**Ancient Microbial DNA in Dental Calculus: A new method for studying rapid human migration events**

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**Abstract:**

Ancient human migrations provide the critical genetic background to historical and contemporary human demographic patterns. However, our ability to infer past human migration events, especially those that occurred over rapid timescales, is often limited. A key example is the peopling of Polynesia, where the timing is relatively well defined, but the exact routes taken during the final stages and the source populations are not. Here, we discuss the technical limitations of current methods for inferring rapid human migration events, using the final stages of Polynesian migration as an example. We also introduce a promising new proxy method to infer human migrations – patterns of bacterial evolution within ancient dental calculus (calcified plaque). While we focus on Polynesia, this method should be applicable to other past migrations, enhancing our understanding of human prehistory and revealing the crucial events that shaped it.

**Keywords:** commensal models, ancient DNA, archaeogenetics, Polynesia, Pacific settlement

**Introduction**

Determining past human migrations (defined here as the movement by people from one place to another with the intention of settling) are of great cultural interest and significance to many people, and can provide a sense of identity and connectedness to one’s culture. The peopling of East Polynesia ~ AD 1000-1300 AD was the last major human migration before the modern era, and one of the most geographically extensive. East Polynesia covers an area larger than North America (Fig. 1), and the colonization of its islands involved the longest maritime migrations in pre-modern history (*i.e.* before AD 1500 AD). Studies of material culture, linguistics, seafaring and climatic simulation, and genetics have disclosed much about the tempo and directions of migration in the western Pacific, notably of the Lapita culture that extended out to Tonga and Samoa by ~800 BC. Despite this, similar research has shown relatively little about the peopling of East Polynesia, as the time span involved is considerably shorter.

The chronological data for East Polynesia remains unsettled, as some archaeologists argue for initial colonization of Central East Polynesia (Society, Cooks, Marquesas, western Tuamotus) or perhaps Hawaii by the late first millennium AD (Athens et al. 2014), while others argue that colonization in these archipelagos began in the 12th century and had reached all the marginal islands, including Easter Island and New Zealand, by the late 13th century (Wilmshurst et al. 2011). Either way, these migrations seem to have been episodic, with an earlier influx to Central East Polynesia, a later movement to Marginal East Polynesia (Hawaii, Easter Island), and a subsequent expansion into the outlying islands around New Zealand. The brevity of these migration pulses, each <200 years in duration, makes it difficult to reconstruct their internal structure, order, and the directions of the movements involved using current methods (archaeological material culture, linguistics, and human genetics).

A new means to infer rapid human migration events has been created through the study of the diverse communities of bacteria (microbiota) contained within the human body. These communities contain thousands of bacterial species (Hooper and Gordon 2001) that are acquired from birth via both vertical inheritance (*i.e.* transmission from parents to offspring) and common environment (diet, cohabitation etc.) (Song et al. 2013; Tims et al. 2013; Goodrich et al. 2016). In addition to the strong element of vertical inheritance, these bacteria replicate quickly – a characteristic that renders the microbiota a promising proxy to infer rapid human migrations. The recent discovery that archaeological dental calculus (calcified plaque) contains ancient microbial DNA (Preus et al. 2011; Adler et al. 2013; Warinner et al. 2014) offers researchers a powerful new tool for reconstructing ancient or historical human movements that remain undocumented. Here, we summarize the existing narrative of Polynesian migrations, noting the issues that remain both unresolved and undetectable by current methods, and explain the merits of a microbial genetics approach.

**Polynesian Migration**

The origins of populations ancestral to Polynesians have been traced back to Island Southeast Asia (ISEA), on the basis of linguistic, archaeological, and genetic evidence ((Anderson et al. 2014; Anderson 2016). Genetic and archaeological evidence suggest a Taiwanese contribution to modern Oceanic populations, along with significant contributions from ISEA and older groups resident in the western Pacific (Papuan) — although recent evidence suggests that Papuan genetic contributions occurred after the initial wave of settlement in western Polynesia (Skoglund et al. 2016). All of the Austronesian languages spoken outside Taiwan belong to the Malayo-Polynesian group, which extends from Madagascar through ISEA and across the Pacific to Easter Island and New Zealand. However, Malayo-Polynesian is not documented as occurring in Taiwan (Blust 2009: 740) and the possibility that it entered ISEA by a different route cannot be ruled out. Further, all of the components of the so-called “transported landscape” of Oceanic populations (Kirch 2000), which included root and tree crops and commensal and domestic animals, originate in ISEA or mainland SEA rather than Taiwan.

Moving eastwards, the development of the Lapita culture ~1400 BC in the Bismarck Archipelago was a major driving process in the peopling of Remote Oceania. The Lapita culture was the first to cross the boundary between Near and Remote Oceania (a 400km stretch of open ocean) to Vanuatu ~1000-1200 BC (Bedford et al. 2006), and moved as far as Samoa and Tonga by ~800 BC (Rieth and Hunt 2008), marking the easternmost edge of Lapita material culture, as Lapita sites further east have not been discovered. Post-Lapita migration reached the outlying islands of West Polynesia, such as Rotuma, Niue, and Pukapuka 200 BC- AD 1 (Anderson et al. 2014: 25), followed much later by colonization of the Society Islands ~ AD 1000-1100 (Wilmshurst et al. 2011), and possibly others in central East Polynesia as distant as Mangareva. Some 100-200 years later (AD ~1200), further eastward migration occurred out to the marginal islands of East Polynesia: Hawaii, Easter Island, then southwards to New Zealand and its outlying islands (i.e. Norfolk Island, Kermadecs, Chathams, Subantarctic islands) (Wilmshurst et al. 2011).

Although some aspects of the chronology of these migration episodes remain uncertain, it is apparent that the general pattern of initial colonization within Polynesia is well known (Fig. 1). However, much less is known about the particular origins of the migrating populations. Some clues are evident in material culture - especially where pottery is included, but pottery was not produced in East Polynesia. Many early East Polynesian artefact assemblages contain types of adzes, fish hooks, and ornaments that are so widely shared, they provide little indication of the sequence of historical relationships amongst them (Kirch 2000: 243-244)**.** Within Central East Polynesia, some specific connections can be established by source identification of basalt adzes, notably those from Marquesan and Samoan sources (Weisler 1997), but two artefacts of tropical marine shell that reached New Zealand cannot be attributed to a specific origin (Davidson et al. 2011; Anderson et al. 2014), and no other specific connections can be drawn between the central and marginal archipelagos. Historical linguistics are similarly constrained. For example, on Captain Cook’s first voyage to the Pacific, Tupaia (a navigator from Raiatea in the Society Islands) was able to act as a translator during contacts with Maori in New Zealand (Cook 2003) because Maori and Tahitian languages had remained mutually intelligible to a large extent despite 500 years of separation; this was also true of Cook Islands Maori, Tahitian-Hawaiian, and so on. However, there was also some regional clustering within East Polynesian languages, which centered upon the Society and Cook Islands to the west and the Marquesas and southeastern Tuamotus-Mangareva to the east, but the extent of difference is insufficient to provide more than a general indication of the routes of migration. For example, it is clear that the Maori language and much of Maori esoteric culture belongs to the Tahitic group, but it has been impossible to use linguistic data to show that migrations to New Zealand originated in the Society Islands, rather than the Cook Islands or the Australs. In short, a means to more finely discriminate human population origins is needed, especially where migrations occurred within short timescales.

***Human genetics***

To date, most human DNA studies have not directly addressed the sequence of colonization within Polynesia, and have instead focused on questions about the earlier phases of Austronesian migration and the extent of admixture between Austronesians and Near Oceanians (Kayser et al. 2008; Soares et al. 2011; Xu et al. 2012; Lipson et al. 2014; Skoglund et al. 2016). Migration within Polynesia, especially East Polynesia, is difficult to study with genetics because the initial founding populations were likely small and underwent drastic and successive bottlenecks during each island migration event, further reducing genetic variation (Murray-McIntosh et al. 1998). Back migrations and modern day admixture also likely rendered genetic diversity more homogeneous, resulting in a loss of dispersal signals. Genetic diversity in Polynesia is also likely to have been further reduced by disease epidemics introduced by early European explorers and colonists, as well as the spread of these diseases in more recent history (*e.g.* 1918 Influenza epidemic) (Kirch and Rallu 2007). As a result, the few studies that have measured modern genetic diversity in East Polynesia found limited genetic variation (Murray-McIntosh et al. 1998; Whyte et al. 2005; Benton et al. 2012). It is possible, however, that future studies examining larger amounts of nuclear DNA through SNP (Single-Nucleotide-Polymorphism) panels or whole genome sequencing may provide greater resolution.

Ancient DNA from human skeletal remains has been used to determine historical human migrations around the globe (Lazaridis et al. 2014; Allentoft et al. 2015; Haak et al. 2015). A major advantage of using ancient DNA is that it circumvents the influence of subsequent back-migration and contemporary genetic admixture on the migratory signal – providing key data both in time and space. However, there are a number of limitations when examining ancient human DNA in Polynesia. Warm, humid climates dominate throughout Polynesia and are known to result in poor DNA preservation (Allentoft et al. 2012). Ethical issues involved with performing destructive sampling and analysis of human remains has also restricted the application of ancient human DNA analysis in the region. Despite this, (Deguilloux et al. 2011) were able to obtain mtDNA from five ~500-year-old samples located in the Gambier Islands, and identified novel polymorphisms in a Near Oceania-associated haplogroup (Q1) and the Polynesian Motif mtDNA haplogroup. In addition, Knapp et al. (2012) applied next-generation sequencing to ~700-year-old human remains in the Wairau Bar site of New Zealand. From the 19 samples screened, DNA was successfully obtained from only four samples from which full mitochondrial genomes were obtained and novel Polynesian mtDNA variation identified. Recently, Skoglund et al. obtained genome-wide ancient DNA data from three individuals from a Lapita site in Teouma (Vanuatu), and one individual from Tonga (Skoglund et al. 2016). Their results suggest that the first wave of humans into Remote Oceania had little to no Papuan ancestry, contrasting the ~25% Papuan genetic contribution found in modern Oceanic populations, and suggesting later population movements introduced Papuan ancestry to Remote Oceania. However, the small number of available ancient samples in Polynesia has limited the use of ancient human genetic data for testing hypotheses regarding later migration routes.

***Genetics of animal proxies***

Polynesians are known to have transported a number of domestic animal taxa on their voyages: dogs (*Canis familiaris*), pigs (*Sus scrofa*), and chickens (*Gallus gallus*), as well as the commensal rodent — the Pacific rat (*Rattus exulans*). The DNA of these animals can be used as a proxy for human migration, with the added bonus that the marine dispersal abilities of these animals were generally poor, limiting natural migration from blurring the human migratory signal (for a review see (Storey et al. 2013). Modern and ancient DNA from these animals have been used as a proxy for human migration into the Pacific. Mitochondrial DNA has been used to trace the dispersal of dogs to Polynesia via a southwestern route through Indonesia (Oskarsson et al. 2011). Ancient mitochondrial DNA from 14 dogs found at the Wairau Bar site identified a small number of haplogroups, suggesting limited genetic variation in the founding population (Greig et al. 2015). Genetic evidence for pig dispersal to Polynesia mirrors that of dogs (Larson et al. 2007, 2010), which is concordant with geometric morphometric analyses of teeth and bones (Dobney et al. 2008). The Pacific rat has been traced back as far as Flores in Indonesia using ancient and modern mitochondrial DNA (Thomson et al. 2014 a), with eastwards dispersal to Polynesia (Matisoo-Smith and Robins 2004). Studies using modern and ancient mitochondrial DNA from chickens suggest an origin in the Philippines with movement eastwards to Polynesia (Dancause et al. 2011; Thomson et al. 2014 b).

These studies have contributed substantially to our understanding of the earlier phases of migration in Oceania, but have yet to be applied to East Polynesia. This may be due to several limitations. Pig, chicken, and dog are distributed patchily in East Polynesia and only the Pacific rat (*R. exulans*) occurred on nearly all islands (Anderson 2009). Again, modern DNA analyses suffer from contemporary admixture, including through European introductions. Ancient DNA analyses of these animals share many of the same limitations as for human specimens, albeit with fewer ethical considerations. As with humans, the increasing and extreme genetic bottlenecks that these organisms experienced through successive island colonization, coupled with the short and rapid timescale of migrations, prevent resolution of the routes taken during the final phases of Polynesian migration. Future recovery of more and better-preserved samples — coupled with genome-scale DNA analyses — may yet provide an improved reconstruction of events.

While constraints in current research approaches to understanding migrations in East Polynesia might well be overcome with future technical advances in existing fields of study, the most promising alternative for the moment is the study of microbial DNA.

***Modern microbial DNA***

The human microbiota contains >100× more genes than the human genome (Hooper and Gordon 2001), which - in combination with the typically fast generation time of bacteria - should provide more information about migration events than can be obtained from human or animal proxy species. While microorganisms have been used to trace large-scale human migrations, they have yet to be tested on rapid migratory events. For example, *Helicobacter pylori* is a bacterium that lives in the stomach of most individuals and is vertically transmitted within families (Rocha et al. 2003). It accompanied humans out of Africa and has thus been used as a proxy for global prehistoric human migration (Falush 2003). *H. pylori* phylogenies have also been used to explore the peopling of Oceania (Moodley et al. 2009), supporting other lines of evidence for two distinct migrations into the Pacific—an earlier one by Australians/Near Oceanians, and a later one by Malayo-Polynesian speaking people (Lapita).

Biogeographic signatures have also been obtained from bacteria within the modern human oral microbiota. Using a single protein-coding gene, *gtf* from *Streptococcus oralis*,Henne el al. (2014) were able to detect remarkable geographic resolution, especially considering its small size (330 bp). Due to the falling costs of DNA sequencing, future studies combining multiple informative genes from many bacterial species may provide the necessary resolution to reconstruct rapid human migrations. However, there are several limitations to using modern microbial DNA for this purpose. For *H. pylori*, sampling involves performing a stomach biopsy on a living individual - an invasive procedure that precludes extensive sampling. While microbial DNA likely has the resolution required to infer rapid human migratory events, there is the potential issue of modern genetic admixture reducing geographic signals. Consequently, a common, robust source of ancient human-associated microbial DNA is needed, and this has now been identified on the teeth of our long-dead ancestors.

**Ancient dental calculus as a new means of tracking human migrations**

Dental plaque is a dense, complex living microbial community that adheres to and grows on the surface of teeth. During the life of an individual, calcium phosphate ions from saliva cause this soft plaque to undergo periodic mineralization events, trapping lower layers in an extremely hard, calcified deposit called dental calculus. The prevalence and robust nature of dental calculus makes it common on the teeth of archaeological human remains. The first definitive observation of calcified bacteria trapped within archaeological dental calculus occurred nearly thirty years ago (Dobney and Brothwell 1986), which led to subsequent research over the next eight years (Dobney and Brothwell 1988; Dobney 1994). It was not until the application of new scientific techniques in archaeology some 13 years later that actual bacterial DNA preserved within ancient dental calculus was observed for the first time via gold-labelled antibody transmission electron microscopy (Preus et al. 2011). This was further verified by the successful extraction, amplification, and sequencing of bacterial DNA from ancient dental calculus (De La Fuente et al. 2013). The first community-level analyses of ancient dental calculus detected changes in human oral microbiota communities likely correlating with dietary changes over 8000 years, from early agriculturalists to the Industrial Revolution (Adler et al. 2013). An increase in the carriage of tooth decay-associated bacteria was also observed through time, likely reflecting the increasing availability of carbohydrates. The composition of the human oral microbiota appeared relatively distinct to each culture and geographic region, highlighting the potential power of microbiota DNA preserved within dental calculus to provide an ancient genetic signal of cultural affinity (Adler et al. 2013). Subsequent studies have reconstructed full genomes of oral pathogens such as *Tannerella forsythia* from medieval specimens (Warinner et al 2014). Collectively, these pioneering studies illustrate that high-resolution investigation of ancient oral microbiota, both at the community and individual bacterium level has the power to provide novel views of human bio-cultural evolution. Protein sequencing has also been applied to ancient calculus, showing that bacterial functions (e.g.,virulence factors) and their interactions with the human host (e.g., immune proteins) are obtainable from ancient dental calculus (Warinner et al. 2014). A further key finding was that DNA from ancient calculus includes signals from food sources such as plants and animals, demonstrating the ability to obtain dietary information from ancient human populations, providing further information to delineate past human lifeways (Warinner et al. 2014).

While the ethics involved in analyzing ancient human DNA can be extremely sensitive, ancient dental calculus is almost entirely microbial in origin (>99.9%), with very limited amounts of human DNA. In addition, dental calculus can be easily removed from teeth, avoiding the destruction of human remains. These two factors ensure ancient calculus can be examined with minimal alteration to valuable museum specimens, potentially allowing large numbers of samples to be collected and analyzed. Practically speaking, ancient dental calculus contains a much higher concentration of DNA than bone (Warinner et al. 2014), which increases the odds of successfully obtaining DNA from poorly preserved ancient specimens. Importantly, the use of oral bacterial DNA in ancient dental calculus also solves the issue of modern genetic admixtures confounding human migration signal. In addition, the oral microbiota exhibits a strong degree of vertical inheritance (Okada et al. 2004; Corby et al. 2007; Li et al. 2007; Ebersole et al. 2014), making it a suitable proxy for human migration. Finally, the large amount of genetic material found in the oral microbiota, coupled with the rapid generation time in bacteria, should provide the resolution required for discerning rapid human migratory events. These characteristics render ancient human dental calculus a promising new means of studying past human population movements.

***Potential methods for analyzing oral bacteria for human migrations***

To track human migrations using ancient dental calculus, genetic mutations within bacterial species must be identified, as bacterial community structure would likely provide insufficient resolution. To examine species and strain level resolution within ancient calculus specimens, several techniques could be applied. The most promising centers around a recently published method called StrainPhlAn (Truong et al. 2017). StrainPhlAn uses species-specific marker genes (Truong et al. 2015) to measure strain-level genetic diversity from metagenomic samples. Briefly, DNA reads from a metagenomic sample are mapped to species-specific markers, and consensus sequences of the dominant strains are constructed. For each species, the consensus sequences are then aligned, concatenated, and used as input for maximum likelihood phylogenetic analysis. This program provides the ability to investigate genetic differences with high resolution between bacterial species from different samples, which may be sufficient to infer past rapid human migrations. In addition, genome level information from strains could be similarly obtained; strain level resolution of commensal species has been recently observed, suggesting that this approach may be feasible for future studies (Weyrich et al. 2017). In either approach, different strains would need to be identified and assessed individually, then, DNA from the dominant strain or multiple strains can be compared and assessed through time as a marker for human migration.

To ensure this method can be utilized, shared bacterial taxa must be identified within each of the samples of interest. This should be a limited issue in dental calculus, as research suggests that many species are shared between individuals within populations, reflecting a ‘core’ calculus microbiota (Welch et al. 2016). The oral microbiome has also been shown to be the one of the most stable and conserved human microbiomes to date (Utter et al. 2016), and many oral strains have been shown to be conserved across hominin species (Weyrich et al. 2017), indicating that shared microbial taxa may be available for this type of analyses across human populations. Strain heterogeneity within a sample might confound basic phylogenetic analysis in some cases, but it is likely that the sample will be dominated by a single strain (Truong et al. 2017), which could be used to infer migratory processes. For example, (Truong et al. 2017) found that for 1500 deeply sequenced gut metagenomes, a single strain typically dominated—even through time—for >70% of the species analyzed, indicating that this approach is plausible within diverse, mixed strain metagenomes.

Another important consideration is the sequencing coverage of the genetic loci to be analyzed (i.e.how many DNA sequences can be obtained for each genetic locus). Insufficient coverage would likely result in the inability to determine if a nucleotide change is due to stochastic effects (e.g., sequencing artefacts, DNA damage, etc.), or if a mixed signal is resulting from strain-level polymorphisms. Obtaining sufficient coverage is especially challenging for ancient DNA due to its fragmented and damaged nature (Dabney et al. 2013). However, one possible solution is to improve the coverage of genetic loci of interest using hybridization-enrichment (Maricic et al. 2010), whereby specific DNA sequences are captured prior to sequencing. This technique drastically lowers the cost of sequencing and increases the coverage obtained, especially for ancient DNA (Schuenemann et al. 2011; Skoglund et al. 2016). Finally, while reference bias towards well-studied strains may make strain-level identification challenging, (Truong et al. 2017) were able to reconstruct strain-level genetic diversities 10-fold higher than were previously available, demonstrating StrainPhlAn’s ability to accommodate incomplete reference data.

A potential issue for using bacteria as a proxy for human migration is horizontal gene transfer (HGT). Bacteria are known to transfer genetic material between each other—even among distantly related species—and such transfers could confound phylogenetic analyses. To circumvent this issue, one can target genetic loci that are conserved, single-copy, and show no or low levels of horizontal gene transfer. Such genes are typically involved in essential informational processes and are parts of large, complex systems (e.g., transcription, translation, tRNA synthetases) (Rivera et al. 1998), and are thought to be recalcitrant to horizontal transfer (Jain et al. 1999; Hao and Golding 2008). The practical use of such loci for bacterial phylogenetic analyses has been previously demonstrated in the literature (Wu and Scott 2012; Wu et al. 2013; Darling et al. 2014; Ankenbrand and Keller 2016). To ensure that these genetic loci are not horizontally transferred, software is available that can detect horizontal gene transfer/recombination in bacterial genomes (Croucher et al. 2015; Martin et al. 2015). One can use these tools to identify putatively horizontally-transferred loci, and once identified, these loci can be discarded from phylogenetic analyses.

To summarize, the approach and tools required for employing dental calculus as a high-resolution proxy of past human migrations are available and feasible, and so is the prospect of being able to infer rapid human migration events from such data.

**Discussion and Conclusions**

As we continue to investigate the origins of migrating populations in Polynesia, it is pertinent to ask which migration events to consider, and why these specific events are of interest to Polynesian prehistory. Below, we have listed five issues for which analysis of ancient microbial DNA could provide critical insights into Polynesian prehistory.

**1.** The initial colonization of West Polynesia, and questions about possible later migrations from Micronesia (Addison and Matisoo-Smith 2010; Davidson 2012), constitute an important unresolved issue. The radiocarbon chronology of colonization sites indicates migration from Fiji, colonized about 900 BC, to Tonga and Samoa by 800 BC (Petchey 2001; Burley et al. 2010). In contrast, the relative frequency of Lapita sites suggests a linear migration through Tonga (where Lapita sites are scarcer to the north) and Samoa (where there is only one Lapita site) (Clark and Anderson 2009). However, it is also possible that Fiji was the direct source of migrants for both Samoa and Tonga, in which case a progressive decay of the migratory pulse would require an alternative explanation (e.g., Rieth et al. 2008; Burley et al. 2011).

**2.** On the same theme, the later colonization of Niue remains unknown. Niue is closer to Tonga than Samoa, and the Niuean language is closer to Tongan. However, Samoan features are strongly evident in the place names and oral traditions. In addition, there is some evidence that Niue was colonized from East Polynesia (Walter and Anderson 2002). Was the initial source population from Tonga or Samoa, and when did East Polynesian influence begin? Identification of either Tonga or Samoa as the source has potentially significant implications for the later colonization of East Polynesia.

**3.** Samoa is commonly referred to as the most likely source of the original migrants to East Polynesia, with suggested voyages through the northern and southern Cook Islands, through the northern Cook Islands to the Society Islands, or perhaps on several routes during the colonizing period (Kirch 2000; Kirch and Green 2001; Wilson 2012; Montenegro et al. 2014, 2016). However, there is a strong linguistic argument for initial migration into East Polynesia through the northern atolls, including the Phoenix and Line Islands, suggesting that a more likely entry to East Polynesia would have been through the Marquesas (Wilson 2012). The different scenarios imply quite different histories of colonization, including the development and spread of language, material culture, and seafaring capabilities.

**4.** Within East Polynesia, there are several cases where alternative source populations of initial migrants are possible, and sorting out the alternatives will help to refine issues of origin, relatedness, and interaction in the region. Hawaii may have been colonized from the Marquesas (Kirch 2000): 291, but it is possible that some colonists came from Tahiti, as marked by a Tahiti cosmogony in Hawaii (Marck 2000:230). Similarly, the colonization of New Zealand was from the Tahitic (western) region of central East Polynesia, but its specific origin remains unknown. Was it directly from the Society Islands? Was it from the Southern Cook Islands or from the Austral Islands? Was it a single population dispersal from one particular island or archipelago or a general movement from across the Tahitic region? These differences can help inform the causes of migration (i.e.,dispersals triggered by specific events mentioned in oral traditions, or a broader event horizon or process). The Moriori people of the Chatham Islands that lie east of New Zealand have linguistic and material culture parallels with New Zealand Maori (Sutton 1980), but their oral traditions are somewhat different (Shand 1911). Modern Moriori prefer the possibility that their ancestors arrived by direct passages from central East Polynesia, and the distance to Chatham Island and New Zealand from the southern Cook Islands is almost exactly the same.

**5.** Accumulating data are once more raising the question about whether some earlier migrants to Easter Island had Amerindian origins. The oldest archaeology that appears on Easter Island clearly has East Polynesian origins, but research in genetics (Moreno-Mayar 2014) and in the origins of monumental architecture (Martinsson-Wallin et al. 2013) has suggested a New World influence soon after initial colonization.

All of these and many other questions about the prehistory of Polynesia exist primarily because our current methodologies do not have sufficient temporal discrimination to trace the movement of people between islands at a centennial scale resolution. Such difficulties are not unique to Polynesia and are observed elsewhere, including the Caribbean and within the American continents (Hofman et al. 2008; Fitzpatrick 2015; Keegan and Hofman 2017). The study of ancient microbial DNA within dental calculus, as outlined here, presents a powerful new tool to identify human cultural signals, track bacterial genome evolution, and ultimately reconstruct human migration patterns. Analysis of the microbiota in dental calculus will provide unprecedented opportunities to trace human movements, thereby enhancing our understanding of human prehistory.

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