2. Ancient and Modern Genetic Variation in the Americas

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Abstract: A number of recent whole mitochondrial genome and nuclear DNA studies support the Beringian Incubation Model as the best description of the demographic history of the population that first entered the Americas approximately 20,000–15,000 B.P., having originated from a single source population located somewhere in Asia. Human presence in the Americas by at least 14,270–14,000 B.P. has been confirmed by archaeological evidence from the Monte Verde site in southern Chile and by the recovery of human coprolites in the Paisley Caves in southern Oregon. A secondary migration or expansion of humans, perhaps from the same source population, introduced additional mtDNA haplogroups into the northernmost areas of North America after the last glacial period. From the initial entrance of humans into the Americas, genetic drift has played a substantial role in shaping the Native American gene pool. On a continental scale, Native Americans exhibit simultaneously the highest measures of both homozygosity and interpopulational genetic distances (a classic example of the Wahlund effect). Genetic variation thus far detected in human remains and human byproducts (e.g., coprolites) that predate 5000 B.P. are consistent with this view, notwithstanding the small sample sizes.

Introduction

As an independent line of evidence to that provided by archaeological, paleontological, and/or morphological studies, molecular data[1] have proven instrumental to our understanding of the emergence and evolution of *Homo sapiens*. Information deciphered from the genomes of countless humans has also aided the reconstruction of the paths that our ancestors followed as they jour-

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neyed across the world. Today, there is a general agreement between the genetic and archaeological evidence for the direction and shape of most of the major population movements[2] (Cavalli-Sforza et al. 1994; Forster 2004; Wells 2006).

The settlement of the Americas marks one of the most recent of these movements. The particular details of this process have been of long-standing anthropological interest (Fewkes et al. 1912) and the focus of a large amount of genetic research (Crawford 1998; Salzano and Callegari-Jacques 1988; Schurr 2004a). Discerning the location in Asia from where proto–Native Americans originated and estimating approximately when humans first colonized the Americas have been goals at the forefront of this research (Goebel et al. 2008; Schurr 2004a).

Intimately tied to the issue of the timing of this event is the route by which humans could have or *must* have entered the Americas from Beringia. The traditionally held view is that the Clovis archaeological complex—recently reassessed to span *maximally* from 13,250–12,800 B.P.[3] (Waters and Stafford 2007), though not all investigators agree with this redating (Haynes et al. 2007)—represents the first presence of humans in the Americas (Fiedel 2000). In this "Clovis-first" model, humans could have walked across the Bering Land Bridge, a landmass present during times of significantly lower sea levels, and then moved south through the ice-free corridor, an opening afforded by the retreating ice sheets that once covered most of the arctic and subarctic regions of North America.

However, evidence for a human presence in Americas south of the Cordilleran and Laurentide ice sheets prior to the opening of the ice-free corridor around 14,000–13,500 B.P. (Goebel et al. 2008) continues to mount. One of the most recent and undeniable pieces of evidence to support this conclusion was the discovery of human coprolites (desiccated human feces) from Paisley 5 Mile Point Cave in southern Oregon that are pre-Clovis in age, dating as early as 14,270–14,000 B.P.[4] (Gilbert, Jenkins, et al. 2008). Even with the associated uncertainty in the radiocarbon dates of these coprolites, the ice-free corridor has been argued to have been inhabitable prior to 13,000 B.P. (Mandryk et al. 2001). Thus the discovery of these coprolites has significantly widened the gap between first occupation of the landmass south of the glaciers and the time that it was possible for humans to enter via a land-based route. In addition, the Monte Verde site in southern Chile shows clear evidence for a pre-Clovis occupation in the Americas around 14,500 B.P. (Dillehay 1999). As it is quite unlikely that archaeologists have sampled from the first few millennia of Native America prehistory (Toth 1991), the date of first occupation of the Americas must be older. An entry prior to the opening of the ice-free corridor implies that humans *must* have initially used boats to enter the Americas along the Pacific coast.[5]

Attention has also been placed on estimating the amount and form of genetic and morphological variation that was brought to the Americas and the closely related topic of how many "waves" of migration contributed to the variation observed today (Eshleman et al. 2003; Schurr 2000, 2002, 2004a, 2004b). To understand the importance of resolving these issues, one can think about the pre-contact Americas as the ultimate "island" a number of humans migrated to but few returned from. In this case, the more precisely one can determine how much variation was carried *to* the Americas, the more precisely one can estimate how

much variation has evolved *within* the Americas. It then becomes quickly apparent that resolving the time at which humans first entered the Americas and the timing of any subsequent "waves" is essential for estimating rates of evolution on the American "island." This would be true not only for genetic and biological evolution but also for rates of linguistic and cultural evolution. Moreover, by establishing the amount and nature of variation brought to the Americas, one can detect homoplasy in DNA sequences (i.e., recurrent mutations), as well as discern independent cultural innovations (as is clearly the case for agriculture in the New and Old Worlds) and biological adaptations (e.g., to high altitude; see Weinstein 2005).

A number of prior studies argued that the genetic variation found in the Americas is best accounted for by multiple human migrations from distinct source populations (see studies reviewed by Eshleman et al. 2003; Schurr 2000, 2002, 2004a, 2004b). Studies of variation in cranial morphology exhibited by Native Americas has also been explained by multiple migration events (see Neves and Hubbe 2005), although other researchers view the patterns of morphological change in the Americas as reflecting a process of adaptation and gene flow (see Gonzalez-Jose et al. 2008).

In line with the theme of the 2008 Twenty-Fifth Visiting Scholar Conference ("Archaeological and Biological Variation in the New World"), the purpose of this chapter is to review both the earliest and most recent studies[6] of genetic variation in Native American populations in order to uncover the trajectory the field has taken with respect to its attention to and appreciation of variation. In this discussion, there will be a particular focus on the variation reported from skeletal remains and other archaeological artifacts that predate 5000 B.P. The most recent ancient and modern DNA studies support an initial entry of humans into the Americas approximately 20,000–15,000 B.P. that emanated from a single source population located somewhere in Asia. This conclusion conflicts with the view held by some skeletal morphologists who maintain that multiple "stocks" of humans contributed biological variation to the Americas.

What Is Mitochondrial DNA?

The vast majority of Native American genetic studies have focused on variation found in the mitochondrial genome. Mitochondrial DNA (mtDNA) is an extranuclear genome found in the mitochondria of cells and has a number of characteristics that are useful in molecular anthropological and phylogenic studies. This genome is a small circular molecule and its entire sequence is known, including the positions of genes. The first whole human mitochondrial genome sequence, and consequently the reference to which all other mitochondrial sequences are compared, is merely 16,569 base pairs (bp) in length (Anderson et al. 1981; Andrews et al. 1999), which is trivial compared to the approximately 3 billion bp found in the nuclear genome. As a consequence of residing in the cytoplasm, human mtDNA is strictly maternally inherited (Giles et al. 1980), meaning that mutations occurring in it reflect only female history and movement. More-

over, mtDNA is particularly useful for discerning maternal ancestor-descendant relationships because it does not recombine during meiosis (Merriwether et al. 1991; Schurr et al. 1990).

The mitochondrial genome has a relatively high mutation rate compared to nuclear genes (Brown et al. 1979; Ingman et al. 2000; Kemp et al. 2007 and reference therein). This high rate generates a sufficient number of mutations to allow one to differentiate new haplotypes or haplogroups in populations that have recently diverged, such as Native Americans. The mitochondrial genome is also not under strong selection (Kivisild et al. 2006). As a result, the distribution of variation in this genome should reflect population history and not natural history (e.g., response to some selective force correlated with latitude such as climate; however, for a contrasting view see Mishmar et al. 2003).

Lastly, there are thousands of copies of the mitochondrial genome in each cell (Wallace 1994), compared with only two copies of each autosome and, in males, a single X and a single Y chromosome. For ancient DNA studies, the high copy number of the mtDNA and its ubiquitous presence in most cells partly compensate for the fact that DNA degrades with time and have made it the primary genetic system for the investigation of DNA from skeletal remains and other ancient human by-products (e.g., coprolites [Poinar et al. 2001]) and artifacts (e.g., quids [LeBlanc et al. 2007]).

Initial Studies of Native American mtDNAs

Native American tribes were some of the first human populations to be studied for mtDNA variation. The earliest of these molecular studies utilized restriction endonucleases (or "restriction enzymes") to screen humans for differences in their mitochondrial genomes. Restriction enzymes are used in nature by bacteria as a defense against invading viruses, which insert their DNAs into the bacterial host in an attempt to seize control of its genetic functions. Each restriction enzyme detects a specific order of nucleotides in a DNA sequence and cleaves the DNA at that particular location upon recognition.

As an example, the restriction enzyme *Hae*III is the third such enzyme purified from the bacterium *Haemophilus aegyptius* and recognizes the sequence GGCC. When this sequence is detected, the enzyme cleaves the DNA between the middle G and C, creating two pieces of DNA with GG at the end of one and CC at the end of the other. Using this example, if one person has the sequence GGCC at some place in her mtDNA and another has GGTC then the enzyme will cut the DNA of the former individual but not that of the latter. Here, two people have been differentiated genetically, and they can be scored as "+" (presence of site = cut) and "-" (absence of site = lack of cut), respectively, at that position in their genomes. This approach to detecting molecular variation is known as restriction fragment length polymorphism (RFLP) analysis.

Using six restriction enzymes[7] to detect mtDNA variation, Wallace and colleagues (1985) found that a certain mtDNA "morph"[8] (or combination of RFLPs) was exhibited by 40 percent of the Pimas (or Akimel O'odham), an indigenous

population of the American Southwest. As this same morph had been previously detected in only one out of fifty-five "Orientals" (Blanc et al. 1983), Wallace and colleagues (1985) concluded that the proto–Native American population experienced a drastic founder effect after leaving Asia and entering the American continents.

Building on the foundation established by this report, Schurr and colleagues (1990) extended the previous sampling of Pimas[9] and added Native American samples from Central America (Maya) and South America (Ticuna). Using these six restriction enzymes[10] and others employed by Cann and colleagues (1987)[11] to survey the samples for known Asian polymorphisms and for the presence of a 9-bp deletion thought to be a marker specific to Asian and Asian-derived populations (Hertzberg et al. 1989), their study revealed that the mtDNAs of these populations could be placed into one of four haplogroups (i.e., groups of closely related haplotypes[12]). From these data, the authors concluded that Native American mtDNAs derive primarily from four Asian maternal lineages. Interestingly, some of the haplotypes were shared across these linguistically and geographically distant populations, suggesting a common origin for all "Amerinds."[13] However, the frequencies of the haplogroups differed substantially between these populations, with the Maya containing haplotypes belonging to all four of the haplogroups and Pimas and Ticuna containing haplotypes belonging to only three of them, albeit not the same three. Recognizing that the microevolutionary force of genetic drift best accounted for this observation, Schurr and colleagues (1990:620) noted that

> The shifting frequencies of founding haplotypes among tribes and the demonstration of the sequential accumulation of multiple new tribal mtDNA mutations indicate that reconstructing the migration patterns of Amerindians through their mtDNA phylogenies should be possible.

Two subsequent papers by Torroni and colleagues (1992, 1993) took mtDNA studies, and those specifically involving Native Americans, to the next level. These studies (1) increased the resolution of mtDNA analysis, (2) increased the number of populations and individuals sampled, and (3) devised a nomenclature for describing mtDNA variation. To the six restriction enzymes used in the studies discussed above, Torroni and colleagues (1992, 1993) added an additional eight,[14] substantially increasing the amount of information that could be obtained from an individual's mitochondrial genome. Analysis with this set of enzymes was estimated to screen approximately 15–20 percent of the genome, or around 2485-3313 bp[15] (Torroni et al. 1993). Additionally, in a subset of samples, they sequenced a 341 bp segment (nucleotide positions [nps] 16,030–16,370) of the control region (or D-loop) of the mtDNA genome, representing most of the first hypervariable region, or HVRI (Torroni et al. 1993).

By 1993, the mtDNA of 383 Native Americans from 17 populations had been examined by high-resolution RFLP analysis (Torroni et al. 1993), and over 97 percent could be placed into one of the four haplogroups originally described by Schurr and colleagues (1990). These haplogroups were given letter designations, beginning at the top of the alphabet. Accordingly, these first mitochondrial hap-

logroups received the names A, B, C, and D[16] (Torroni et al. 1992). The HVRI sequences obtained in 38 of these samples were partitioned into three haplogroups (A, B, and C), with members of haplogroup D clustering somewhat less distinctly, due to the lack of haplogroup D defining markers in HVRI.

As discovered by Schurr and colleagues (1990), most "Amerind" populations contained some frequency of at least three of these haplogroups.[17] In contrast, the Dogrib and Navajo (grouped linguistically into "Na-Dene"[18]) were almost fixed for haplogroup A,[19] and contained an appreciable frequency (approximately 27 percent) of a form of haplogroup A that exhibited a *Rsa*I site loss at np 16,329, caused by an A→G transition at np 16,331. Torroni and colleagues (1992, 1993) argued that this was evidence for the independent origins of Na-Dene Indians and Amerinds, who entered the Americas in two distinct migrations. Considering the intrahaplogroup variation reported in Amerinds, Torroni and colleagues (1993) argued that each haplogroup was introduced into the Americas by a single haplotype, the "founding haplotype" of the haplogroup. This deduction supported the observation of Wallace and colleagues (1985) that the proto–Native American population underwent an extreme founder effect.

With the genetic evidence available at the time, Torroni and colleagues (1993:581) concluded that "[t]he current study, together with previous studies . . . , confirms that *all* Native American mtDNAs fall into four distinct haplogroups (A–D)" [emphasis ours]. This statement would come to have a profound effect on the future of Native American mtDNA studies, locking the mind-set of many researchers into what might best be termed the "four-founding lineage paradigm."[20] As will be discussed below, in retrospect, the boldness of this statement was unwarranted given that between 2.6 percent (Torroni 1993:Table 2) and 6 percent (Torroni 1993:Table 4) of the samples they analyzed belonged to unknown haplogroups, which they attributed to non–Native American admixture. In retrospect, Torroni and colleagues (1993) should have emphasized, as did Schurr and colleagues (1990:613) a few years prior in their abstract, that "Amerind mtDNAs derived from *at least* four primary lineages" [emphasis ours]. Nonetheless, the four-founding lineage paradigm had been established, and what largely followed was a time when less, not more, mtDNA variation was surveyed in Native American populations.

The Four-Founding Lineage Paradigm

While researchers such as Wallace, Schurr, Torroni, and their colleagues were conducting high-resolution RFLP analyses of Native American mtDNAs, others were focused solely on sequence variation in the first (HVRI) or second (HVRII) hypervariable regions of the genome (e.g., Ginther et al. 1993; Ward et al. 1991, 1993). However, after it was "concluded" that only four haplogroups were present in the founding American population, high-resolution studies quickly fell out of favor. The cost of conducting such an intensive mutation detection process also limited its widespread use. Thus, in the 1990s, a researcher

might have been left to ask, "Why look for variation beyond the markers that define haplogroups A, B, C, or D?"

More to the point, one could simply sequence the HVRI of a particular mtDNA to determine an individual's haplogroup, as demonstrated possible for at least haplogroups A, B, and C by Torroni and colleagues (1993). In sum, this meant that a study could benefit by being able to analyze more samples for known markers at the cost of conducting high-resolution RFLP analysis. Moreover, this approach meant that one could retrieve adequate comparative data from degraded DNA sources, such as frozen archived blood fractions (an approach called "Freezer Anthropology" [Merriwether 1999]) or even highly degraded DNA sources, such as ancient skeletal remains (e.g., Stone and Stoneking 1993, 1998).

The abandonment of the high-resolution RFLP approach for methods involving the genotyping of samples for haplogroup defining markers and/or sequencing the hypervariable regions of the D-loop resulted in the accumulation of numerous data sets from a variety of different Native American populations. These data revealed that the vast majority of mtDNAs in the Americas did belong to haplogroups A–D. However, the nonuniformity of haplotype definition in these studies generated interpretations of the resulting data that were sometimes inconsistent with each other.[21]

Terminology for mtDNA Variants

At this point, it might be useful to review the history of the terminology used to discuss mtDNA variation in Native American populations. Different descriptions of mtDNA variants over the past 15 years have sometimes created confusion about the units of genetic analysis in these studies and the relative comparability of mtDNA genotypes defined by RFLP analysis and HVRI sequencing and, hence, the number of founding haplotypes and lineages that have been brought to the Americas.

Because of the different approaches to detecting mtDNA haplogroups and characterizing haplotypes, parallel terminologies for describing these variants were employed. In the studies carried out in the Wallace lab, the terms *haplogroup* and *lineage* were used interchangeably to denote a distinct cluster of phylogenetically related mtDNAs. The smaller branches of a haplogroup were called *subhaplogroups* or *sublineages*. In addition, a unique mtDNA genotype defined by RFLP analysis was called a *haplotype*. A haplotype encompassed all of the mutations identified through this method, given that all of the nucleotides in the mtDNA genome are linked.

However, other researchers used different terms to describe Native American mtDNAs. While recognizing haplogroups on the basis of the defining RFLPs in these studies, the term *lineage,* not *haplotype*, was used to describe each unique HVRI sequence in a given population. Every set of related lineages was then called a *cluster* or sometimes a *clade*, with the nodal or root types of the clusters being considered their founding lineages.

In this regard, it should be reemphasized here that one can usually ascertain the mtDNA haplogroup to which a given HVRI sequence belongs by noting which combination of mutations is present in the mtDNA being analyzed. In other words, most of the founding haplogroups have a unique sequence "motif" that allows one to quickly delineate a particular maternal lineage from the rest. However, a RFLP haplotype may also have more than one HVRI sequence associated with it because of the more rapid mutation rate of the HVRI compared to the coding region in which the RFLPs occur. Thus, RFLP haplotypes and HVRI haplotypes/sequences may or may not identify the exact same mtDNA.

Therefore, while largely producing the same general picture of variation, RFLP analysis and HVRI sequencing generate slightly different assessments of haplotype and lineage diversity in human populations. On the other hand, as will be discussed below, over the past five years research employing whole mtDNA genome sequencing has enlarged our understanding of mtDNA variation in the Americas, both in terms of the number of haplogroups present in Native American populations and the number of distinct haplotypes that were brought by their founders.

In this chapter, we will use the following nomenclature to describe mtDNA variants and the maternal lineages to which they belong: (1) a *haplogroup* is a large cluster of phylogenetically related mtDNAs defined by mutations in the coding region; (2) a *subhaplogroup* is a smaller, derived clade within a haplogroup; and (3) a *haplotype/lineage* is a mtDNA that is distinct from another by one or more mutations.

Moving Beyond the Four-Founding Lineage Paradigm

A few early ancient DNA studies (e.g., Hauswirth et al. 1994; Pääbo et al. 1988) made claims of discovering new Native American founding lineages but suffered from failure to sufficiently demonstrate the authenticity of their data.[22] However, the assertion of the existence of new founding lineages was not limited to ancient DNA studies (see studies reviewed by Smith et al. 1999). For example, Easton and colleagues (1996) reported that additional haplogroups, which they called "X6" and "X7," were present among the Yanomama of Brazil and Venezuela. Putatively similar mtDNAs had also been identified in other Native American populations (Merriwether et al. 1994, 1995; Santos et al. 1996; Torroni et al. 1993). These X6/X7 haplotypes had the +*Dde*I 10,394 and +*Alu*I 10,397 sites that occur in Asian macrohaplogroup M but otherwise lacked the diagnostic RFLPs of haplogroups C and D, while differing between themselves by the presence or absence of the *Hae*III 16,517 site.

However, when the HVRI sequences from X6/X7 mtDNAs were analyzed with those from other Native American populations, they clustered within haplogroups C and D (Schurr and Wallace 1999; Stone and Stoneking 1998). These results suggested that X6/X7 mtDNAs were autochthonous haplogroup C and D mtDNAs that had lost the diagnostic markers of these maternal lineages (-*Hinc*II

13,259 and -*Alu*I 5,176, respectively) through back mutations, rather than belonging to an additional founding lineage deriving from haplogroup M. Furthermore, all subsequent studies of Yanomama mtDNA (Hunley et al. 2008; Merriwether et al. 2000) failed to corroborate this discovery, which may have originated from laboratory-based errors.[23]

Nevertheless, it was not clear that all of the nonhaplogroup A–D mtDNAs (or "others") in Native American populations resulted from back mutations, admixture, or lab errors. The bona fide existence of a fifth founding mtDNA haplogroup was demonstrated when Forster and colleagues (1996) discovered that 11 percent of the Nuu-chah-nulth and 5 percent of the Yakima belonged to haplogroup X[24] and, shortly after, similar haplotypes were reported at a frequency of 25 percent in the Ojibwa (Scozzari et al. 1997). Brown and colleagues (1998) further confirmed the presence of haplogroup X mtDNAs in native North Americans by comparing these haplotypes with putatively related mtDNAs from European and Central Asian populations. In addition, from a collection of 70 North American Indian samples previously determined to represent "others," Smith and colleagues (1999) discovered that 32 of them belonged to haplogroup X. These 32 individuals represented members of diverse linguistic groups residing in the Northeast (Algonquian), the Plains (Siouan), the Southwest (Kiowa-Tanoan), and California (Hokan).

While the wide distribution of haplogroup X in North America strongly suggested that it was an additional founding haplogroup, its presence in European populations left open the possibility that it was introduced through admixture after A.D. 1492. However, the form of haplogroup X in the Americas (X2a) was shown to be quite distinct from those found in Europe and Central Asia (X2e) (for nomenclature in haplogroup X, see Reidla et al. 2003), and the diversity within Native American populations suggested that the haplogroup was quite ancient in North America (Brown et al. 1998). Haplogroup X in the Americas prior to European contact was finally demonstrated by its presence in the 1340 ± 40 ^{14}C B.P. (3340 B.P.) human remains discovered near Vantage, Washington (Malhi and Smith 2002).[25] Thus, haplogroup X is now viewed as an additional founder lineage along with haplogroups A, B, C, and D, albeit a "minor" one that has an unusual geographic distribution compared to the other four (Brown et al. 1998; Dornelles et al. 2005; Perego et al. 2009; Smith et al. 1999).

Reevaluating the Five-Founding Lineage Paradigm

Even with the confirmation of haplogroup X in North American Indians, there were still some mtDNAs that did not belong to one of the five founding haplogroups and that required further explanation. In a study of mtDNA variation among the Cayapa of Ecuador, Rickards and colleagues (1999) claimed to have detected an additional Native American founding lineage. They found that approximately 22 percent of the population exhibited an HVRI sequence that did not appear to belong to haplogroups A, B, C, or D, and according to the

authors, the lineage might have been specific to the Cayapa.[26] Their claim failed to find much support, in part because they did not screen the samples for the Native American haplogroup defining RFLPs, excepting the 9-bp deletion (Schurr 2004a). The "Cayapa haplotype" was later reported in one Brazilian (Alves-Silva et al. 2000), among northern Mexicans (Green et al. 2000) and Chilean Yaghan and Mapuche (Moraga et al. 2000) and, through RFLP analysis, was shown to belong to haplogroup D. However, as noted, its associated HVRI sequence was distinct from others typically seen for mtDNAs belonging to this haplogroup. Thus, it appeared to represent a subbranch of haplogroup D that had dramatically diverged in the Americas or possibly another founding haplotype for this haplogroup. However, the evidence was not considered conclusive, leaving this proposed founder haplotype in limbo (Bandelt et al. 2003).

The Cayapa haplotype made a very important appearance some years later in the 10,300-year-old On Your Knees Cave (OYKC) skeletal remains from Prince of Wales Island in southeastern Alaska (Kemp et al. 2007). The discovery of this sequence in remains of such antiquity alone strengthened the case for this being another founder haplotype. In addition, a nearly exhaustive search of published mtDNA data from extant and prehistoric Native Americans available at the time revealed that this haplotype and closely related derivative forms occurred in a mere 47 of 3,286 individuals (1.4 percent), including some from the Chumash, Nahua, and Quechua (see Kemp et al. 2007:Figure 1). Most of these 47 individuals resided near the coast or were located squarely in the West, a pattern that may have resulted from an ancient coastal migration. A number of haplogroup A haplotypes also have a coastal bias in North America, which may also be a signature of humans having migrated along this route (Eshleman et al. 2004).

Regarding the relationships between these individuals, the OYKC individual occupied the central location in a haplotype network, consistent with it being the ancestral form in the Americas (see Kemp et al. 2007:Figure 2). A similar haplotype was also detected in Asia, albeit in only 1 out of 3,824 Asian individuals screened, a Han Chinese from Qingdao (Yao et al. 2002). This evidence satisfied the criteria of Torroni and colleagues (1993:581–582) that founding haplotypes should (1) "… be widespread within the Amerinds and should be shared between tribes because they preceded the Amerind tribal differentiation," (2) ". . . be central to the branching of their haplogroup in the phylogenetic analysis, because all new haplotypes would have originated from them," and (3) " . . . still be possible to detect . . . in East Asian and Siberian populations, because they originated in Asia." The OYKC DNA study added an additional criterion in that "[t]he older a lineage, the higher its probability of representing, or closely resembling, the founding haplotype of the haplogroup of which it is a member" (Kemp et al. 2007:616).

While establishing the "OYKC/Cayapa" haplotype as the founder of a second American D subhaplogroup pushed the field to reexamine the nature of diversity within haplogroups A–D and X, Kemp and colleagues (2007) were unable to determine precisely where the haplotype fit into the ever-changing mtDNA nomenclature.[27] It has since been determined to belong to subhaplogroup "D4h3a" based on whole mtDNA genome sequencing (Perego et al. 2009).

Return to High Resolution mtDNA Studies: Whole Mitochondrial Genome Sequencing

Recently, Native American mtDNA studies have reembraced the high-resolution approach of the late 1980s and early 1990s.[28] However, the new approach represents an improvement over the high-resolution RFLP method for detecting variation in that whole mitochondrial genomes are now being routinely sequenced. To state the obvious, once the entire mtDNA sequence is known, there is no more information to be gained from this genome. This rekindled appreciation for variation likely stems from the discovery of additional founding haplogroups (X2a) and subhaplogroups (D4h3a). In addition, others noted mtDNA structure within the Americas that was suggestive, but not demonstrable proof, of additional Native American founding haplotypes (e.g., Malhi et al. 2002). Lastly, continued improvement in sequence chemistry and analysis meant that longer sequences could be read with more accuracy and processed faster and more cheaply than ever before.

The first of the recent whole mitochondrial DNA studies conducted by Tamm and colleagues (2007) was rather elegant. The authors began with the observations that humans were present in northeastern Siberia at the Yana Rhinoceros Horn Site around 30,000 B.P. (Pitulko et al. 2004) and in South America around 15,000 B.P. (Dillehay 1999). From these observations, they proposed two simple scenarios for the peopling of the Americas: (1) the proto–Native American population resided in Beringia, being isolated from central Asian populations, until conditions permitted entering the Americas (this is called the "Beringian Incubation Model," or BIM[29]) or (2) the proto–Native American population did not reach Beringia until just prior to 15,000 B.P. and subsequently entered the Americas (the "Direct Colonization Model," or DCM).

Each of these scenarios makes predictions as to what should be observed in the genetic record of Native Americans. Under BIM, humans would have carried lineages that accumulated unique mutational changes while in Beringia, that is, prior to entering the Americas. Alternatively, DCM predicts that Native American founding lineages will be essentially identical to haplotypes found in Asian populations. In addition, the structure of mtDNA variation in the Americas would be indicative of the speed at which humans initially spread throughout the continents. If humans spread rapidly, then one would expect to find founding haplotypes shared far and wide. However, if the movement were more protracted, then one would expect to find nested sets of variation in different geographic locations within the American continents.

Through the compilation of 599 earlier published complete mitochondrial genomes from Asia and the Americas and 27 novel sequences from Native Americans, Tamm and colleagues (2007) found evidence that previously unrecognized variation was exhibited by the first humans who entered the Americas. In addition, their analysis helped place the Native American mitochondrial haplotypes in the nomenclature being developed for worldwide populations. They noted that haplogroups A, B, and X were founded by single haplotypes that defined subhaplo-

groups A2, B2, and X2a, respectively. Haplogroup C was founded by three, or possibly four, independent haplotypes defining subhaplogroups C1b, C1c, C1d, and C4c. The last haplotype was detected in only two Ijka speakers from Colombia and, as Tamm and colleagues (2007) suggest, deserves further attention.[30] Haplogroup D was brought to the Americas on two haplotype backgrounds, those of subhaplogroups D1 and D4h3 (for haplogroup, subhaplogroup, and founding haplotype defining mutation, see Tamm et al. 2007:Table 1). More recently, Perego and colleagues (2009) have further clarified on the structure of D4h3 through the analysis of 44 whole genomes belonging to this subhaplogroup. Their study revealed a deep division between the sequences exhibited by Native Americans and that from a single Han Chinese individual (as discussed above, originally discovered in Asia by Yao et al. 2002), respectively referred to as clades D4h3a and D4h3b.

In line with the discussion above (Terminology for mtDNA Variants), Tamm and colleagues (2007) found evidence for five haplogroups present in indigenous American populations (A–D, X) that were introduced by nine founding lineages/haplotypes, each representing one of nine subhaplogroups or clades of haplogroups A, B, C, D, and X. As eight of these subhaplogroups are found widespread in the Americas, these data are consistent with a very rapid spread of humans throughout the American continents. Interestingly, the American haplotypes were a few mutational steps away from their sister Asian haplotypes. That the Native American mutations have not been identified in Asian matrilines lends support to the idea that the proto–Native American population was isolated for some time from other Asian populations, during which it accumulated these new variants (i.e., during an "incubation" period). Tamm and colleagues (2007) also estimated an age of approximately 17,000–10,000 B.P. for A2, B2, C1b, C1c, C1d, and D1, given the amount of diversity observed in these subhaplogroups today. Two additional subhaplogroups, D2a and D3, which possibly arose in the same source population (as will be discussed below), were introduced much later into the Americas, and subhaplogroup A2a was carried back into western Siberia, where it is found today among the Evenks, Koryaks, and Sel'kups (Schurr et al. 1999; Tamm et al. 2007). Overall, their study provided compelling evidence that the initial founders of the Americas emerged from a single Beringian source population.

Within six months[31] of the publication of the Tamm and colleagues (2007) findings, three independent research teams reported results from the collection and/or analysis of whole mitochondrial genomes that generally supported the BIM and/or a single origin for the first Americans. Kitchen and colleagues (2008) compiled sets of previous published data[32] on which they conducted Bayesian skyline plot analyses (Drummond et al. 2005). Based on the coalescent, this analytical tool infers effective population size backward through time from a set of sequence data and has been demonstrated to be an effective means for reconstructing population dynamics. In an updated version of this analysis,[33] Mulligan and colleagues (2008) found that the proto–Native American population was isolated from central Asian populations for 7,000–15,000 years, during which it experienced little or no growth wherever it resided, which was suggested by the authors to have been in Beringia (Kitchen et al. 2008). This part of the demographic profile corresponds to the "incubation" phase proposed by Tamm and

colleagues (2007), during which lineages would have accumulated novel mutations that would differentiate them from other Asian populations. Following this period, the population underwent a bottleneck upon entering the Americas approximately 17,000–16,000 B.P., after which a major reexpansion occurred (Mulligan et al. 2008), likely as a consequence of entering a large landmass that was filled with megafauna and the like but, importantly, not occupied by other humans. Note that while the degree of this bottleneck was likely exacerbated by the paleoclimatic conditions in Beringia, this founder effect, recognized more than twenty years prior by Wallace and colleagues (1985) represents just one of a series of founder effects that shaped the human gene pool since our species exited Africa (Ramachandran et al. 2005).

Similarly, Fagundes, Kanitz, and Bonatto (2008) produced 58 new whole mitochondrial genome sequences from populations in North and South America. Combined with 28 previously published sequences the authors found that subhaplogroups A2, B2, C1, D1, and X2a contained similar levels of variation and are, thus, of similar antiquity in the Americas. Moreover, the study identified Native American "exclusive mutations" in these subhaplogroups that differentiated them from similar haplotypes found in Asia. Again, these findings were consistent with an "incubation" period experienced by the proto–Native American population in Asia. The authors used a mutation rate from Mishmar and colleagues (2003) that estimated the incubation period to have lasted at least 5,000 years. Employing the same Bayesian skyline plot analysis as did Kitchen and colleagues (2008), Fagundes, Kanitz, and Bonatto (2008) further found that proto–Native Americans ended their initial differentiation from Asian populations by undergoing a population bottleneck around 23,000–19,000 B.P. and later rebounded in a population expansion in the Americas 18,000–15,000 B.P.[34]

A study conducted by Achilli and colleagues (2008) provided an additional 14 novel Native American whole mitochondrial sequences. Compared with 107 previously published whole genome sequences and 47 coding region[35] sequences, the authors found that the "common" subhaplogroups A2, B2, C1, and D1 were founded by six haplotypes (A2, B2, C1b, C1c, C1d, and D1). [36] Identical to that noted above, they also discovered that these founding haplotypes were a few mutational steps from similar types in Asian populations, again suggesting that an incubation period preceded the entrance of humans into the Americas. Today, these subhaplogroups exhibit a fair degree of "star-likeness" which resulted from a population expansion, presumably the one that followed shortly after humans first entered the Americas. With one exception (C1d),[37] Achilli and colleagues (2008) estimated the founder haplotypes to be about 18,000–21,000 years in age.

The results of these four recent studies are consistent with BIM. [38] In addition, all of the studies support the concept of a single source population having given rise to most, if not all, of the first inhabitants of the Americas. This consensus stands in opposition to the conclusion that the Americas were populated by multiple "waves of migrations" reached by some, but not all, of the earliest mtDNA studies (see Eshleman et al. 2003; Schurr 2000, 2002, 2004a, 2004b). The inferences drawn from these whole mitochondrial genome studies differ most dramatically in population event dates and age estimates of the founding haplo-

types/haplogroups in the Americas. Nevertheless, the age of current estimates (approximately 20,000–15,000 B.P.) is more in line with the available archaeological data than estimates of 40,000–20,000 B.P. for the entrance of humans into the Americas provided by earlier mtDNA studies (see Eshleman et al. 2003; Schurr 2004a). This result is likely due, in part, to their better approximations of the number of haplotypes present in the founding American population.

Using sequence data to date events in human prehistory continues to be a questionable practice. However, the ability to do so should improve with consideration of the fact that the observed rate of molecular evolution may not be constant over time (Ho et al. 2007; Kemp et al. 2007) and with the use of proper points of calibration (Endicott and Ho 2008). For instance, Ho and Endicott's (2008) reanalysis of the Fagundes and colleagues (2008) and Achilli and colleagues (2008) data using calibration points internal to the human mitochondrial tree produced an average age of 14,000 B.P. for the founding lineages, in this case removing approximately 6,000 years from the original estimates.

The "Other" Genome: Thinking Outside the Mitochondrial DNA Genome

Investigations of Y chromosomal and autosomal variation among Native Americans are also consistent with an origin from a single source population and the Beringian Incubation Model.[39] The Y chromosome is passed intergenerationally through only males and can, therefore, be considered an analogue to mtDNA for tracing male movement/history. By contrast, autosomal DNA (i.e., nonsex chromosomal DNA found in the nucleus) is biparentally inherited and, therefore, reflects both male and female history.

There is a limited amount of Y chromosomal variation exhibited by Native American males compared to males in Asian populations (Bortolini et al. 2003; Karafet et al. 1999, 2001; Lell et al. 2002; Zegura et al. 2004), which is consistent with the notion that the peopling of the Americas was accompanied by a population bottleneck. Similar to mtDNA, Y chromosomal variation is affected more strongly by genetic drift (randomness) than is autosomal variation, because of the smaller effective population size of the Y chromosome. Currently, only Y chromosome haplogroups C and Q are believed to be indigenous to the Americas, with other haplogroups most likely being the product of postcontact admixture (Bolnick et al. 2006; Malhi et al. 2008; Zegura et al. 2004). However, Lell and colleagues (2002) and Bortolini and colleagues (2003) have suggested that certain R1-M173 haplotypes may also have been brought to the Americas by the proto–Native American migrants. R1-M173 haplotypes appear at low frequencies in only indigenous populations of North and Central America, including the Athapaskan-speaking Chipewyans (Bortolini et al. 2003; Lell et al. 2002). Although quite diverse in eastern Siberia, there is some debate as to whether those seen in Native Americans are indigenous in origin, owing to their also being commonly observed in European populations (Lell et al. 2002; Tarazona-Santos and Santos 2002).

One form of haplogroup Q in the Americas, Q3-M242*, is shared with Asian populations. Equivalent to the Native American derived mtDNA lineages, a subbranch of haplogroup Q3-M242*, called Q3-M3*, is widespread in the Americas (Bortolini et al. 2003; Karafet et al. 1999, 2001; Lell et al. 2002; Underhill et al. 1996; Zegura et al. 2004). The defining mutation of this haplogroup likely arose during the peopling of the Americas or slightly beforehand, perhaps during the incubation period, and is known to be at least 10,300 years old in the Americas (Kemp et al. 2007). Q3-M3* is also the most widespread Y-chromosome type in the American continents (Bolnick et al. 2006; Malhi et al. 2008; Underhill et al. 1996; Zegura et al. 2004), and its distribution is consistent with the notion that all Native American males originated from a single source population. Haplogroup Q3-M3* has also been observed among the Chukchi, Evenks, and Siberian Eskimos and was probably introduced into northeastern Siberia through gene flow back across the Bering Strait (Karafet et al. 1997; Lell et al. 1997).

A recently described autosomal marker is also consistent with an origin from a single source population for all Native Americans. Through an analysis of 377 microsatellite markers[40] from the HGPD-CEPH[41] human genome diversity cell line panel, Zhivotovsky and colleagues (2003) discovered that a 275-bp allele at D9S1120[42] was present in all of the Native American populations sampled (Colombian, Karitiana, Maya, Pima, and Surui) yet absent from the other 47 worldwide populations represented in the panel. This allele has nine repeats of the sequence TAGA and, hence, has been nicknamed the "9 repeat allele" (9RA).

In order to better characterize the distribution of the 9RA, Schroeder and colleagues (2007) screened an additional 13 North American populations[43] representing members of "Na-Dene," Aleut-Eskimo, and North American "Amerinds,"[44] in addition to seven Altaic populations.[45] Their results indicated that the 9RA was ubiquitous among Native American populations, being present in approximately 31.7 percent of their samples. They also detected the 9RA in approximately 24 percent of the Chukchi and 18 percent of Koryaks, a finding that again suggested possible gene flow back across the Bering Strait, as discussed above for Y-chromosome haplogroup Q3-M3* and mtDNA subhaplogroup A2a. These results suggested that the 9RA arose during the incubation phase of a single source population, perhaps the "Beringian" population suggested by the mtDNA researchers. More recently, Schroeder and colleagues (2009) found that 91 percent of chromosomes that exhibit the 9RA are flanked by an identical genetic background that spans some 76.26 kilobases, a haplotype they coined the "American Model Haplotype" (AMH). As the remaining 9RA chromosomes share a portion of the AMH, it appears that all chromosomes carrying the marker are identical by descent. Importantly, this indicates that all Native American populations share a large portion of their ancestry, again supporting the notion of them having arisen from a single source population.

While mtDNA subhaplogroups D2a and D3 were later introduced into the Americas and are largely associated with Athapaskans and Aleut-Eskimos (Rubicz et al. 2003; Starikovskaya et al. 1998; Tamm et al. 2007; Zlojutro et al. 2006), 23–39 percent of the individuals in these populations exhibit the 9RA (Schroeder et al. 2007). This evidence lends itself to two possible scenarios: (1) very few women introduced D2a and D3 into the northernmost parts of the Americas such

that they did not disrupt the frequency of the already established 9RA, or (2) the women who carried D2a and D3 to the Americas also carried the 9RA, probably having originated from the same source population that gave rise to the first Americans and the mtDNA and Y-chromosome founding haplotypes.

In explaining the distribution of the 9RA, only models that emphasized substantial isolation of Native American populations from the Old World resulted in similar theoretical expectations for the unusual distribution of such a marker (Schroeder et al. 2009). This view stands in interesting opposition to the recent models presented by Ray and colleagues (2010) that emphasize the importance of gene flow with Asia in shaping the Native American gene pool. Presently, it is unclear how much these observations are affected by having studied Native American populations with substantial non–Native American admixture or, more important, if it can explain the difference between the findings of the two research teams as they studied different sets of populations (Ray et al. 2010; Schroeder et al. 2009). Nevertheless, large-scale modeling such as that presented in Ray and colleagues (2010) will continue to add novel insights about the entrance of humans into the Americas.

Recently, Halverson and Bolnick (2008) have found patterns of ABO blood group[46] variation in pre-Columbian Native American populations to be consistent with a population bottleneck at the time that humans entered the Americas. In a study of the distribution of ABO alleles in contemporary Native American populations from Mesoamerica and South America, Estrada-Mena and colleagues (2010) drew a similar conclusion. As it has long been recognized that most Native American populations are nearly fixed for the O allele (Mourant 1976), the most interesting observation drawn by Estrada-Mena and colleagues (2010) is that a derived O allele, called $O^{1v(G542A)}$, is found in every Native American population screened to date at the sequence level (Nahua, Mazahua, Maya, Mexican mestizos, Cayapa, and Bolivian Aymara). Thus far, this allele has not been observed in Asian populations screened at the same level (Chinese, Japanese, and Korean). Thus, despite the current limited sampling of both Asian and Native American populations, the $O^{1v(G542A)}$ allele may end up being confirmed as yet another Native American specific marker that evolved during the "Beringian Incubation."

Neither of these recent studies on the ABO system in Native Americans has sufficiently addressed the observation that Eskimos and some Athapaskan populations exhibit appreciable frequencies of the A and B alleles (Szathmary 1979). These observations may very well be the product of a secondary expansion into the Americas as described above. Nevertheless, advances in our knowledge of variation in the ABO system at the sequence level (Estrada-Mena et al. 2010) among Native Americans mirrors the attention now placed on whole mtDNA sequencing.

Based on genotyping 36 markers in the vicinity of the 44 exon of the dystrophin gene, Bourgeois and colleagues (2009) described six common X-chromosome haplotypes, called B001–B006 found in worldwide populations. Comparatively, Native American populations have much higher frequencies of haplotypes B004 and B006 and lack haplotype B005. Thus it appears that X-chromosome variation among Native Americans has been similarly shaped by the founder effect associated with the peopling of the Americas (Bourgeois et al. 2009).

Limited but Highly Structured Variation in the Americas

Having examined the evidence concerning the extent of genetic variation that was initially brought to the Americas, it is also worthwhile to explore what has happened to that variation over the past 15,000 years or so. In North America, for example, one finds remarkable mtDNA structure according to geographic regions (Figure 2-1). As argued above, if the proto–Native American population originated from a single source population, then the frequencies depicted on this map suggest that genetic drift was a dominant force shaping mtDNA variation in Native Americans, most likely when populations were very small prior to the Formative period in North America. This structured pattern is also consistent with the idea that "tribalization" began early in the prehistory of the Americas (Malhi et al. 2002; Torroni et al. 1993).

Genetic structure in the nuclear genomes of Native Americans supports just such a scenario. Through a comparison of variation at 678 autosomal microsatellite markers in 422 indigenous individuals from North, Central, and South American populations to thousands of others from global populations, Wang and colleagues (2007:Table 1) found that Native Americans exhibit the highest levels of homozygosity and the largest interpopulation genetic distances (evaluated by F_{ST}). This pattern indicates that, compared with other continental "populations," the Native American gene pool contains the least amount of genetic variation while simultaneously being the most highly structured. This outcome is consistent with the scenario that the proto–Native American population underwent a genetic bottleneck, probably upon entry into the Americas and, after colonizing these continental regions, has been highly subject to genetic drift. The patterning of the continent-wide genomic diversity among Native Americans is consistent with a single major colonization that first entered the Americas via the coast (Wang et al. 2007).

Ancient DNA Studies: Genetic Variation in the Americas Prior to 5000 B.P.

If one could somehow study directly the remains of a population of the first Americans, one would be provided with an unparalleled opportunity to measure the amount of genetic variation carried to the Americas and to test directly for the hypothetical founder lineages. However, no such population has been identified. The finite number of remains that have been discovered in the Americas that are greater than 5,000 years old *and* available for destructive analysis, as is necessary for ancient DNA (aDNA) studies, have traditionally been the limiting factors in this line of scientific inquiry. Moreover, many of the oldest Native American remains no longer contain DNA (Smith et al. 2005).

Even though the physical remains of the very first Americans have not been discovered, and most likely will never be, genetic data recovered from human remains and artifacts that represent ancient populations of great antiquity allow one to get closer and closer to measuring directly the amount of genetic variation that

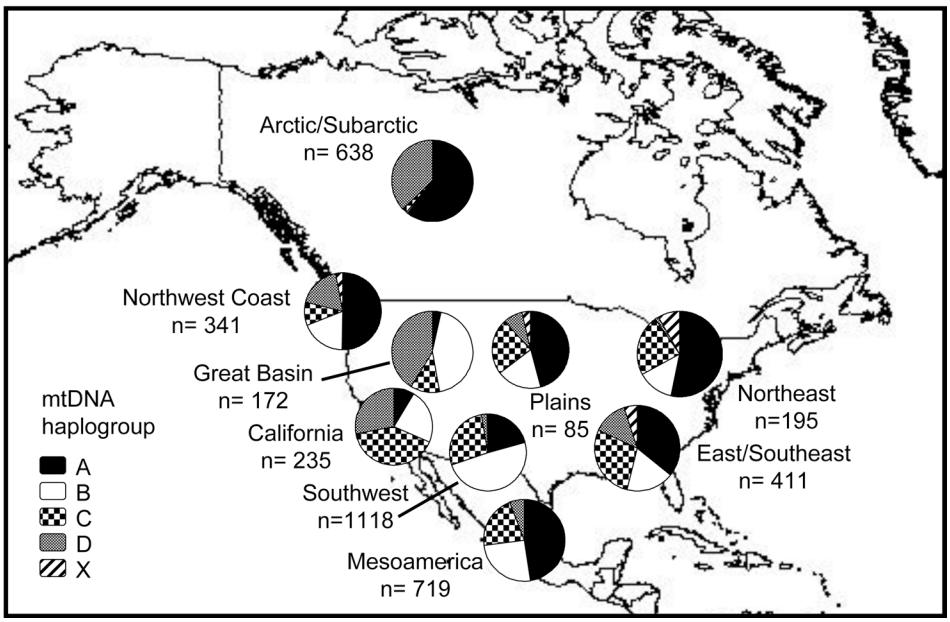

Figure 2-1. *Distribution of mtDNA haplogroup frequencies across North America from various previous studies (Bolnick and Smith 2003; Budowle et al. 2002; Carlyle et al. 2000; De la Cruz et al. 2008; Eshleman 2002; González-Oliver et al. 2001; Green et al. 2000; Huoponen et al. 1997; Johnson and Lorenz 2006; Kaestle and Smith 2001; Kemp et al. 2005, 2010; Lorenz and Smith 1996; Malhi 2001; Malhi et al. 2003, 2004; Merriwether, et al. 1995; Merriwether and Ferrell 1996; Monroe et al. 2006; Parr et al. 1996; Rubicz et al. 2003; Schurr et al. 1990; Scozzari et al. 1997; Shields et al. 1993; Smith et al. 1999; Stone and Stoneking 1998; Torroni et al. 1992, 1993, 1994; Ward et al. 1991, 1993).*

was present in the founding population. This is precisely the reason that Kemp and colleagues (2007) suggested an additional criterion be added to those proposed by Torroni and colleagues (1993) for establishing a lineage as a founder (discussed above under Reevaluating the Five-Founding Lineage Paradigm). In this case, it is important to discuss the available data from remains that predate 5000 B.P., which will be done in chronological order starting with the oldest evidence.[47]

Pleistocene DNA

The oldest human DNA from the Americas has come not from bones or teeth but rather from ancient feces (coprolites). Recently, Gilbert, Jenkins, and colleagues (2008) were able to determine the mitochondrial haplogroups of a number of humans that defecated in Paisley 5 Mile Point Cave in south-central Oregon, on the edge of the Great Basin. Three samples dating around 12,300 ^{14}C B.P. (approximately 14,270–14,000 cal yr B.P.) have revealed the presence of sub-haplogroups A2 and B2. These are extremely important data points as they place

humans in the Americas prior to the Clovis period and prior to the opening of the ice-free corridor. An additional coprolite from the caves, dated to approximately 11,000–10,000 ^{14}C B.P., also revealed the presence of subhaplogroup B2.

Early Holocene DNA (10,300–8000 B.P.)

As previously noted, the earliest presence of D4h3 in the Americas was found in the 10,300–year-old OYKC remains from the northern tip of Prince of Wales Island in southeastern Alaska (Kemp et al. 2007). In addition, molecular sex determination indicated that the individual was male and his Y chromosome belonged to haplogroup Q3-M3*, establishing a minimum date for the emergence of the defining marker of this haplogroup (Kemp et al. 2007). Kaestle and Smith (2001) determined that the 9200 ± 60 ^{14}C B.P. (11,020 B.P.) individual[48] discovered at the Wizards Beach site in western Nevada belonged to haplogroup C. The HVRI sequence of the Wizards Beach individual represents any one of the three proposed founder haplotypes of subhaplogroup C1 (Kaestle 1998; Tamm et al. 2007). The individual found in Hourglass Cave in the Rocky Mountains in Colorado who died approximately 8000 B.P. was determined to be a member of haplogroup B (Stone and Stoneking 1996). Molecular sex typing confirmed the morphological assessment that this individual was male. As in the case of the Wizards Beach individual, the HVRI sequence of Hourglass Cave man also represented the proposed founder haplotype for his haplogroup (Stone and Stoneking 1996; Tamm et al. 2007).

Mid-Holocene DNA (8000–5000 B.P.)

An additional coprolite from Paisley Caves, dated to approximately 6600 ^{14}C B.P. was determined to have been made by a member of subhaplogroup B2 (Gilbert, Jenkins, et al. 2008). At 4950 ± 170 ^{14}C B.P. (6950 B.P.), two individuals from the China Lake site in the British Columbia interior were determined to belong to a form of haplogroup M that has yet to be identified in any extant Native American population (Malhi et al. 2007). As these individuals both exhibited a very rare supratrochlear spur on their left humeri, it was believed by the physical anthropologists who examined the remains that these males were full siblings and possibly twins (see Cybulski et al. 2004, this volume). This haplotype possibly represents a sixth founding haplogroup, one that may have gone extinct in the last 5,000 years or so. However, further analysis of this mtDNA is required to rule out prehistoric gene flow from the Beringian region, where haplogroup M derived lineages not seen in the Americas are common. Given the profound effect that genetic drift has had on the Native American gene pool (Wang et al. 2007), this haplotype may still exist in unstudied populations.

In total, eleven samples that predate 5000 B.P. have yielded well-preserved mtDNA and/or sex chromosomal DNA. Notwithstanding the small sample size and the fact that none of these data represent that of a "population," some trends are worth noting. One of the eleven samples (9.1 percent) belongs to subhaplogroup D4h3, a sublineage or clade that today is exhibited by only 1.4 percent of Native Americans. More noteworthy are the two samples that may represent a haplogroup that has possibly become extinct in the Americas. These observations are consistent with the notion that genetic drift has long been a powerful evolu-

tionary force in the Americas and further indicate that additional genetic variation may be found in the ancient Americas. In fact, this process could account for the observation of "other" haplogroups in many aDNA studies in the Americas conducted in the past 20 years.

However, to date, most of the putative founder lineages and haplotypes suggested in the past have been shown to be either derivatives of haplogroups A–D and X or haplotypes introduced by admixture or the by-products of contamination. Nevertheless, continued aDNA research on very ancient Native American remains and/or coprolites will be crucial for addressing the possibility of additional founding lineages being present in the Americas. It is imperative that when aDNA researchers discover a previously unrecognized form of DNA in the Americas they authenticate the finding and do not simply place it in an "other" category.

An Asian Origin for Certain, but Where? [49]

While all of the genetic studies discussed in this chapter point to an Asian origin for Native Americans, one could ask whether the precision of this conclusion is an improvement over those that were held almost 100 years ago. For example, based on physical characteristics, Hrdlicka (1912:11) argued for an East Asian origin, but noted that

> . . . difficulties arise when we endeavor to assign the origin of the Indian to some particular branch of the yellowish-brown population. We find that he stands quite as closely related to some of the Malaysian peoples as to Tibetans, the Upper Yenisei natives, and some of the northeastern Asiatics.

Prior to the discovery of mitochondrial haplogroup X in the Americas, mtDNA studies suggested either a south-central Siberian (Torroni et al. 1993) or a Mongolian (Kolman et al. 1996; Merriwether et al. 1996) origin for Native Americans, as these populations exhibited haplogroups A, B, C, and D and, importantly, the founder Native American haplotypes of these haplogroups proposed by Torroni and colleagues (1993). The reasoning here was simple in that researchers were looking for the least geographically distant population from the Americas that contained all of the haplogroups exhibited by Native Americans.

After haplogroup X was determined to be an additional Native American founding haplogroup (as discussed above), researchers began looking for a population in Asia that contained all five haplogroups. Derenko and colleagues (2001) discovered haplogroup X in populations of the Altai mountain region in southern Siberia, where haplogroups A, B, C, and D were also present. However, the forms of haplogroup X found in the Altaians (X2e) were intermediate between the sublineages found in North America (X2a) and Europe (also see Brown et al. 1998), being at least two mutational steps away from those in the former and at least one mutational step away from the latter (see Derenko et al. 2001:Figure 1). Nevertheless, the Lake Baikal–Altai mountain region stood as the most probable origin for Native Americans (see, for example, Schurr 2004a:Figures 6 and 7).

The recent whole mitochondrial, nuclear, and Y-chromosomal DNA studies, in concert with the ancient DNA research discussed above, have made it more difficult to determine a precise Asian origin for Native Americans than when less data were available. Ironically, the field has come full circle back to the statement made by Hrdlicka in 1912, as the specific place of origin in Asia remains uncertain. The difference is that, today, we are more confident than ever in our uncertainty. No Asian populations sampled to date contain all of the founder Native American mtDNA and Y-chromosome haplogroups, the 9RA at D9S1120, and the $O^{1v(G542A)}$ allele. To complicate matters further, the closest relatives to the 9RA "AMH" described by Schroeder and colleagues (2009) are the Baloch, Brahui, Burusho, Pathan, and Sindhi populations of southern Asia (Afghanistan, Iran, and Pakistan) that have AMHs with 12 repeats (or 12RA). In total, these observations are hardly surprising, given the strong support for the Beringian Incubation Model discussed above. If this model is correct, then the proto–Native American population accumulated unique mutations when in isolation from other Asian groups prior to entering the Americas, leaving behind populations lacking these genetic variants.

In light of this evidence, the logical question arises as to the fate of this "Beringian" population, the answer to which is far from certain. Did all the members of this population enter the Americas? Is that population now extinct? Either of these scenarios seems unlikely. The best chance of identifying a population in Asia from which Native Americans originated will come from aDNA studies of remains that date around 20,000–15,000 B.P. The view that some population in Asia today will resemble the proto–Native American population is not plausible, as it ignores the fact that populations on the other side of the Bering Strait have undergone an equal degree of genetic evolution since their separation.

Conclusions

The flurry of recent whole mitochondrial genome studies support the Beringian Incubation Model as the best description of the demographic history of the population that would first enter the Americas approximately 20,000–15,000 B.P., having originated from a single source population located somewhere in Asia. Variation exhibited by Native Americans in their nuclear genomes likewise supports this scenario. A human presence in the Americas by at least 14,270–14,000 B.P. has been confirmed by the recovery of human coprolites in the Paisley Caves in southern Oregon. These data indicate that humans must have initially entered the Americas along the Pacific Coast, as the ice-free corridor was not open at this time.

A secondary migration or expansion of humans introduced additional mtDNA haplogroups into the northernmost areas of North America after the last glacial period. However, these migrants either originated from the same source population as did the initial migrants or were so few in number that they did not substantially disrupt the existing pattern of nuclear genetic variation (i.e., at D9S1120).

Since the time that humans entered the Americas, genetic drift has played a substantial role in shaping the Native American gene pool. On a continental

scale, Native Americans exhibit the highest measure of homozygosity, and this is likely the by-product of having undergone a major population bottleneck around the time of settlement of the Americas. Native Americans also exhibit the highest interpopulation genetic distances, probably as a result of maintaining relatively small populations sizes during the Paleo-Indian period in conjunction with the early process of tribalization. Genetic variation thus far detected in human remains and human by-products (e.g., coprolites) that predate 5000 B.P. are consistent with this view, notwithstanding the small sample sizes.

As for how these conclusions can be reconciled with studies of cranial morphological variation found in the Americas is not certain. It may be that the only way to directly test for the presence of an earlier migration that did not contribute genes to extant Native Americans is through aDNA studies.

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Notes

1. Most of the genetic markers targeted for reconstructing population history are effectively neutral, having little or no effect on the phenotype.

2. However, major debate continues over the proper use of the molecular clock to date these events (see recent debate between Bandelt [2008] and Howell and colleagues [2008]).

3. The minimal span of the Clovis archaeological complex is now dated from 13,125–12,925 B.P., a mere 200 years (Waters and Stafford 2007). Note that all dates in this chapter are given in calendar years, unless otherwise noted.

4. While pre-Clovis human remains have yet to be discovered, the feces they left in dry rock shelters are apparently quite abundant. The oldest human remains thus far discovered in the Americas, the Arlington Springs "Man" from Santa Rosa Island off the coast of Santa Barbara, California, are Clovis in age (Johnson et al. 2002).

5. Ironically, the location of Arlington Springs "Man," the oldest skeletal remains discovered in the Americas, suggests that Native Americans must have used boats in Clovis times to be able to reach the Channel Islands.

6. Studies that have been conducted since the time of the last major review papers on the peopling of the Americas from a genetic perspective (Eshleman et al. 2003; Mulligan et al. 2004; Schurr 2000, 2002, 2004a).

7. *Ava*II, *Bam*HI, *Hae*II, *Hinc*II, *Hpa*I, and *Hpa*II

8. *Hinc*II morph 6

9. Schurr and colleagues (1990) noted that the Pima sample also included some Papago (or Tohono O'odham), a genetically and linguistically closely related population (Kemp et al. 2010; Malhi et al. 2003). This fact was not noted by Wallace and colleagues (1985), despite their using the same collection of samples.

10. These were the same six enzymes used by Wallace and colleagues (1985), with the exception that *Msp*I was used in place of *Hpa*II. As these two enzymes recognize and cut the same sequence CCGG, there is no difference to the approach of Schurr and colleagues (1990).

11. These enzymes included *Alu*I, *Dde*I, *Hha*I, *Hinf*I, *Mbo*I, *Rsa*I, and *Taq*I.

12. In this study, *haplotype* was defined as a unique combination of restriction enzyme recognition sites and length variants at the COII/tRNALys intergenic region. Haplotypes that shared phylogenetically important mutations (RFLPs) were assigned to a specific "haplotype group," or haplogroup for short. Interestingly, Schurr and colleagues (1990) grouped haplotype "AM4" with others that exhibited the 9-bp deletion, which today are known to belong to haplogroup B. Subsequent analysis indicated that this haplotype should actually be placed in the haplogroup containing haplotypes "AM6–9" (confirmed in the hypervariable region sequence data reported by Torroni and colleagues [1993]), namely, haplogroup A. Therefore, haplotype AM4 is a member of haplogroup A that independently gained the 9-bp deletion. The last twenty years of research has demonstrated that the 9 bp deletion is prone to homoplasy, having arisen multiple times in unrelated mtDNA lineages around the world (see studies discussed by Schurr and Wallace [2002] and Kemp and colleagues [2005]).

13. *Amerind* is a proposed linguistic macrogroup comprised of all Native American languages that do not belonging to Na-Dene or Aleut-Eskimo (Greenberg et al. 1986). This classification is not supported by all linguists (Campbell 1997; Nichols 1990), and molecular studies have failed to find genetic unity of populations classified as Amerind, to the exclusion of those classified as Na-Dene or Aleut-Eskimo (Bolnick et al. 2004; Hunley and Long 2005). While we use the term *Amerind* here to contextualize the earliest Native American mtDNA studies, the category of Amerind is not a meaningful genetic unit for Native American populations and we recommend that its use should be otherwise abandoned.

14. For a total of 14 restriction enzymes: *Alu*I, *Ava*II, *Bam*HI, *Dde*I, *Hae*II, *Hae*III, *Hha*I, *Hinf*I, *Hinc*II, *Hpa*I, *Hpa*II/*Msp*I, *Mbo*I, *Rsa*I, and *Taq*I.

15. A rough calculation, given the 16,569 bp length of the reference sequence (Anderson et al. 1981; Andrews et al. 1999).

16. Torroni and colleagues (1992) used the letters A, B, C, and D, but called them "clusters," whereas Torroni and colleagues (1993) later adopted the term *haplogroup*, which is the preferred term today. Naming mitochondrial DNA lineages has come a long way in the past 20 years, but still suffers from inconsisten-

cies in nomenclature. The high-resolution data obtained through the continued collection of whole genome sequences from worldwide populations will likely alleviate this problem by facilitating adoption of a standardized nomenclature system, such as that used to name Y-chromosome lineages (YCC 2002).

17. The Kuna of Panama were the only Amerind population to contain fewer than three haplogroups, being fixed for haplogroup A (Torroni et al. 1993).

18. *Na-Dene* is another questionable linguistic grouping, particularly with the inclusion of Haida (Campbell 1997), with equivocal evidence of being genetically distinct (Malhi et al. 2004 and references therein. See note 19 about admixture in the Haida.). However, Na-Dene as Athapaskan-Tlingit-Eyak has found support among linguists as having possible links to the Yeniseic language family in Siberia (Vajda 2010) and, thus, is far less controversial than *Amerind*.

19. It is interesting to note that in their abstract, Torroni and colleagues (1993:563) state "[t]his analysis revealed the presence of four haplotype groups (haplogroups A, B, C, and D) in the Amerind but only *one* haplogroup (A) in the Na-Dene, and confirmed the independent origins of the Amerinds and the Na-Dene" [emphasis ours]. However, they observed (1993:Table 2, Table 4) that, in addition to exhibiting haplogroup A, (1) Navajo contain haplogroup B, (2) Apache contain haplogroups B, C, and D, and (3) Haida contain haplogroup D. Torroni and colleagues (1993) suggested that these haplotypes were acquired by the Haida, Navajo, and Apache through admixture with Amerind populations. Indeed, subsequent work confirmed that many of the nonhaplogroup A mtDNAs were introduced into the southern Athapaskans (Navajo and Apache) through gene flow with indigenous Southwest populations (Malhi et al. 2003, 2008; Smith et al. 2000). However, one of the Apache haplogroup D samples sequenced by Torroni and colleagues (1993) belongs to subhaplogroup D2, a mtDNA lineage originating from the north. This type is found among Aleuts and Eskimos (Derbeneva et al. 2002; Rubicz et al. 2003; Starikovskaya et al. 1998) and has most recently been discovered in the 3,500–4,500-year-old frozen hair of a Palaeo-Eskimo from Greenland (Gilbert, Kivisild, et al. 2008). Therefore, the Na-Dene populations are not genetically monotypic with regard to mitochondrial haplogroups, as originally argued by Torroni and colleagues (1993).

20. Here a lineage is referred to as a *haplotype*. In the example of the study conducted by Torroni and colleagues (1993), four founding mtDNA haplotypes (maternal lineages) that just happened to represent four different haplogroups were detected. Today there are still four "major" haplogroups in the Americas, plus a minor one, X. However, multiple founding haplotypes representing subhaplogroups of the five founding lineages appear to have been carried to the Americas (Tamm et al. 2007). The distinction is subtle but extremely important, as the accuracy in reconstructing the evolutionary history of Native Americans hinges partly on the estimated amount of variation introduced into the Americas. In this case, it is important to know that haplogroup D was originally introduced in the form of more than one haplotype, as the mutational steps that separate them would have occurred in Asia, not in the Americas (Kemp et al. 2007).

21. See, for example, the necessary measures taken by Kemp and colleagues (2007) to compare D-loop sequences collected over a 14-year period.

22. From the results of the Little Salt Springs study (Pääbo et al. 1988), it is clear which haplogroup the young woman did not belong to rather than which haplogroup she did. None of the Windover sequences produced by Hauswirth and colleagues (1994) resemble those of Native Americans or those derived from Asia; instead they most closely resemble European mtDNA types, probably as a result of modern DNA contamination.

23. The possibility that X6 and X7 were the result of laboratory error is suggested here because of the overall poor quality of sequence data presented in the research report, which was scientifically scrutinized by Bandelt and colleagues (2002).

24. Not to be confused with X6 and X7 reported by Easton and colleagues (1996).

25. Prior to the Malhi and Smith (2002) study, Stone and Stoneking (1998) noted that one individual from the ancient Norris Farms Oneota had a HVRI sequence that probably belonged to haplogroup X. However, they did not confirm this by screening the individual's mtDNA genome for the coding region mutations that define haplogroup X.

26. The basic HVRI motif of the Cayapa haplotype is 16223(T), 16241(G), 16301(T), 16342(C), and 16362(C), relative to the reference sequence (Anderson et al. 1981; Andrews et al. 1999).

27. For the reasons discussed in note 16.

28. There were only a few studies between 2007 and the late 1980s and early 1990s that went beyond simply screening the haplogroup defining markers and/or sequencing variable portions and lengths of the D-loop (e.g., Bandelt et al. 2003; Derbeneva et al. 2002; Silva Jr. et al. 2002).

29. The Beringian Incubation Model was inspired by the works of Emöke J. E. Szathmary, as cited by Bonatto and Salzano (1997).

30. The status of C4c as an additional founder lineage is strengthened by its uniqueness to those C4 haplotypes found in Asia (Tamm et al. 2007). It has recently been detected among the Shuswap of British Columbia (Malhi et al. 2010).

31. Tamm and colleagues (2007) published on September 5, Kitchen and colleagues (2008) on February 13, Fagundes and colleagues (2008) on February 28, and Achilli and colleagues (2008) on March 12. It was a truly amazing occurrence that all four of these research teams published virtually simultaneously on a single scenario for the peopling of the Americas.

32. These data consisted of 77 coding region sequences.

33. Fagundes, Kanitz, and Bonatto (2008) found that the use of admixed individuals by Kitchen and colleagues (2008) had skewed their analysis. In this case, Mulligan and colleagues (2008) removed these data and reconducted the Bayesian skyline analysis.

34. While the scenario proposed by Fagundes and colleagues (2008) is similar to that of Kitchen and colleagues (2008), the dates do conflict. The likely sources of these discrepancies are the different data sets and priors used in their respective analyses.

35. Representing the mitochondrial genome sans the D-loop sequence.

36. At first glance, it may appear that Achilli and colleagues (2008) dismiss C4c, D4h3, and X2a as founder lineages because they are not mentioned in the abstract as "successful" founders. However, upon closer inspection, their study focused on exploring diversity in the more common haplogroups. The corresponding author of the study, Antonio Torroni, confirmed that their research team consider C4c, D4h3, and X2a "successful" founders, pointing out that those haplotypes highlighted in red in their Figure 1 are all considered founders (personal communication, August 30, 2008).

37. Achilli and colleagues (2008) estimated an age of 10,900 ± 2900 B.P. for C1d, which is similar to the 9500 ± 3400 B.P. age estimated by Tamm and colleagues (2007).

38. At the 2008 Society for American Archaeology conference in Vancouver, British Columbia, many archaeologists were not swayed by these recent molecular studies and cautioned that there is no evidence for this occupation of Beringia. Notwithstanding much of Beringia today is under water, "Beringia" as part of the "Beringian Incubation Model" need not mean Beringia proper. The molecular data suggest only that the proto–Native American population that resided somewhere in Asia *must* have been isolated. To geneticists, Beringia seemed too perfect a homeland.

39. Evidence from the nuclear genome will not be discussed in the same detail as was the mtDNA evidence.

40. Microsatellites, also known as short tandem repeats (STRs), are genetic markers consisting of repeated short sequences, such as GATA/GATA/GATA. Alternate alleles, or forms, of these markers are recorded by their lengths and/or repeat number, which ultimately dictate their length. Microsatellites exhibit numerous alleles and are, therefore, useful for identification purposes, as it is unlikely that two humans will have the same repeat profiles over a number of these markers. STRs are the basis for DNA profiling.

41. Human Genome Diversity Project-Centre d'Etude du Polymorphisme Humain

42. D9S1120 stands for DNA 9th-Chromosome Segment 1120.

43. Aleut, Inuit, Apache, Dogrib, Cherokee, Chippewa, Huichol, Mixtec, Northern Paiute, Sioux, Seri, Jemez, and Creek.

44. Schroeder and colleagues (2007) sought to test whether the allele crosscut the linguistic divisions proposed by Greenberg and colleagues (1986). See note 13 for a discussion about *Amerind*.

45. Chukchi, Koryaks, Evenk, "Southern Altai," "Northern Altai," Altai Kazakh, and Mongolians.

46. The ABO blood groups are coded for by alternate alleles at a genetic locus on the ninth chromosome.

47. In this section of the paper, we report dates as they are found in the original publications. That is, we have not done our own conversions from radiocarbon years to calendar years. In this case, for example, we discuss the On Your Knees Cave remains that date to 10,300 B.P. (Kemp et al. 2007) prior to those of the Wizards Beach remains that date to 9200 ± 60 14C B.P. (Kaestle and Smith 2001), even though in calendar years the latter may be of equivalent or greater antiquity.

48. The Wizards Beach individual was identified as Museum ID Ahur 2023 (Kaestle and Smith 2001:Table 3).

49. For a more detailed discussion of genetic evidence available by 2001 for the Asian origin(s) of Native Americans, see Schurr (2004b).

References

Achilli, Alessandro, Ugo A. Perego, Claudio M. Bravi, Michael D. Coble, Qing-Peng Kong, Scott R. Woodward, Antonio Salas, Antonio Torroni, and Hans-Jürgen Bandelt
2008 The Phylogeny of the Four Pan-American mtDNA Haplogroups: Implications for Evolutionary and Disease Studies. PLoS ONE 3(3):e1764. doi:10.1371/journal.pone.0001764.

Alves-Silva, Juliana, Magda da Silva Santos, Pedro E. M. Guimarães, Alessandro C. S. Ferreira, Hans-Jürgen Bandelt, Sérgio D. J. Pena, and Vania Ferreira Prado
2000 The Ancestry of Brazilian mtDNA Lineages. *American Journal of Human Genetics* 67:444–461.

Anderson, S., A. T. Bankier, B. G. Barrel, M. H. L. De Bruijn, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, P. H. Schreier, A. J. H. Smith, R. Staden, and I. G. Young
1981 Sequence and Organization of the Human Mitochondrial Genome. *Nature* 290: 457–465.

Andrews, Richard M., Iwona Kubacka, Patrick F. Chinnery, Robert N. Lightowlers, Douglass M. Turnbull, and Neil Howell
1999 Reanalysis and Revision of the Cambridge Reference Sequence for Human Mitochondrial DNA. *Nature Genetics* 23:147.

Bandelt, Hans-Jürgen
2008 Clock Debate: When Times Are A-changin': Time Dependency of Molecular Rate Estimates: Tempest in a Teacup. *Heredity* 100:1–2.

Bandelt, Hans-Jürgen, Corinna Herrnstadt, Yong-Gang Yao, Qing-Pong Kong, Toomas Kivisild, Chiara Rengo, Rosaria Scozzari, M. Richards, Richard Villems, Vincent Macaulay, Neil Howell, Antonio Torroni and Ya-Ping Zhang
2003 Identification of Native American Founder mtDNAs Through the Analysis of Complete mtDNA Sequences: Some Caveats. *Annals of Human Genetics* 67:512–524.

Bandelt, Hans-Jürgen, Lluís Quintana-Murci, Antonio Salas, and Vincent Macaulay
2002 The Fingerprint of Phantom Mutations in Mitochondrial DNA Data. *American Journal of Human Genetics* 71:1150–60.

Blanc, Hugues, Kuang-Ho Chen, Melanie A. D'Amore, and Douglas C. Wallace
1983 Amino Acid Change Associated With the Major Polymorphic Hinc II Site of Oriental and Caucasian Mitochondrial DNAs. *American Journal of Human Genetics* 35:167–76.

Bolnick, Deborah A., Daniel I. Bolnick, and David Glenn Smith
2006 Asymmetric Male and Female Genetic Histories among Native Americans from Eastern North America. *Molecular Biology and Evolution* 23:2161–2174.

Bolnick, Deborah A., Beth A. Shook, Lyle Campbell, and Ives Goddard
2004 Problematic Use of Greenberg's Linguistic Classification of the Americas in Studies of Native American Genetic Variation. *American Journal of Human Genetics* 75:519–522.

Bolnick, Deborah. A., and David. G. Smith
2003 Unexpected Patterns of Mitochondrial DNA Variation among Native Americans from the Southeastern United States. *American Journal of Physical Anthropology* 122:336-354.
Bonatto, Sandro L., and Francisco M. Salzano
1997 A Single and Early Migration for the Peopling of the Americas Supported by Mitochondrial DNA Sequence Data. *Proceedings of the National Academy of Sciences of the United States of America* 94:1866–1871.
Bortolini, Maria C., Francisco M. Salzano, Mark G. Thomas, S. Stuart, S. P. Nasanen,
C. H. Bau, M. H. Hutz, Z. Layrisse, Maria Luiza Petzl-Erler, Luiza T. Tsuneto,
Kim Hill, Ana M. Hurtado, D. Castro-de-Guerra, M. M. Torres, H. Groot, R. Michalski,
P. Nymadawa, Gabriel Bedoya, N. Bradman, Damian Labuda, and Andres Ruiz-Linares
2003 Y-chromosome Evidence for Differing Ancient Demographic Histories in the Americas. *American Journal of Human Genetics* 73:524–539.
Bourgeois, S., V. Yotova, Sijia Wang, S. Bourtoumieu, C. Moreau, R. Michalski,
J. P. Moisan, Kim Hill, Ana M. Hurtado, Andres Ruiz-Linares, and Damian Labuda
2009 X-Chromosome Lineages and the Settlement of the Americas. *American Journal of Physical Anthropology* 140:417–428.
Brown, Michael D., Seyed H. Hosseini, Antonio Torroni, Hans-Jürgen Bandelt, Jon C.
Allen, Theodore G. Schurr, Rosaria Scozzari, Fulvio Cruciani, and Douglas C. Wallace
1998 mtDNA Haplogroup X: An Ancient Link Between Europe/Western Asia and North America? *American Journal of Human Genetics* 63:1852–1861.
Brown, Wesley M., Matthew George Jr., and Allan C. Wilson
1979 Rapid Evolution of Animal Mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America* 74:1967–1971.
Budowle, Bruce, Marc W. Allard, Constance L. Fisher, Alice R. Isenberg, Keith L.
Monson, John E. B. Stewart, Mark R. Wilson, and Kevin W. P. Miller
2002 HVI and HVII Mitochondrial DNA Data in Apaches and Navajos. *International Journal of Legal Medicine* 116:212–215.
Campbell, Lyle
1997 *American Indian Languages*. Oxford University Press, New York.
Cann, Rebecca L., Mark Stoneking, and Allan C. Wilson
1987 Mitochondrial DNA and Human Evolution. *Nature* 325:31–36.
Carlyle, Shawn W., Ryan L. Parr, M. Geoffrey Hayes, and Dennis H. O'Rourke
2000 Context of Maternal Lineages in the Greater Southwest. *American Journal of Physical Anthropology* 113:85–101.
Cavalli-Sforza, L. Luca, Paolo Menozzi, and Alberto Piazza
1994 *The History and Geography of Human Genes*. Abridged paperback edition. Princeton University Press, Princeton, New Jersey.
Crawford, Michael H.
1998 *The Origins of Native Americans: Evidence from Anthropological Genetics*. Cambridge University Press, Cambridge.
Cybulski, Jerome S., Harold Harry, Alan D. McMillan, and Scott Cousins
2004 The China Lake and Big Bar Projects: Community Based Research in Physical Anthropology and Mortuary Archaeology. Ms. 4503, Library and Archives, Canadian Museum of Civilization, Gatineau.
De la Cruz, Isabel, Angelica González-Oliver, Brian M. Kemp, Juan A. Román,
David G. Smith, and Alfonso Torre-Blanco
2008 Sex Identification of Infants Sacrificed to the Ancient Aztec Rain Gods in Tlatelolco. *Current Anthropology* 49:519–526.

Derbeneva, Olga A., Rem I. Sukernik, Natalia V. Volodko, Seyed H. Hosseini, Marie T. Lott, and Douglas C. Wallace
2002 Analysis of Mitochondrial DNA Diversity in the Aleuts of the Commander Islands and Its Implictions for the Genetic History of Beringia. *American Journal of Human Genetics* 71:415–421.

Derenko, Miroslava V., Tomasz Grzybowski, Boris A. Malyarchuk, Jakub Czarny, Danuta Miscicka-Sliwka, and Ilia A. Zakharov
2001 The Presence of Mitochondrial Haplogroup X in Altaians from South Siberia. *American Journal of Human Genetics* 69:237–241.

Dillehay, Tom D.
1999 Late Pleistocence Cultures of South America. *Evolutionary Anthropology* 7:206–216.

Dornelles, Claudia L, Sandro L. Bonatto, Loreta B. de Freitas, and Francisco M. Salzano
2005 Is Haplogroup X Present in Extant South American Indians? *American Journal of Physical Anthropology* 127:439–448.

Drummond, Alexei. J., Andrew Rambaut, Beth Shapiro, and Oliver G. Pybus
2005 Bayesian Coalescent Inference of Past Population Dynamics from Molecular Sequences. *Molecular Biology and Evolution* 22:1185–1192.

Easton, Ruth D., D. Andrew Merriwether, Douglas E. Crews, and Robert E. Ferrell
1996 mtDNA Variation in the Yanomami: Evidence for Additional New World Founding Lineages. *American Journal of Human Genetics* 59:213–225.

Endicott, Phillip, and Simon Y. Ho
2008 A Bayesian Evaluation of Human Mitochondrial Substitution Rates. *American Journal of Human Genetics* 82:895–902.

Eshleman, Jason A.
2002 Mitochondrial DNA and Prehistoric Population Movements in Western North America. Ph.D. dissertation, Department of Anthropology, University of California, Davis.

Eshleman, Jason A., Ripan S. Malhi, John R. Johnson, Frederika A. Kaestle, Joseph Lorenz, and David Glenn Smith
2004 Mitochondrial DNA and Prehistoric Settlements: Native Migrations on the Western Edge of North America. *Human Biology* 76:55–75.

Eshleman, Jason A., Ripan S. Malhi, and David Glenn Smith
2003 Mitochondrial DNA Studies of Native Americans: Conceptions and Misconceptions of the Population Prehistory of the Americas. *Evolutionary Anthropology* 12:7–18.

Estrada-Mena, Benito, Javier Estrada, Raúl Ulloa-Arvizu, Miriam Guido, Rocío Méndez, Ramón Coral, Thelma Canto, Julio Granados, Rodrigo Rubí-Castellanos, Héctor Rangel-Villalobos, and Alejandro García-Carrancá
2010 Blood Group O Alleles in Native Americans: Implications in the Peopling of the Americas. *American Journal of Physical Anthropology* 142:85–94.

Fagundes, Nelson J., Ricardo Kanitz, and Sandro L. Bonatto
2008 A Reevaluation of the Native American mtDNA Genome Diversity and Its Bearing on the Models of Early Colonization of Beringia. PLoS ONE 3(9):e3157. doi:10.1371/journal.pone.0003157.

Fagundes, Nelson J. R., Ricardo Kanitz, Roberta Eckert, Ana C. S. Valls, Mauricio R. Bogo, Francisco M. Salzano, David Glenn Smith, Wilson A. Silva Jr., Marco A. Zago, Andrea K. Ribeiro-dos-Santos, Sidney E. B. Santos, Maria Luiza Petzl-Erler, and Sandro L. Bonatto
2008 Mitochondrial Population Genomics Supports a Single Pre-Clovis Origin with

a Coastal Route for the Peopling of the Americas. *American Journal of Human Genetics* 82:583–592.

Fewkes, J. Walter, Ales Hrdlicka, William H. Dall, James W. Gidley, Austin Hobart Clark, William H. Holmes, Alice C. Fletcher, Walter Hough, Stansbury Hagar, Paul Bartsch, Alexander F. Chamberlain, and Roland B. Dixon

1912 The Problems of the Unity or Plurality and the Probable Place of Origin of the American Aborigines. *American Anthropologist* 14:1–59.

Fiedel, Stuart J.

2000 The Peopling of the New World: Present Evidence, New Theories, and Future Directions. *Journal of Archaeological Research* 8:39–103.

Forster, Peter

2004 Ice Ages and the Mitochondrial DNA Chronology of Human Dispersals: A Review. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 359:255–264.

Forster, Peter, Rosalind Harding, Antonio Torroni, and Hans-Jürgen Bandelt

1996 Origin and Evolution of Native American mDNA Variation: A Reappraisal. *American Journal of Human Genetics* 59:935–945.

Gilbert, M. Thomas P., Dennis L. Jenkins, Anders Götherström, Nuria Naveran, Juan J. Sanchez, Michael Hofreiter, Philip Francis Thomsen, Jonas Binladen, Thomas F. G. Higham, Robert M. Yohe II, Robert Parr, Linda Scott Cummings, and Eske Willerslev

2008 DNA from Pre-Clovis Human Coprolites in Oregon, North America. *Science* 320:786–789.

Gilbert, M. Thomas P., Toomas Kivisild, Bjarne Grønnow, Pernille K. Andersen, Ene Metspalu, Maere Reidla, Erika Tamm, Erik Axelsson, Anders Götherström, Paula F. Campos, Morten Rasmussen, Mait Metspalu, Thomas F. G. Higham, Jean-Luc Schwenninger, Roger Nathan, Cees-Jan De Hoog, Anders Koch, Lone Nukaaraq Møller, Claus Andreasen, Morten Meldgaard, Richard Villems, Christian Bendixen, and Eske Willerslev

2008 Paleo-Eskimo mtDNA Genome Reveals Matrilineal Discontinuity in Greenland. *Science* 320:1787–1789.

Giles, Richard E., Hugues Blanc, Howard M. Cann, and Douglas C. Wallace

1980 Maternal Inheritance of Human Mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America* 77:6715–6719.

Ginther, C., D. Corach, G. A. Penacino, J. A. Rey, F. R. Carnese, M. H. Hutz, A. Anderson, J. Just, Francisco M. Salzano, and M. C. King

1993 Genetic Variation among the Mapuche Indians from the Patagonian Region of Argentina: Mitochondrial DNA Sequence Variation and Allele Frequencies of Several Nuclear Genes. *Exs* 67:211–219.

Goebel, Ted, Michael R. Waters, and Dennis H. O'Rourke

2008 The Late Pleistocene Dispersal of Modern Humans in the Americas. *Science* 319:1497–1502.

Gonzalez-Jose, Rolando, Maria C. Bortolini, F. R. Santos, and Sandro L. Bonatto

2008 The Peopling of America: Craniofacial Shape Variation on a Continental Scale and Its Interpretation from an Interdisciplinary View. *American Journal of Physical Anthropology* 137:175–187.

González-Oliver, Angelica, Lourdes Marquez-Morfin, Jose C. Jimenez, and Alfonso Torre-Blanco

2001 Founding Amerindian Mitochondrial DNA Lineages in Ancient Maya from Xcaret, Quintana Roo. *American Journal of Physical Anthropology* 116:230–235.

Green, Lance D., James N. Derr, and Alec Knight
2000 mtDNA Affinities of the Peoples of North-Central Mexico. *American Journal of Human Genetics* 66:989–998.
Greenberg, Joseph H., Christy G. Turner II, and Stephan L. Zegura
1986 The Settlement of the Americas: A Comparison of the Linguistic, Dental, and Genetic Evidence. *Current Anthropology* 27:477–497.
Halverson, Melissa S., and Deborah A. Bolnick
2008 An Ancient DNA Test of a Founder Effect in Native American ABO Blood Group Frequencies. *American Journal of Physical Anthropology* 137:342–347.
Hauswirth, William W., Cynthia D. Dickel, D. J. Rowold, and A. Hauswirth
1994 Inter- and Intrapopulation Studies of Ancient Humans. *Experientia* 50:585–591.
Haynes, Gary, David G. Anderson, Reid Ferring, Stuart J. Fiedel, Donald K. Grayson, C. Vance Haynes Jr., Vance T. Holliday, Bruce B. Huckell, Marcel Kornfeld, David J. Meltzer, Julie Morrow, Todd Surovell, Nicole M. Waguespack, Peter Wigand, and Robert M. Yohe II
2007 Comment on "Redefining the Age of Clovis: Implications for the Peopling of the Americas." *Science* 317:320; author reply 320.
Hertzberg, M., K. N. Mickleson, S. W. Serjeantson, J. F. Prior, and R. J. Trent
1989 An Asian-Specific 9-bp Deletion of Mitochondrial DNA Is Frequently Found in Polynesians. *American Journal of Human Genetics* 44:504–510.
Ho, Simon Y., and Phillip Endicott
2008 The Crucial Role of Calibration in Molecular Date Estimates for the Peopling of the Americas. *American Journal of Human Genetics* 83:142–146; author reply 146–147.
Ho, Simon Y. W., Beth Shapiro, Matthew J. Phillips, Alan Cooper, and Alexei J. Drummond
2007 Evidence for Time Dependency of Molecular Rate Estimates. *Systematic Biology* 56:515–522.
Howell, Neil, Corrina Howell, and Joanna L. Elson
2008 Molecular Clock Debate: Time Dependency of Molecular Rate Estimates for mtDNA: This Is Not the Time for Wishful Thinking. *Heredity* 101:107–108.
Hrdlicka, Ales
1912 The Bearing of Physical Anthropology on the Problems Under Consideration. *American Anthropologist* 14:8–12.
Hunley, Keith, and Jeffrey C. Long
2005 Gene Flow Across Linguistic Boundaries in Native North American Populations. *Proceedings of the National Academy of Sciences of the United States of America* 102:1312–1317.
Hunley, Keith L., J. E. Spence, and D. Andrew Merriwether
2008 The Impact of Group Fissions on Genetic Structure in Native South America and Implications for Human Evolution. *American Journal of Physical Anthropology* 135:195–205.
Huoponen, Kirsi, Antonio Torroni, P. R. Wickman, Daniele Sellitto, D. S. Gurley, Rosaria Scozzari, and Douglas. C. Wallace
1997 Mitochondrial DNA and Y Chromosome-Specific Polymorphisms in the Seminole Tribe of Florida. *European Journal of Human Genetics* 5:25–34.
Ingman, Max, Henrik Kaessmann, Svante Pääbo, and Ulf Gyllensten
2000 Mitochondrial Genome Variation and the Origin of Modern Humans. *Nature* 408:708–713.
Johnson, John R., and Joseph G. Lorenz
2006 Genetics, Linguistics, and Prehistoric Migrations: An Analysis of California In-

dian Mitochondrial DNA Lineages. *Journal of California and Great Basin Anthropology* 26(1):31–62.

Johnson, John R., Thomas W. Stafford, Henry O. Aije, and Don P. Morrise
2002 Arlington Springs Revisited. *Proceedings of the Fifth California Islands Symposium*, edited by David R. Browne, Kathryn L. Mitchell, and Henry W. Chaney, pp. 541–545. Santa Barbara Museum of Natural History.

Kaestle, Frederika Ann
1998 Molecular Evidence for Prehistoric Native American Population Movement: The Numic Expansion. Ph.D. dissertation, Department of Anthropology, University of California, Davis.

Kaestle, Frederika A., and David Glenn Smith
2001 Ancient Mitochondrial DNA Evidence for Prehistoric Population Movement: The Numic Expansion. *American Journal of Physical Anthropology* 115:1–12.

Karafet, Tatiana, L. Xu, R. Du, W. Wang, S. Feng, R. S. Wells, Alan J. Redd, Stephen L. Zegura, and Michael F. Hammer
2001 Paternal Population History of East Asia: Sources, Patterns, and Microevolutionary Processes. *American Journal of Human Genetics* 69:615–628.

Karafet, Tatiana M., Stephen L. Zegura, Olga Posukh, Ludmila Osipova, A. Bergen, Jeffery Long, D. Goldman, William Klitz, Shinji Harihara, Peter de Knijff, Victor Wiebe, R. C. Griffiths, Alan R. Templeton, and Michael F. Hammer
1999 Ancestral Asian Source(s) of New World Y-Chromosome Founder Haplotypes. *American Journal of Human Genetics* 64:817–831.

Karafet, Tatiana, Stephen L. Zegura, Jennifer Vuturo-Brady, Olga Posukh, Ludmila Osipova, Victor Wiebe, Francine Romero, Jeffery C. Long, Shinji Harihara, Feng Jin, Bumbein Dashyam, Tudevdagva Gerelsaikhan, Keiichi Omoto, and Michael F. Hammer
1997 Y Chromosome Markers and Trans-Bering Strait Dispersals. *American Journal of Physical Anthropology* 102:301–314.

Kemp, Brian M., Angélica González-Oliver, Ripan S. Malhi, Cara Monroe, Kari Britt Schroeder, John McDonough, Gillian Rhett, Andres Resendéz, Rosenda I. Peñaloza-Espinosa, Leonor Buentello-Malo, Clara Gorodesky, and David Glenn Smith
2010 Evaluating the Farming/Language Dispersal Hypothesis with Mitochondrial and Y-chromosomal DNA Variation in the Southwest and Mesoamerica. *Proceedings of the National Academy of Sciences of the United States of America* 107:6759–6764.

Kemp, Brian M., Ripan S. Malhi, John McDonough, Deborah A. Bolnick, Jason A. Eshleman, Olga Rickards, Cristina Martinez-Labarga, John R. Johnson, Joesph G. Lorenz, E. James Dixon, Terence E. Fifield, Timothy H. Heaton, Rosita Worl, and David Glenn Smith
2007 Genetic Analysis of Early Holocene Skeletal Remains from Alaska and Its Implication for the Timing of the Peopling of the Americas. *American Journal of Physical Anthropology* 132:605–621.

Kemp, Brian M., Andrés Reséndez, Juan Alberto Román Berrelleza, Ripan S. Malhi, and David Glenn Smith
2005 An Analysis of Ancient Aztec mtDNA from Tlatelolco: Pre-Columbian Relations and the Spread of Uto-Aztecan. In *Biomolecular Archaeology: Genetic Approaches to the Past*, edited by David M. Reed, pp. 22–46. Occasional Paper No. 32. Center for Archaeological Investigations, Southern Illinois University Carbondale.

Kitchen, Andrew, Michael M. Miyamoto, and Connie J. Mulligan
2008 A Three-Stage Colonization Model for the Peopling of the Americas. PLoS ONE 3(2):e1596. doi:10.1371/journal.pone.0001596.

Kivisild, Toomas, Peidong Shen, Dennis P. Wall, Bao Do, Raphael Sung, Karen Davis, Giuseppe Passarino, Peter A. Underhill, Curt Scharfe, Antonio Torroni, Rosaria Scozzari, David Modiano, Alfredo Coppa, Peter de Knijff, Marcus Feldman, Luca L. Cavalli-Sforza, and Peter J. Oefner

2006 The Role of Selection in the Evolution of Human Mitochondrial Genomes. *Genetics* 172:373–387.

Kolman, Connie J., Nyamkhishig Sambuughin, and Eldredge Bermingham

1996 Mitochondrial DNA Analysis of Mongolian Populations and Implications for the Origin of New World Founders. *Genetics* 142:1321–1334.

LeBlanc, Steven A., Lori Kreisman, Brian M. Kemp, Shawn W. Carlyle, Anna Dhody, Francis Smiley, and Thomas Benjamin

2007 Quids and Aprons: Ancient DNA from Artifacts from the American Southwest. *Journal of Field Archaeology* 32:161–175.

Lell, Jeffrey T., Michael D. Brown, Theodore G. Schurr, Rem I. Sukernik, Yelena B. Starikovskaya, Antonio Torroni, Lorna G. Moore, Gary M. Troup, and Douglas C. Wallace

1997 Y Chromosome Polymorphisms in Native American and Siberian Populations: Identification of Native American Y Chromosome Haplotypes. *Human Genetics* 100:536–543.

Lell, Jeffrey T., Rem I. Sukernik, Yelena B. Starikovskaya, Bing Su, Li Jin, Theodore G. Schurr, Peter A. Underhill, and Douglas C. Wallace

2002 The Dual Origin and Siberian Affinities of Native American Y Chromosomes. *American Journal of Human Genetics* 70:192–206.

Lorenz, Joseph G., and David Glenn Smith

1996 Distribution of Four Founding mtDNA Haplogroups among Native North Americans. *American Journal of Physical Anthropology* 101:307–323.

Malhi, Ripan S.

2001 Investigating Prehistoric Population Movements in North America with Ancient and Modern DNA. Ph.D. dissertation, Department of Anthropology, University of California, Davis.

Malhi, Ripan S., Katherine E. Breece, Beth A. Schultz Shook, Frederika A. Kaestle, James C. Chatters, Steven Hackenberger, and David Glenn Smith

2004 Patterns of mtDNA Diversity in Northwestern North America. *Human Biology* 76:33–54.

Malhi, Ripan S., Jerome S. Cybulski, Raul Y. Tito, Jesse Johnson, Harold Harry, and Carrie Dan

2010 Brief Communication: Mitochondrial Haplotype C4c Confirmed as a Founding Genome in the Americas. *American Journal of Physical Anthropology* 141:494–497.

Malhi, Ripan S., Jason A. Eshleman, Jonathan A. Greenberg, Deborah A. Weiss, Beth A. Schultz Shook, Frederika A. Kaestle, Joseph G. Lorenz, Brian M. Kemp, John R. Johnson, and David G. Smith

2002 The Structure of Diversity Within the New World Mitochondrial DNA Haplogroups: Implications for the Prehistory of North America. *American Journal of Human Genetics* 70:905–919.

Malhi, Ripan Singh, Angelica Gonzalez-Oliver, Kari Britt Schroeder, Brian M. Kemp, Jonathan A. Greenberg, Solomon Z. Dobrowski, David Glenn Smith, Andres Resendéz, Tatiana Karafet, Michael Hammer, Stephen Zegura, and Tatiana Brovko

2008 Distribution of Y Chromosomes among Native North Americans: A Study of Athapaskan Population History. *American Journal of Physical Anthropology* 137:412–424

Malhi, Ripan S., Brian M. Kemp, Jason A. Eshleman, Jerome Cybulski, David Glenn Smith, Scott Cousins, and Harold Harry
2007 Haplogroup M Discovered in Prehistoric North America. *Journal of Archaeological Science* 34:642–648.

Malhi, Ripan S., Holly M. Mortenson, Jason A. Eshleman, Brian M. Kemp, Joseph G. Lorenz, Frederika A. Kaestle, John R. Johnson, Clara Gorodezky, and David Glenn Smith
2003 Native American mtDNA Prehistory in the American Southwest. *American Journal of Physical Anthropology* 120:108–124.

Malhi, Ripan S., and David Glenn Smith
2002 Brief Communication: Haplogroup X Confirmed in Prehistoric North America. *American Journal of Physical Anthropology* 119:84–86.

Mandryk, Carole A. S., Heiner Josenhans, Daryl W. Fedje, and Rolf W. Mathewes
2001 Late Quaternary Paleoenvironments of Northwestern North America: Implications for Inland Versus Coastal Migration Routes. *Quaternary Science Review* 20:301–314.

Merriwether, D. Andrew
1999 Freezer Anthropology: New Uses for Old Blood. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 354:121–129.

Merriwether, D. Andrew, Andrew G. Clark, Scott W. Ballinger, Theodore G. Schurr, Himla Soodyall, Trefor Jenkins, Stephen T. Sherry, and Douglas C. Wallace
1991 The Structure of Human Mitochondrial DNA Variation. *Journal of Molecular Evolution* 33:543–555.

Merriwether, D. Andrew, and Robert E. Ferrell
1996 The Four Founding Lineage Hypothesis for the New World: A Critical Reevaluation. *Molecular Phylogenetics and Evolution* 5:241–246.

Merriwether, D. Andrew, William W. Hall, Anders Vahlne, and Robert E. Ferrell
1996 mtDNA Variation Indicates Mongolia May Have Been the Source for the Founding Population for the New World. *American Journal of Human Genetics* 59:204–212.

Merriwether, D. Andrew, Brian M. Kemp, Douglas E. Crews, and James V. Neel
2000 Gene Flow and Genetic Variation in the Yanomama as Revealed by Mitochondrial DNA. In *America Past, America Present: Genes and Languages in the Americas and Beyond*, edited by Colin Renfrew, pp. 89–124. McDonald Institute for Archaeological Research, Cambridge, England.

Merriwether, D. Andrew, Francisco Rothhammer, and Robert E. Ferrell
1994 Genetic Variation in the New World: Ancient Teeth, Bone, and Tissue as Sources of DNA. *Cellular and Molecular Life Sciences* 50:592–601.
1995 Distribution of the Four Founding Lineage Haplotypes in Native Americans Suggests a Single Wave of Migration for the New World. *American Journal of Physical Anthropology* 98:411–430.

Mishmar, Dan, Eduardo Ruiz-Pesini, Pawel Golik, Vincent Macaulay, Andrew G. Clarke, Seyed Hosseini, Martin Brandon, Kirk Easley, Estella Chen, Michael D. Brown, Rem I. Sukernik, Antonel Olckers, and Douglas C. Wallace
2003 Natural Selection Shaped Regional mtDNA Variation in Humans. *Proceedings of the National Academy of Sciences of the United States of America* 100:171–176.

Monroe, Cara, Brian M. Kemp, and David Glenn Smith
2006 Mitochondrial DNA Variation of Yuman Speaking Populations. Poster presented at the Languages and Genes conference, University of California, Santa Barbara.

Moraga, Mauricio L., P. Rocco, J. F. Miquel, F. Nervi, Elena Llop, R. Chakraborty, Francisco Rothhammer, and P. Carvallo
2000 Mitochondrial DNA Polymorphisms in Chilean Aboriginal Populations: Im-

plications for the Peopling of the Southern Cone of the Continent. *American Journal of Physical Anthropology* 113:19–29.

Mourant, Arthur E.
1976 *The Distribution of the Human Blood Groups and Other Polymorphism*. Oxford University Press, London.

Mulligan, Connie J., Keith Hunley, Suzanne Cole, and Jeffrey C. Long
2004 Population Genetics, History, and Health Patterns in Native Americans. *Annual Review of Genomics and Human Genetics* 5:295–315.

Mulligan, Connie J., Andrew Kitchen, and Michael M. Miyamoto
2008 Updated Three-Stage Model for the Peopling of the Americas. PLoS ONE 3(9):e3199. doi:10.1371/journal.pone.0003199.

Neves, Walter A., and Mark Hubbe
2005 Cranial Morphology of Early Americans from Lagoa Santa, Brazil: Implications for the Settlement of the New World. *Proceedings of the National Academy of Sciences of the United States of America* 102:18309–18314.

Nichols, Johanna
1990 Linguistic Diversity and the First Settlement of the New World. *Language* 66:475–521.

Pääbo, Svante, John A. Gifford, and Allan C. Wilson
1988 Mitochondrial DNA Sequences from a 7000-Year Old Brain. *Nucleic Acids Research* 16:9775–9788.

Parr, Ryan L., Shawn W. Carlyle, and Dennis H. O'Rourke
1996 Ancient DNA Analysis of Fremont Amerindians of the Great Salt Lake Wetlands. *American Journal of Physical Anthropology* 99:507–518.

Perego, Ugo A., Alessandro Achilli, Norman Angerhofer, Matteo Accetturo, Maria Pala, Anna Olivieri, Baharak Hooshiar Kashani, Kathleen H. Ritchie, Rosaria Scozzari, Qing-Peng Kong, Natalie M. Myres, Antonio Salas, Ornella Semino, Hans-Jürgen Bandelt, Scott R. Woodward, and Antonio Torroni
2009 Distinctive Paleo-Indian Migration Routes from Beringia Marked by Two Rare mtDNA Haplogroups. *Current Biology* 19:1–8.

Pitulko, Volodya V., P. A. Nikolsky, E. Y. Girya, A. E. Basilyan, V. E. Tumskoy, S. A. Koulakov, S. N. Astakhov, E. Y. Pavlova, and M. A. Anisimov
2004 The Yana RHS Site: Humans in the Arctic Before the Last Glacial Maximum. *Science* 303:52–56.

Poinar, Hendrik N., Melanie Kuch, Kristin D. Sobolik, Ian Barnes, Arthur B. Stankiewicz, Tomasz Kuder, W. Geofferey Spaulding, Vaughn M. Bryant, Alan Cooper, and Svante Pääbo
2001 A Molecular Analysis of Dietary Diversity for Three Archaic Native Americans. *Proceedings of the National Academy of Sciences of the United States of America* 98:4317–4322.

Ramachandran, Sohini, Omkar Deshpande, Charles C. Roseman, Noah A. Rosenberg, Marcus W. Feldman, and L. Luca Cavalli-Sforza
2005 Support from the Relationship of Genetic and Geographic Distance in Human Populations for a Serial Founder Effect Originating in Africa. *Proceedings of the National Academy of Sciences of the United States of America* 102:15942–15947.

Ray, Nicolas, D. Wegmann, Nelson J. Fagundes, Sijia Wang, Andres Ruiz-Linares, and Laurent Excoffier
2010 A Statistical Evaluation of Models for the Initial Settlement of the American Continent Emphasizes the Importance of Gene Flow with Asia. *Molecular Biology and Evolution* 27:337–345.

Reidla, Maere, Toomas Kivisild, Ene Metspalu, Katrin Kaldma, Kristiina Tambets, Helle-Viivi Tolk, Jüri Parik, Eva-Liis Loogvali, Miroslava V. Derenko, Boris Malyarchuk, Marina Bermisheva, Sergey Zhadanov, Erwan Pennarun, Marina Gubina, Maria Golubenko, Larisa Damba, Sardana Fedorova, Vladislava Gusar, Elena Grechanina, Ilia Mikerezi, Jean-Paul Moisan, André Chaventre, Elsa Khusnutdinova, Ludmila Osipova, Vadim Stepanov, Mikhail Voevoda, Alessandro Achilli, Chiara Rengo, Olga Rickards, Gian Franco De Stefano, Surinder Papiha, Lars Beckman, Branka Janicijevic, Pavao Rudan, Nicholas Anagnou, Emmanuel Michalodimitrakis, Slawomir Koziel, Esien Usanga, Tarekegn Geberhiwot, Corinna Herrnstadt, Neil Howell, Antonio Torroni, and Richard Villems
2003 Origin and Diffusion of mtDNA Haplogroup X. *American Journal of Human Genetics* 73:1178–1190.

Rickards, Olga, Cristina Martínez-Labarga, J. Koji Lum, Gian Franco De Stefano, and Rebecca L. Cann
1999 mtDNA History of the Cayapa Amerinds of Ecuador: Detection of Additional Founding Lineages for the Native American Populations. *American Journal of Human Genetics* 65:519–530.

Rubicz, Rohina, Theodore G. Schurr, Paul L. Babb, and Michael H. Crawford
2003 Mitochondrial DNA Variation and the Origins of the Aleuts. *Human Biology* 75:809–835.

Salzano, Francisco M., and Sidia M. Callegari-Jacques
1988 *South American Indians: A Case Study in Evolution*. Clarendon, Oxford.

Santos, Sidney E., Andrea K. Ribeiro-Dos-Santos, D. Meyer, and Marco A. Zago
1996 Multiple Founder Haplotypes of Mitochondrial DNA in Amerindians Revealed by RFLP and Sequencing. *Annals of Human Genetics* 60:305–319.

Schroeder, Kari B., Mattias Jakobsson, Michael H. Crawford, Theodore G. Schurr, Simina M. Boca, Donald F. Conrad, Raul Y. Tito, Ludmilla P. Osipova, Larissa A. Tarskaia, Sergey I. Zhadanov, Jeffrey D. Wall, Jonathan K. Pritchard, Ripan S. Malhi, David G. Smith, and Noah A. Rosenberg
2009 Haplotypic Background of a Private Allele at High Frequency in the Americas. *Molecular Biology and Evolution* 26:995–1016.

Schroeder, Kari B., Theodore G. Schurr, Jeffrey C. Long, Noah A. Rosenberg, Michael H. Crawford, Larissa A. Tarskaia, Ludmila P. Osipova, Sergey I. Zhadanov, and David G. Smith
2007 A Private Allele Ubiquitous in the Americas. *Biology Letters* 3:218–223.

Schurr, Theodore G.
2000 Mitochondrial DNA Variation in Native Americans and Siberians, and Its Implications for the Peopling of the New World. *American Scientist* 88:246–253.
2002 A Molecular Anthropological View of the Peopling of the Americas. *Athena Review* 3(2):59–77.
2004a The Peopling of the New World: Perspectives from Molecular Anthropology. *Annual Review of Anthropology* 33:551–583.
2004b Molecular Genetic Diversity in Siberians and Native Americans Suggests an Early Colonization of the New World. In *Entering America: Northeast Asia and Beringia Before the Last Glacial Maximum*, edited by David B. Madsen, pp. 187–238. University of Utah Press, Salt Lake City.

Schurr, Theodore G., Scott W. Ballinger, Yik-Yuen Gan, Judith A. Hodge, D. Andrew Merriwether, Dale N. Lawrence, William C. Knowler, Kenneth M. Weiss, and Douglas C. Wallace
1990 Amerindian Mitochondrial DNAs Have Rare Asian Mutations at High Fre-

quencies, Suggesting They Derived from Four Primary Maternal Lineages. *American Journal of Human Genetics* 46:613–623.

Schurr, Theodore G., Rem I. Sukernik, Yelena B. Starikovskaya, and Douglas C. Wallace
1999 Mitochondrial DNA Variation in Koryaks and Itel'men: Population Replacement in the Okhotsk Sea-Bering Sea Region During the Neolithic. *American Journal of Physical Anthropology* 108:1–39.

Schurr, Theodore G., and Douglas C. Wallace
1999 mtDNA Variation in Native Americans and Siberians, and Its Implications for the Peopling of the New World. In *Who Were the First Americans?: Proceedings of the 58th Annual Biology Colloquium*, edited by Robson Bonnichsen, pp. 41–77. Corvallis: Center for the Study of the First Americans, Corvallis, Oregon.
2002 Mitochondrial DNA Diversity in Southeast Asian Populations. *Human Biology* 74:431–452.

Scozzari, Rosaria, Fulvio Cruciani, Piero Santolamazza, Daniele Sellitto, David E. C. Cole, Laurence A. Rubin, Damian Labuda, Elisabetta Marini, Valeria Succa, Guiseppe Vona, and Antonio Torroni
1997 mtDNA and Y Chromosome-Specific Polymorphisms in Modern Ojibwa: Implications about the Origin of Their Gene Pool. *American Journal of Human Genetics* 60:241–244.

Shields, Gerald F., Andrea M. Schmeichen, Barbara L. Frazier, Alan Redd, Mikhail I. Voevoda, Judy K. Reed, and Ryk H. Ward
1993 mtDNA Sequences Suggest a Recent Evolutionary Divergence for Beringian and Northern North American Populations. *American Journal of Human Genetics* 53:549–562.

Silva, Wilson A., Jr., Sandro L. Bonatto, Adriano J. Holanda, Andrea K. Ribeiro-dos-Santos, Beatriz M. Paixao, Gustavo H. Goldman, Kiyoko Abe-Sandes, Luis Rodriguez-Delfin, Marcela Barbosa, Maria Luiza Paco-Larson, Maria Luiza Petzl-Erler, Valeria Valente, Sidney E. B. Santos, and Marco A. Zago
2002 Mitochondrial Genome Diversity of Native Americans Supports a Single Early Entry of Founder Populations into America. *American Journal of Human Genetics* 71:187–192.

Smith, David Glenn, Joseph Lorenz, Becky K. Rolfs, Robert L. Bettinger, Brian Green, Jason Eshleman, Beth Schultz, and Ripan Malhi
2000 Implications of the distribution of Albumin Naskapi and Albumin Mexico for New World Prehistory. *American Journal of Physical Anthropology* 111:557–572.

Smith, David Glenn, Ripan S. Malhi, Jason A. Eshleman, Frederika A. Kaestle, and Brian M. Kemp
2005 Mitochondrial DNA Haplogroups of Paleoamericans in North America. In *Paleoamerican Origins: Beyond Clovis*, edited by Robson Bonnichsen, Bradley T. Lepper, Dennis Stanford, and Michael R. Waters, pp. 243–254. Texas A&M University Press, College Station.

Smith, David Glenn, Ripan S. Malhi, Jason Eshleman, Joseph G. Lorenz, and Frederika A. Kaestle
1999 Distribution of mtDNA Haplogroup X Among Native North Americans. *American Journal of Physical Anthropology* 110:271–284.

Starikovskaya, Yelena B., Rem I. Sukernik, Theodore G. Schurr, Andres M. Kogelnik, and Douglas C. Wallace
1998 mtDNA Diversity in Chukchi and Siberian Eskimos: Implications for the Genetic History of Ancient Beringia and the Peopling of the New World. *American Journal of Human Genetics* 63:1473–1491.

Stone, Anne C., and Mark Stoneking
1993 Ancient DNA from a Pre-Columbian Amerindian Population. *American Journal of Physical Anthropology* 92:463–471.
1996 Genetic Analysis of an 8000 Year-Old Native American Skeleton. *Ancient Biomolecules* 1:83–87.
1998 mtDNA Analysis of a Prehistoric Oneota Population: Implications for the Peopling of the New World. *American Journal of Human Genetics* 62:1153–1170.

Szathmary, Emöke J. E.
1979 Blood Groups of Siberians, Eskimos, Subarctic, and Northwest Coast Indians: The Problem of Origins and Genetic Relationships. In *The First Americans: Origins, Affinities, and Adaptations*, edited by William S. Laughin and Albert B. Harper, pp. 185–209. Gustav Fischer, New York.

Tamm, Erika, Toomas Kivisild, Maere Reidla, Mait Metspalu, David Glenn Smith, Connie J. Mulligan, Claudio M. Bravi, Olga Rickards, Cristina Martinez-Labarga, Elas K. Khusnutdinova, Sardana A. Fedorova, Maria V. Golubenko, Vadim A. Stepanov, Marrina A. Gubina, Sergey I. Zhadanov, Ludmila P. Osipova, Larisa Damba, Mikhail I. Voevoda, Jose E. Dipierri, Richard Villems, and Ripan S. Malhi
2007 Beringian Standstill and Spread of Native American Founders. PloS ONE 2(9):e829. doi:10.1371/journal.pone.0000829.

Tarazona-Santos, Eduardo, and Fabrício R. Santos
2002 The Peopling of the Americas: A Second Major Migration? *American Journal of Human Genetics* 70:1377–1380.

Torroni, Antonio, Yu-Sheng Chen, Ornella Semino, Augusta Silvana, Beneceretti Santachiara, C. Ronald Scott, Marie T. Lott, Marcus Winter, and Douglas C. Wallace
1994 mtDNA and Y-Chromosome Polymorphisms in Four Native American Populations from Southern Mexico. *American Journal of Human Genetics* 54:303–318.

Torroni, Antonio, Theodore G. Schurr, Margaret F. Cabell, Michael D. Brown, James V. Neel, Merethe Larsen, David G. Smith, Carlos M. Vullo, and Douglas C. Wallace
1993 Asian Affinities and Continental Radiation of the Four Founding Native American mtDNAs. *American Journal of Human Genetics* 53:563–590.

Torroni, Antonio, Theodore G. Schurr, Chin-Chuan Yang, Emöke J. E. Szathmary, Robert C. Williams, Moses S. Schanfield, Gary A. Troup, William C. Knowler, Dale N. Lawrence, Kenneth M. Weiss, and Douglas C. Wallace
1992 Native American Mitochondrial DNA Analysis Indicates That the Amerind and the Nadene Populations Were Founded by Two Independent Migrations. *Genetics* 130:153–162.

Toth, Nicholas
1991 The Material Record. In *The First Americans: Search and Research*, edited by Thomas D. Dillehay and David J. Meltzer, pp. 53–76. CRC Press, Boca Raton, Florida.

Underhill, Peter A., Li Jin, Rachel Zemans, Peter J. Oefner, and L. Luca Cavalli-Sforza
1996 A Pre-Columbian Y Chromosome-Specific Transition and Its Implications for Human Evolutionary History. *Proceedings of the National Academy of Sciences of the United States of America* 93:196–200.

Vajda, Edward
2010 A Siberian Link with Na-Dene Languages. *Archaeological Papers of the University of Alaska* 5:75–156.

Wallace, Douglas C.
1994 Mitochondrial DNA Sequence Variation in Human Evolution and Disease. *Proceedings of the National Academy of Sciences of the United States of America* 91:8739–8746.

Wallace, Douglas C., Katherine Garrison, and William C. Knowler
1985 Dramatic Founder Effects in Amerindian Mitochondrial DNAs. *American Journal of Physical Anthropology* 68:149–156.

Wang, Sijia, Cecil M. Lewis Jr., Mattias Jakobsson, Sohini Ramachandran, Nicolas Ray, Gabriel Bedoya, Winston Rojas, Maria V. Parra, Julio A. Molina, Carla Gallo, Guido Mazzotti, Giovanni Poletti, Kim Hill, Ana M. Hurtado, Damian Labuda, William Klitz, Ramiro Barrantes, Maria Catira Bortolini, Francisco M. Salzano, Maria Luiza Petzl-Erler, Luiza T. Tsuneto, Elena Llop, Francisco Rothhammer, Laurent Excoffier, Marcus W. Feldman, Noah A. Rosenberg, and Andres Ruiz-Linares
2007 Genetic Variation and Population Structure in Native Americans. PLoS Genet 3(11):e185. doi:10.1371/journal.pgen.0030185.

Ward, Ryk H., Barbara L. Frazier, Kerry Dew-Jager, and Svante Pääbo
1991 Extensive Mitochondrial Diversity Within a Single Amerindian Tribe. *Proceedings of the National Academy of Sciences of the United States of America* 88: 8720–8724.

Ward, Ryk H., Alan Redd, Diana Valencia, Barbara Frazier, and Svante Pääbo
1993 Genetic and Linguistic Differentiation in the Americas. *Proceedings of the National Academy of Sciences of the United States of America* 90:10663–10667.

Waters, Michael R., and Thomas W. Stafford Jr.
2007 Redefining the Age of Clovis: Implications for the Peopling of the Americas. *Science* 315:1122–1126.

Weinstein, Karen J.
2005 Body Proportions in Ancient Andeans from High and Low Altitudes. *American Journal of Physical Anthropology* 128:569–585.

Wells, Spencer
2006 *Deep Ancestry: Inside the Genographic Project*. National Geographic, Washington, D.C.

Yao, Yong-Gang, Qing-Peng Kong, Hans-Jürgen Bandelt, Toomas Kivisild, and Ya-Ping Zhang
2002 Phylogeographic Differentiation of Mitochondrial DNA in Han Chinese. *American Journal of Human Genetics* 70:635–651.

YCC
2002 A Nomenclature System for the Tree of Human Y-Chromosomal Binary Haplogroups. *Genome Research* 12:339–348.

Zegura, Stephen L., Tatiana M. Karafet, Lev A. Zhivotovsky, and Michael F. Hammer
2004 High-Resolution SNPs and Microsatellite Haplotypes Point to a Single, Recent Entry of Native American Y Chromosomes into the Americas. *Molecular Biology and Evolution* 21:164–175.

Zhivotovsky, Lev A., Noah A. Rosenberg, and Marcus W. Feldman
2003 Features of Evolution and Expansion of Modern Humans, Inferred from Genomewide Microsatellite Markers. *American Journal of Human Genetics* 72:1171–1186.

Zlojutro, Mark, Rohina Rubicz, Eric J. Devor, Victor A. Spitsyn, Sergei V. Makarov, Kristin Wilson and Michael H. Crawford
2006 Genetic Structure of the Aleuts and Circumpolar Populations Based on Mitochondrial DNA Sequences: A Synthesis. *American Journal of Physical Anthropology* 129:446–464.