P family; K-M9*(xP256, P79, M175)	0.163	0.333	0.500	0.125	0.143
K3-P79	0.047			0.063	0.048
M-P256*(xM4, M353, P117)	0.093			0.063	
M1 family-M4	0.302		0.125	0.063	
M2 family-M353	0.070	0.333			
M3-P117				0.063	
C2-M38*(xM208)	0.256	0.083	0.125	0.125	

Characterizations of haplogroups as Asian, Asian-descended, Polynesian, and Near Oceanic Melanesian are based on Kayser et al. (2006) and Delfin et al. (2012).

Table 4. mtDNA Haplogroup Frequencies for Five Fijian Island Populations

Haplogroup	Population				
	Viti Levu (*n* = 22)	Vanua Levu (*n* = 21)	Kadavu (*n* = 21)	Lau Islands (*n* = 22)	Rotuma (*n* = 39)
Frequencies of Asian and Asian-descended haplogroups					
B4b1					0.077
B4a1a1	0.136		0.095	0.136	0.051
B4a1a1a	0.636	0.762	0.619	0.682	0.821
Frequencies of Melanesian haplogroups					
P1e		0.095			
Q1		0.095		0.045	
Q1a2	0.045				
Q2	0.136	0.048		0.091	
M28				0.045	
M28a	0.045		0.286		0.051

Characterizations of haplogroups as Asian, Asian-descended, and Melanesian are based on Friedlaender et al. (2007) and Van Oven and Kayser (2009).

405-bp fragment from the mtDNA HVS1 region. Sequencing of the new samples was performed by the UAGC. The forward and reverse fragments were visualized using Sequencher (version 4.8; Gene Codes Corp.) and aligned to the revised Cambridge Reference Sequence. Substitutions within each sequence were examined to ensure proper sequence calling, and a consensus sequence was constructed by merging the two fragments. mtDNA haplogroups were assigned based on substitutions identified in the literature (Friedlaender et al. 2007; Van Oven and Kayser 2009), and each haplogroup was characterized as either Asian or Melanesian based on the origin of the lineage rather than the location where the particular haplogroup may have arisen. For example, we characterized haplogroups B4a1a1 and B4a1a1a as Asian because the B4 lineage originated in Asia, even though B4a1a1 itself may have arisen in Melanesia among Asian-descended peoples (Mirabal et al. 2012) and B4a1a1a is very strongly associated with Polynesia (Redd et al. 1995). Additional mtDNA HVS1 sequences for

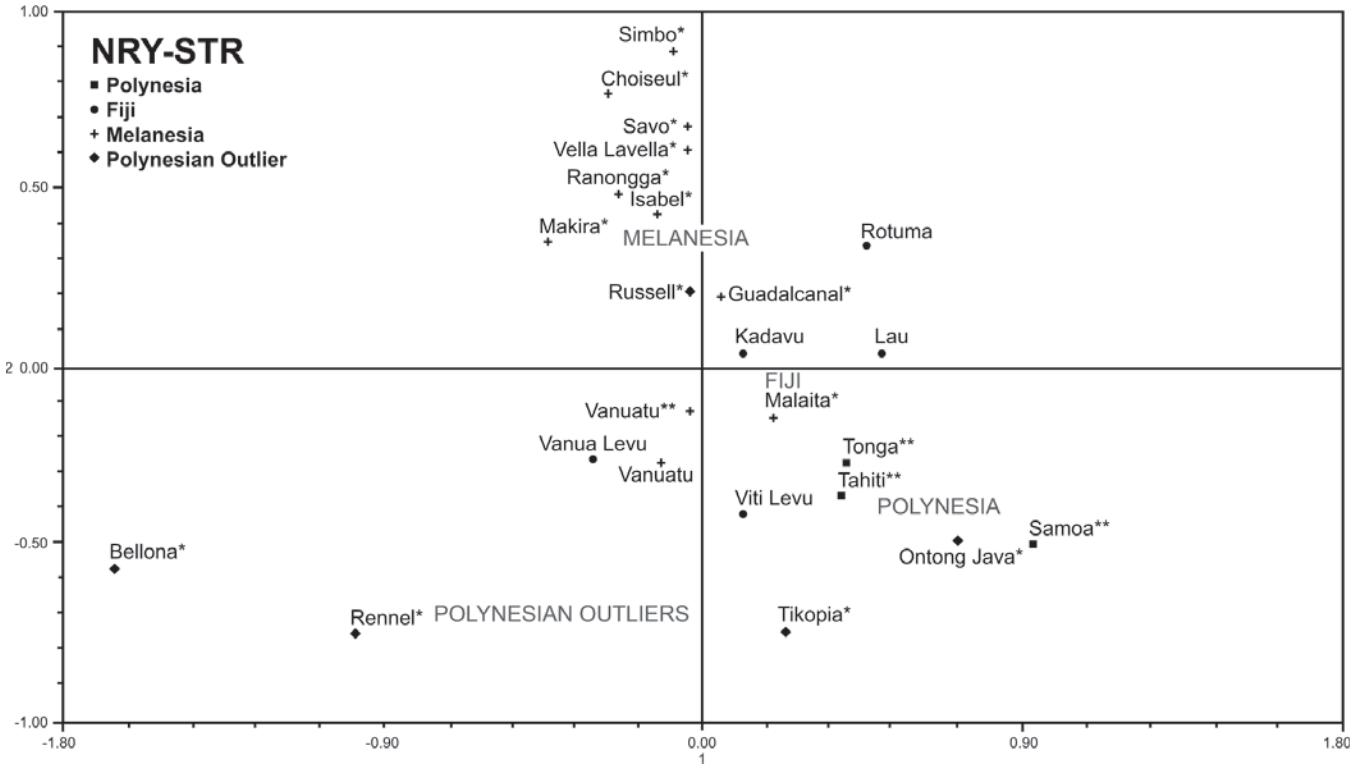

FIGURE 2. Two-dimensional monotonic MDS plot of Slatkin's linearized FST genetic distance values based on nine NRY-STRs: 19, 385a, 385b, 389I, 389II, 390, 391, 392, and 393. Single asterisks (*) denote NRY-STR data from the literature (Delfin et al. 2012); double asterisks (**) denote NRY-STR data provided by A.J.R. Final stress = 0.15; r = 0.90.

various Melanesian islands were obtained from GenBank (accession nos. JN017205–JN017907). These sequences were 340 bp long, so the Fijian sequences were correspondingly trimmed to facilitate analysis. The mtDNA HVS1 sequences were analyzed in the same manner as the NRY-STR data. Again, to facilitate comparison, the mtDNA haplogroup frequencies for Melanesia and Polynesia (from Delfin et al. 2012) shown in Table 2 were limited to the same individual haplogroups and haplogroup families determined for the five Fijian island populations as shown in Table 4.

Results

NRY-STR genetic distance data (Table 2) and the resulting multidimensional scaling (MDS) plot (Figure 2) show four of the five Fijian populations grouped relatively intermediately between the Polynesian and Melanesian clusters. However, the four central Fijian populations were neither as clearly intermediate between the Polynesian and Melanesian clusters nor as tightly clustered themselves for the NRY-STRs as they were for the mtDNA HVS1 region. The additional Rotuman samples included in the present study shifted Rotuma somewhat closer to the Lau Islands and the Fijian centroid compared with prior results (Shipley et al. 2015). Rotuma had the second lowest number of different haplotypes (17), the lowest gene diversity (0.53 ± 0.30), and the lowest mean number of pairwise differences (4.75 ± 2.4). These results are almost identical to those for Samoa: 17, 0.53 ± 0.30, and 4.77 ± 2.43, respectively. AMOVA of the NRY-STR haplotypes of the Fijian populations including Rotuma showed significant genetic structure (p = 0.00) and 5.59% variation among populations, and AMOVA excluding Rotuma still showed significant genetic structure (p = 0.04) and 3.89% variation among populations. The Lau Islands had the lowest number of different haplotypes (16), the second lowest gene diversity (0.70 ± 0.38), and the second lowest mean number of pairwise differences (6.18 ± 3.08).

NRY-SNP haplogroup frequencies (Table 3) show that, among specific Asian haplogroups, the O3 family had the highest frequency (accounting for 51.2% of Asian haplogroups), while among specific Melanesian haplogroups, the Melanesian M1 family had the highest frequency at 27.2%. However, an examination of individual island

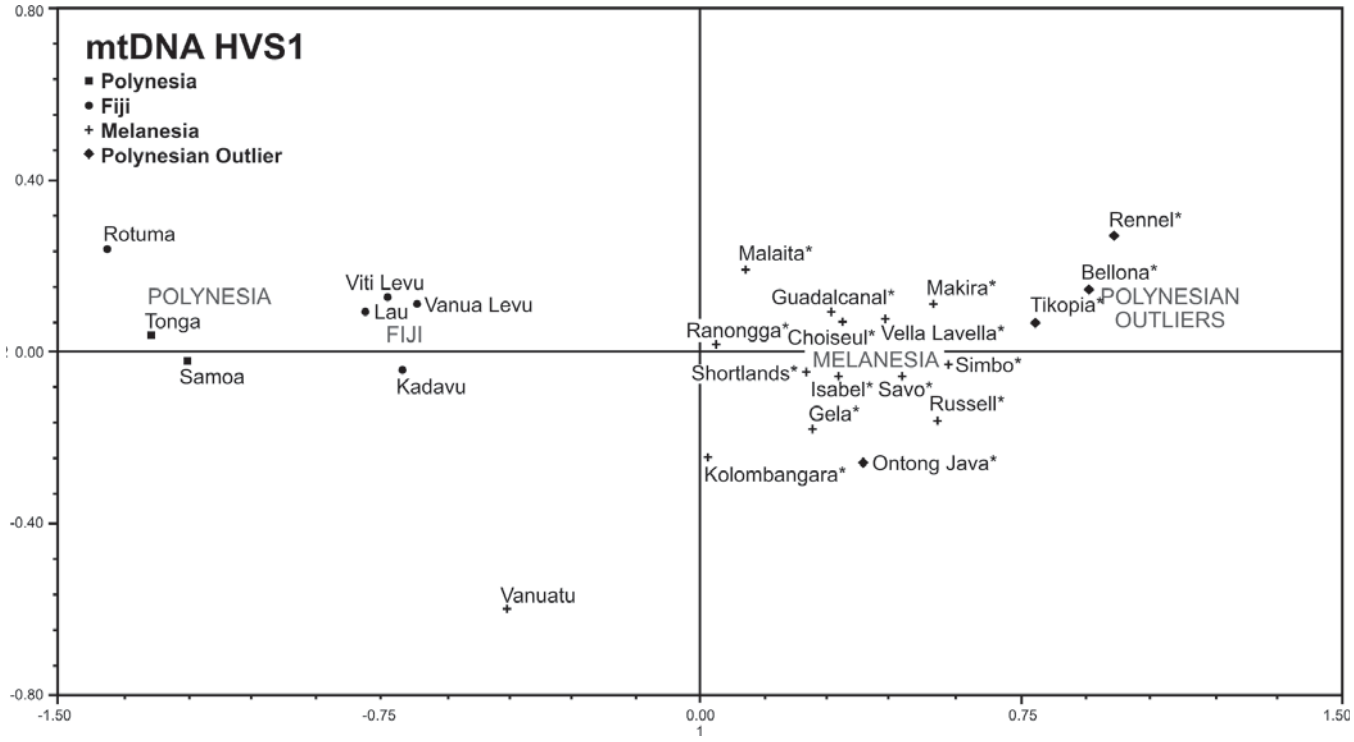

FIGURE 3. Two-dimensional monotonic MDS plot of Slatkin's linearized FST genetic distances based on mtDNA HVS1 sequences. Asterisks denote mtDNA data from the literature (Delfin et al. 2012). Final stress = 0.10; r = 0.89.

populations revealed substantial heterogeneity, with the western islands of Viti Levu (7.0% Asian, 93.0% Melanesian), Vanua Levu (25.0% Asian, 75.0% Melanesian), and Kadavu (25.0% Asian, 75.0% Melanesian) exhibiting relatively higher frequencies of Melanesian NRY-SNP haplogroups and the northern island of Rotuma (81.0% Asian, 19.0% Melanesian) and the eastern Lau Islands (50.0% Asian, 50.0% Melanesian) exhibiting relatively higher frequencies of Asian NRY-SNP haplogroups. Further, Viti Levu exhibited a remarkably high frequency, 30.2%, of Melanesian M1 family NRY-SNP haplogroups, while Rotuma exhibited a remarkably high frequency, 76.2%, of Asian O3 family NRY-SNP haplogroups.

mtDNA HVS1 genetic distance data (Table 2) and the resulting MDS plot (Figure 3) show four of the five Fijian populations grouped clearly intermediately between the Polynesian and Melanesian clusters, with the Rotumans clearly grouping with the Polynesians. In particular, the four core Fijian island 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 NRY-STRs. The additional Rotuman samples did not significantly shift Rotuma relative to the Fijian centroid compared with prior results (Shipley et al. 2015). Specifically, Rotuma grouped strongly with Polynesia (Rotuma-Polynesia F_{ST} = 0.00, Rotuma-Melanesia F_{ST} = 0.46), while the other four Fijian populations formed a close group between Polynesia and Melanesia (Fijian group–Polynesia F_{ST} = 0.10, Fijian group–Melanesia F_{ST} = 0.25). AMOVA of the mtDNA HVS1 haplotypes of the Fijian populations including Rotuma showed significant genetic structure (p = 0.03) and 3.91% variation among populations, but AMOVA excluding Rotuma did not show significant genetic structure (p = 0.45) and –0.29% variation among populations.

mtDNA haplogroup frequencies (Table 4) show that, among specific Asian haplogroups, B4a1a1a had the highest frequency (accounting for 85.6% of all Asian haplogroups), while among specific Melanesian haplogroups, Q2 had the highest frequency (accounting for 51.5% of Melanesian haplogroups). Again, however, an examination of individual island populations revealed substantial heterogeneity, with the western islands of Viti Levu (77.3% Asian, 22.7% Melanesian), Vanua Levu (76.2% Asian, 23.8% Melanesian), and Kadavu (71.4% Asian, 28.6% Melanesian) exhibiting relatively higher frequencies of Melanesian NRY-SNP

haplogroups and the northern island of Rotuma (94.9% Asian, 5.1% Melanesian) and the eastern Lau Islands (81.8% Asian, 18.2% Melanesian) exhibiting relatively higher frequencies of Asian mtDNA haplogroups. Further, Kadavu exhibited a remarkably high frequency, 28.6%, of the Melanesian M28a mtDNA haplogroup, while Rotuma exhibited the highest frequency, 82.1%, of the Asian B4a1a1a mtDNA haplogroup.

Discussion

We present the most comprehensive genetic characterization to date of five Fijian island populations, Viti Levu, Vanua Levu, Kadavu, the Lau Islands, and Rotuma, including NRY and mtDNA haplotypes and haplogroups (summarized in Table 2). Our findings confirm that, as a whole, Fijians are genetically intermediate between Melanesians and Polynesians, which reflects a settlement process involving genetic admixture over time. Our data also show that individual Fijian island populations exhibit significant genetic structure reflecting different settlement experiences in which Rotumans and the Lau Islanders were more heavily genetically influenced by Polynesians while Viti Levuans, Vanua Levuans, and Kadavuans were more heavily genetically influenced by Melanesians. In particular, Rotumans and Lau Islanders have, respectively, 81.0% and 50.0% Asian (or Asian-descended) or Polynesian NRY-SNP haplogroups, which is more similar to Polynesians (77.0%; Delfin et al. 2012), while Viti Levuans, Vanua Levuans, and Kadavuans have, respectively, 7.0%, 25.0%, and 25.0% Asian (or Asian-descended) or Polynesian NRY-SNP haplogroups, which is more similar to Near Oceanic Melanesians (15.5%; Delfin et al. 2012). Further, Rotumans and Lau Islanders have, respectively, 94.9% and 81.8% Asian (or Asian-descended) mtDNA haplogroups, which is again more similar to Polynesians (96.4%; Delfin et al. 2012), while Viti Levuans, Vanua Levuans, and Kadavuans have, respectively, 77.3%, 76.2%, and 71.4% Asian mtDNA haplogroups, which is again more similar to Near Oceanic Melanesians (60.6%; Delfin et al. 2012). Additionally, Rotuman mtDNA haplotypes group much more strongly with Polynesian mtDNA haplotypes than with any other Fijian island population. On the other hand, Rotuman NRY-STR haplotypes group closer to Melanesian than to Polynesian mtDNA haplotypes. However, this anomaly is likely because genetic drift has an inherently stronger effect on the Y chromosome.

Nongenetic evidence of the relative influences of Melanesia and Polynesia on Fiji as a whole and on the various Fijian island populations, and nongenetic evidence for modifying the regional boundary relative to Fiji were discussed in the introductory remarks. Our findings support the notion that Fiji is a special place—a "between place" (Kirch 2000: 156)—between two great regions and support nongenetic evidence and arguments for adjusting the Melanesia-Polynesia boundary line and, in the interest of completeness, creating an intermediate or "Central Oceania" region within the NRO scheme to bring it into greater accordance with the field of genetic anthropology. As summarized in Figure 4, for the MPM scheme our data support moving the boundary to include Viti Levu, Vanua Levu, and Kadavu in Melanesia and Rotuma and the Lau Islands in Polynesia. For

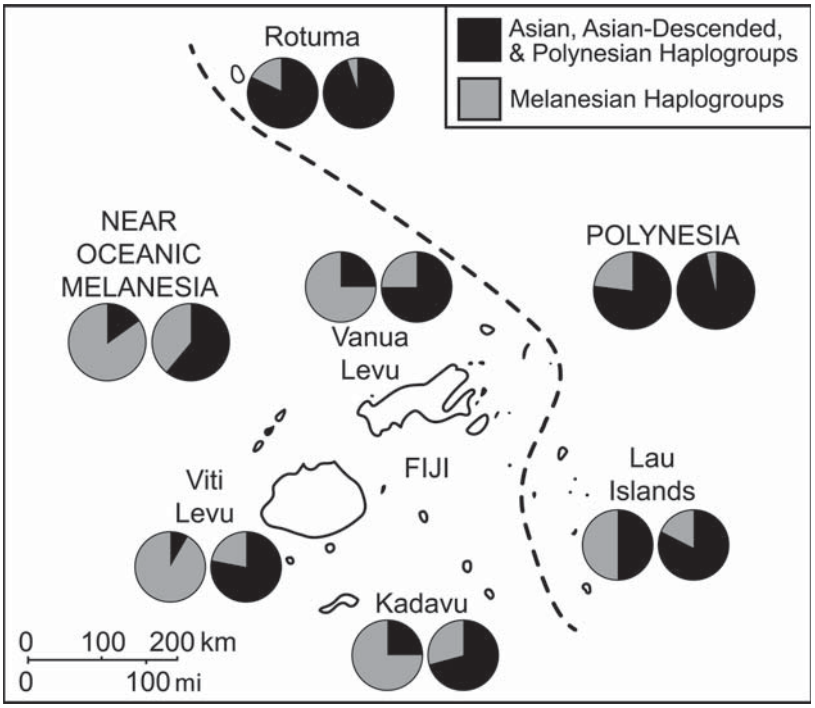

FIGURE 4. Proposed change to the boundary between Melanesia and Polynesia relative to Fiji (or the eastern boundary of a proposed Central Oceania region), showing NRY-SNP (left pie charts) and mtDNA (right pie charts) haplogroup frequencies for five Fijian island populations. Near Oceanic Melanesian and Polynesian data are from Delfin et al. (2012).

the NRO scheme, our data support locating Viti Levu, Vanua Levu, and Kadavu in Central Oceania (which would presumably extend westward to abut the traditional Near Oceania border) and locating Rotuma and the Lau Islands in Remote Oceania. This remedies the untenable situation of, for example, categorizing the populations of the Santa Cruz Islands, Vanuatu, and western Fiji with the genetically very different populations of the eastern Pacific. More work is needed to more accurately characterize the genetics of the Pacific, and the adjustment we espouse may be further supplemented based on subsequent work. For example, while Rotuma is clearly genetically very similar to Polynesia, the Lau Islands are somewhat more genetically intermediate between Fiji and Polynesia, and additional work could better determine its regional relationships and affiliation. Equally clear, however, is that regional definitions that do not take genetics into account will not accurately reflect all anthropological evidence.

We also note that, while examining parental origins to identify samples for analysis, we found what appears to be exceptionally strong patrilocality among at least two of the Fijian populations. Specifically, of all the samples for which we knew both the mother's and father's birthplaces, for Kadavuans 100% of fathers but only 42% of mothers were from Kadavu, and for Lau Islanders 100% of fathers but only 57% of mothers were from the Lau Islands. In contrast, for Viti Levuans 83% of fathers and 74% of mothers were from Viti Levu, and for Vanua Levuans 96% of fathers and 91% of mothers were from Vanua Levu. For Rotumans, 98% of both fathers and mothers were from Rotuma, but this may reflect Rotuma's greater geographical isolation and correspondingly lower access to partners from other populations.

Our study has several limitations. First, although we increased our sample size for Rotuma, sample sizes for other populations could be larger. For the NRY-STR analysis in particular, $n < 20$ for four of the five island populations, and $n = 10$ for the Kadavuans. Second, most of our samples were collected from individuals on the campus of the University of the South Pacific in Suva, and these individuals may not fully genetically represent their home island populations. Third, our examination was limited to specific genetic markers on the Y chromosome and the HVS1 region of the mtDNA genome, and an examination of other markers and other regions of the human genome might yield different results. For example, we note that A16247G in the HVS1 region, which we used to distinguish between the B4a1a1 and B4a1a1a haplogroups, has been found to backmutate (Duggan and Stoneking 2013; Duggan et al. 2014), so some small portion of the participants identified as belonging to B4a1a1 may actually belong to B4a1a1a. Even if this is the case, it would not change our conclusions, but sequencing and analyzing other portions of the mtDNA genome would clarify these haplogroup assignments. Relatedly, the diagnostic mutation for B4a1a1a recently changed to A6905G, but this is not within the HVS1 region, so we have continued to use A16247G. Further, more work needs to be done to characterize autosomal markers in Pacific populations. With regard to Fiji, only one study has examined autosomal STRs, and it found that Fiji as a whole was most similar to Samoa, Hawaii, and Pohnpei (Lum et al. 1998), which supports to our broader findings and conclusions.

The results of this study contribute to understanding genetic structure among the Fijian island populations and the process of settling the region. Although prior studies treated the Fijians as genetically homogeneous, we found important genetic differences among the various island populations that support nongenetic evidence for moving regional boundaries to within the Fijian archipelago. Data collection that does not take these differences into account could yield unreliable results, and regional boundaries that do not take them into account will not reflect all anthropological evidence. Thus, our findings support the continuing need for additional examination of individual island populations within Fiji to better understand the process of settling Fiji and of the surrounding regions.

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