

***Homo sapiens* in the Americas. Overview of the earliest human expansion in the New World**

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Summary - *Although it is widely recognised that America was the last continent to be populated by our species, researchers' views on various aspects of this process (e.g. the period in which it occurred, the area from which the colonizing populations came, the number of dispersal waves and the routes taken by these migrations) differ significantly. In this paper, we review both classical data and more recent findings from various research fields – including geology, paleoecology, archaeology, skeletal biology, and genetics – that may shed light on the dynamics of the colonization of the American continent, according to a critical reappraisal of the various hypotheses and models that have been advanced over time to explain this process.*

Keywords - *Early modern humans, Migratory routes, Models of dispersal, Last Glacial Maximum, America.*

Introduction

The dispersal of human populations in the American continent may be viewed as a part of the worldwide process of expansion of our species which, as consistently demonstrated by fossil and genetic data (e.g., Cann *et al.*, 1987; Stringer & Andrews, 1988; Manzi *et al.*, 1994; Harpending *et al.*, 1998; Stringer, 2002; McDougall *et al.*, 2008; Endicott *et al.*, 2010; Manzi, 2012), first appeared in Africa around 200 thousand years ago (200 ka).

Homo sapiens started spreading out of Africa between 120 and 60 ka, gradually reaching the landmasses found elsewhere on the planet at various times; the Americas were the last continent to be inhabited in this expansion process (Cavalli-Sforza & Feldman, 2003; Endicott *et al.*, 2003; Foster & Matsumura, 2005; Makaulay *et al.*, 2005; Tangerang *et al.*, 2005; Mellars, 2006; Rose *et al.*, 2011). Ever since the 16th century, the colonization of the American continent has attracted the interest of many intellectuals,

including the Jesuit Jose de Acosta (1589) and the naturalist Georges-Louis Leclerc de Buffon (1749), who were among the first to speculate on the origin of the ancestors of the Native American populations (Mazieres, 2011). Thus, in the 20th century numerous hypotheses were advanced to describe the first settlement of the Americas in the light of archaeological, anthropological and (more recently) genetic data.

In this review, we consider both classical data and recent findings from various research fields - such as geology, paleoecology, archaeology, skeletal biology, and genetics – that have been taken into account to explain the dynamics of the colonization of the American continent. We also briefly discuss the various hypotheses and models that have been proposed over time to describe this process, including: the Clovis-first/single origin hypothesis (Hrdlicka, 1937; Haynes, 2002; Fagan, 2004), the Blitzkrieg/overkill model (Martin, 1973, 1984), the Three-wave migration/tripartite model (Greenberg *et al.*, 1986), the Solutrean hypothesis (Bradley &

Stanford, 2004), the Two main biological components/stock hypothesis (Neves & Pucciarelli, 1991; Pucciarelli *et al.*, 2003), the Beringian incubation/source population model (Szathmáry, 1981, 1996; Foster, 1996; Bonatto & Salzano, 1997; Bodner, 2012), the Three-stage colonization model (Kitchen *et al.*, 2008, Mulligan *et al.*, 2008), the Single migration hypothesis (Fagundes *et al.*, 2008), the Dual migration hypothesis (Torroni, 1992; Schurr & Sherry, 2004; Perego *et al.*, 2009), and the Recurrent gene flow model (Ray *et al.*, 2009; De Azevedo *et al.*, 2011). Nevertheless, we believe that such a puzzling anthropological issue may be better understood by combining a multidisciplinary approach with a large scale genetic analysis, as a recent study (Reich *et al.*, 2012) suggests.

At the end of the Pleistocene

The paths that may have been followed by the human populations that first colonized the Americas were influenced by the climatic and environmental effects of the last glaciation (Marine Isotopic Stages 4-2), which occurred during the Late Pleistocene (Guthrie, 2001; Mandryk *et al.*, 2001, Peltier, 2002; Meltzer, 2009; Dixon, 2011).

In particular, during the Last Glacial Maximum (LGM) around 21 ka (Clark & Mix, 2002), most of present-day Canada and the northern United States were covered by two main glaciers: the Laurentide glacier (which covered approximately 13.4 million km² and extended from the Atlantic coast to the foothills of the Rocky Mountains) and the Cordilleran glacier (which covered about 2.4 million km² and extended from the Pacific coast to the western edge of the Rocky Mountains) (Meltzer, 2009). During the coldest phases of the Late Pleistocene the sea level was as much as 120 meters lower than it is today (Peltier, 2002), and Asia and America were connected by a land bridge known as Beringia, corresponding to what is now the Bering Strait (Fig. 1). Fossil records indicate that, around the time of the LGM, Beringia was

a wide plain free of ice, containing a rich variety of plant species, herds of herbivorous mammals (mammoth, musk oxen, horses, camels, bison) as well as their predators (lions, short-faced bears, sabertoothed cats) (Guthrie, 2001).

Moreover, stone artefacts used on the skeletal remains of extinct mammals have recently been described at Yana Rhinoceros Horn (north-western Siberia), demonstrating that Beringia was inhabited by human groups from at least 32 ka (Pitulko *et al.*, 2004). In recent years, other archaeological sites have been described in both Alaska and eastern Siberia; these sites include: i) Swan Point (central Alaska), where artefacts consisting of both microblades and burins dated to 14 ka were found; ii) Nenana (central Alaska) and Ushki (south-western Siberia), which yielded small blades and flakes dated to between 13.8 and 13 ka; iii) Sluiceway-Tuluq (north-western Alaska), where lanceolate bifaces dated to between 13.4 and 13 ka were brought to light; and (iv) Nohagabara I (western Alaska), where bifaces and microblades cores dated to between 13.8 and 12.7 ka were found (reviewed in Goebel *et al.*, 2008; Dillehay, 2009).

However, human populations were presumably unable to reach southern Alaska, during the extensive marine regression that characterized the LGM, because the Laurentide and Cordilleran glaciers formed a continuous ice mass that extended from the Pacific to the Atlantic coasts (Meltzer, 2009). Paleoclimate studies have shown that the first accessible route for human populations was probably along the Pacific coast, which became free of ice around 15 ka, while an inland passage (“ice-free corridor”) opened when the glaciers began to separate at the foot of the Rocky Mountains, though this did not probably occur until 13.5 ka (Mandryk *et al.*, 2001; Dixon, 2011).

The archaeological evidence

The first archaeological evidence of prehistoric human settlements in the Americas was discovered in 1927 at Folsom in New Mexico, where some spearheads associated with the remains of a



Fig. 1 - Map showing the main physical characteristics of the American continent during the Last Glacial Maximum (LGM), with a selection of the prehistoric sites mentioned in the text. The ice-free corridor opened approximately after 13.5 ka. Adapted from Meltzer (2009).

Late Pleistocene bison were found, and in 1933 at Clovis, which is also in New Mexico, where other less sophisticated, and therefore probably earlier, spearheads were discovered near the remains of a mammoth (Howard, 1933). In the following years lithic artefacts, made with what was to become known as Clovis technology, were found in many archaeological sites in North and Central America. The Clovis technology thus

proved to have been the most widespread cultural tradition on the continent, though more recent dates obtained for several Clovis sites have shown that it only spanned approximately 400 years, from 13.2 to 12.8 ka (Waters Jr. & Stafford, 2007), after which it was replaced by other cultural traditions.

The typical elements of the Clovis culture are fluted projectile points, made with siliceous

rocks such as chert, jasper, chalcedony and obsidian, modelled by removing large blades from a nucleus and refinished so as to obtain a laminar shape that is tapered on either side (Goebel *et al.*, 2008).

Following the discovery of Clovis artefacts, it was assumed for many decades that the first people to reach the American continent were hunter-gatherer groups using this technology, who had come from Asia at the end of the LGM and had populated the entire continent, from the Hudson Bay to Tierra del Fuego, over a period of a thousand years: a scenario referred to as the Clovis-first/single origin hypothesis (Hrdlicka, 1937; Haynes, 2002; Fagan, 2004).

However, while artefacts produced with the Clovis technology have been described in many North American archaeological sites, the archaeological record for South America is very different, with no single material culture dominating this sub-continent. South American sites that are contemporary with the Clovis phase are characterized by a marked degree of regional cultural diversification, with at least six sites being identified (Cerro Tres Tetras, Cueva Casa del Minero and Piedra Museo in Argentina, and Fell's Cave, Quebrada Santa Julia and Quebrada Jaguay in Chile) in which a wide range of flake tools, bifacial points and, in some cases, distinctive Fishtail points have been described (reviewed in Goebel *et al.*, 2008). Some authors have ascribed this cultural diversity to geographical barriers that hampered human movements (such as the Andean highlands and Amazon river) as well as to climatic and ecological changes between areas at different latitudes (Rothhammer & Dillehay, 2009).

Late Pleistocene extinctions

Clovis spearheads have frequently been associated with skeletal remains of proboscideans, there being at least 14 sites dating from the Clovis period in which mammoths and mastodons were killed and butchered (Meltzer, 2009). Since the American Late Pleistocene is characterized by the extinction of 35 genera of large

mammals, in both North and South America (Greyson & Meltzer, 2003), some researchers believe that the extinction of these species was the result of hunting on a massive scale practised by the Clovis people, in what has been called the Blitzkrieg/overkill model (Martin, 1973, 1984).

However, this assumption has been repeatedly questioned in recent years because there is no archaeological evidence of the killing of animals other than mammoths and mastodons during the Clovis age, and because both the Late Pleistocene and the Pleistocene/Holocene transition are characterized by numerous extinctions, even in regions in which human populations are known not to have been present (Greyson & Meltzer, 2003).

Whether or not it was due to anthropogenic causes, the extinction of these species is very likely to have had an important impact on the history of the American continent. Indeed, according to the hypothesis that human infectious diseases evolved as the result of the interaction between humans and domestic animals (Diamond, 2000), Native Americans populations were deprived of the opportunity to develop any immunity over time owing to the rapid disappearance of many potentially domestic species. When Native Americans subsequently came into contact with Europeans in the late 15th century, they suffered a high mortality caused by infectious diseases such as smallpox, tuberculosis, measles, rubella and syphilis, as archaeological and historical records indicate. Moreover, a recent genetic study (O'Fallon & Fehren-Schmitz, 2011) indicates that Native American populations were subject to a significant contraction in population size around 500 years ago, when the number of females is known to have decreased by around 50%.

In search of greater antiquity

The identification of pre-Clovis archaeological sites proved to be an arduous task owing to the difficulty encountered in many cases in understanding whether chipped stones and crushed mammal bones were the result of human

activity or were merely due to natural processes (Lyman *et al.*, 1998). In other cases, establishing the age of a site proved to be difficult owing to the lack of reliable radiometric dates (Meltzer, 2009). In view of these difficulties, many archaeological sites that had until the mid-1990s been considered to point to an early colonization of the Americas (approx. 50-30 ka), such as those at Calico (California), Pedra Furada (Brazil), Pendejo Cave (New Mexico), Pikimachay Cave (Peru), Tlapacoya (Peru) and Tule Springs (Nevada) (reviewed in Goebel *et al.*, 2008), are no longer considered a robust evidence and are generally disregarded.

The first archaeological site accepted by the vast majority of researchers as pre-Clovis is Monte Verde in southern Chile, dated to 14.6 ka, where the exceptional state of preservation of organic materials, interpreted as the remains of a human encampment, provided a precious insight into the daily lives of the inhabitants of the Americas at the very end of the Pleistocene (Dillehay, 1997; Dillehay *et al.*, 2008).

Many other pre-Clovis sites have been described in recent years in North America. Those generally accepted by the majority of authors include: i) the Debra L. Friedkin Site (Texas), where more than 15,000 artefacts, consisting mainly of small blades, and a block of hematite, probably used to obtain red pigment, dating from between 15.5 and 13.2 ka, were discovered (Waters *et al.*, 2011); ii) Meadowcraft Rockshelter (Pennsylvania), which contained stone tools dating from between 15.2 and 13.4 ka, but that may be even older (Adovasio & Pedler, 2004); iii) Schaefer and Hebior (Wisconsin), with stone artefacts and skeletal remains of mammoth with cutmarks, dating from between 14.5 and 14.2 ka (Joyce, 2006); iv) Page-Ladson (Florida), dating to 14.4 ka, where artefacts associated with bones of extinct Pleistocene animals, including a mastodon tusk with cutmarks where it was joined to the skull, were found (Webb, 2005).

Other archaeological evidence, besides that found at Monte Verde, pre-dating the Clovis period exists in South America: at Santana

do Riacho and Lapa do Boquete (Brasil), dating from around 14 ka, and at Taima-Taima (Venezuela), dating from 13.2 ka (reviewed in Dillehay, 2009).

In brief, as we shall see below, the body of evidence yielded by different fields of research allows us to state with some confidence that the hunter-gatherers who developed the Clovis technology were not the first inhabitants of America (Waters Jr. & Stafford, 2007).

Looking for a multidisciplinary approach

The first attempt to use a multidisciplinary approach to describe the earliest arrival of humans in the Americas dates back to the late 1980s and is known as the Three-wave migration/tripartite model (Greenberg *et al.*, 1986).

This model is mainly based on studies by the linguist Joseph Greenberg who, upon comparing the languages spoken by Native American groups, grouped them into three families: Amerindian, Na-dene (or Athabascan) and Eskimo-Aleut. According to Greenberg, the populations of each of these language families were descended from three groups that had reached the Americas at different times: the Amerindians around 11 ka, the Na-Denes 9 ka and the Eskimo-Aleuts 4 ka (Greenberg *et al.*, 1986) (Fig. 2a).

On the basis of the frequency of 28 dental traits spread among Native American populations, the physical anthropologist Christy Turner II also came to the conclusion that the early settlers of the Americas could be subdivided into three groups, i.e. the Eskimo-Aleuts, Greater Northwest Coast Indians and Macro-Indians. According to Christy Turner II, the Macro-Indians (who corresponded to the Amerindians of Greenberg) had reached America around 14 ka; the order of the other two groups instead differed from Greenberg's order insofar as Christy Turner II considered the Eskimo-Aleuts to have arrived about 11 ka, and the Greater Northwest Coast Indians (who only partially corresponded to the Na-denes) to have reached America last (Greenberg *et al.*, 1986).

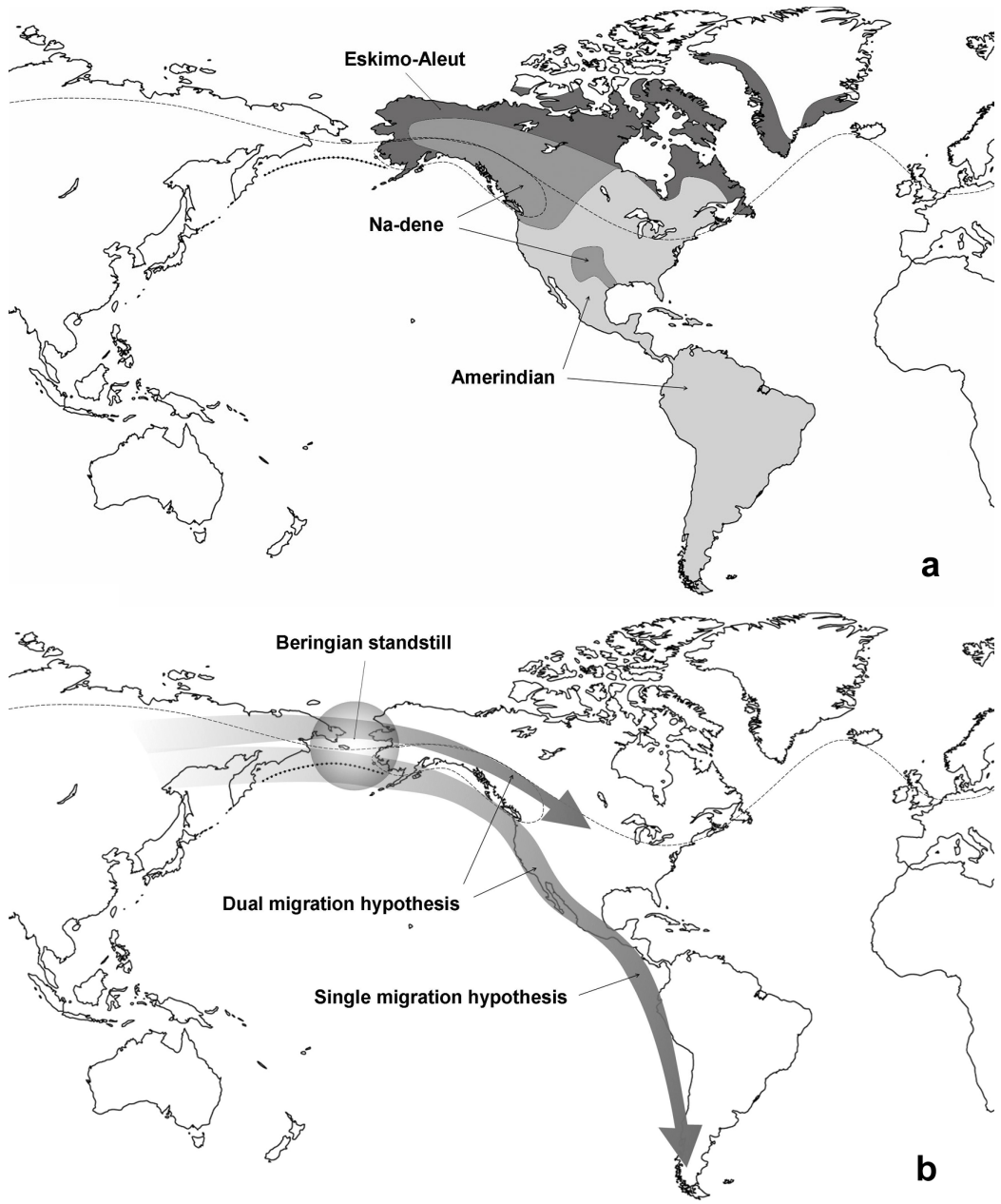


Fig. 2 – a) According to the Three-wave migration/tripartite model, which combined linguistic, dental and genetic evidence, Native Americans descended from three groups of migrants belonging to distinct linguistic families: Amerindians, Na-denes and Eskimo-Aleutians. b) The Single migration hypothesis states that America was settled through one migration route along the Pacific coast, whereas two independent migration routes were followed according to the Dual migration hypothesis. In addition, some genetic models suggest that colonization from Asia was interrupted by a long-term settlement in Beringia, as originally pointed out by E. Szathmáry (1981).

When the geneticist Stephen Zegura joined Greenberg and Turner II, the genetic analyses available at the time were based exclusively on classical genetic markers (blood groups, serum proteins, enzyme polymorphisms) (Cavalli-Sforza *et al.*, 1994, among others), whose resolution was not sufficient to separate Native Americans into different groups. As the only marker that appeared to confirm the three-wave migration model was the distribution of the allotypes of the immunoglobulin G (Williams *et al.*, 1985), Zegura concluded that the genetic data available could not be used to support the results yielded by the linguistic and dental analyses (Greenberg *et al.*, 1986).

Colonization from Europe?

When the Clovis artefacts first appeared, numerous archaeologists were struck by their resemblance to those of the Solutrean culture of the European upper Paleolithic, dated to between 23.5 and 20 ka (Straus *et al.*, 2005). For example, the archaeologist Arthur Jelinek (1971) described the artefacts from the Franco-Cantabrian area as being highly similar to the Clovis artefacts, although a historical relationship between these two technologies was, in his view, unlikely because they are separated temporally by more than 6,000 years and geographically by the Atlantic Ocean.

In 2004, however, the experimental archaeologists Bruce Bradley and Dennis Stanford, having probably been influenced by the discovery in North America of some individuals with the X haplogroup of mitochondrial DNA (mtDNA), which also occurs in European populations (Brown *et al.*, 1998), argued that such a hypothesis deserved to be reconsidered (Meltzer, 2009). Bradley and Stanford founded their Solutrean hypothesis on the view that, despite being highly complex, the Clovis lithic technology cannot be traced to other technologies in either Siberia or Alaska but is, in their view, closely related to the Solutrean technology. In order to explain how some European populations might have reached

America, they speculated that people had crossed the Atlantic from Spain during the LGM, when North America and Europe were connected by a bridge of land and continental glaciers. They claimed that their hypothesis was supported by the fact that the only archaeological sites considered at the time to be of pre-Clovis age – i.e. Meadowcroft Rockshelter (Pennsylvania), Cactus Hill (Virginia) and Page Ladson (Florida) – were all located on the eastern side of North America (Bradley & Stanford, 2004).

Most researchers now consider the similarity between the Solutrean and Clovis lithic technologies to be due to cultural convergence (Straus *et al.*, 2005); moreover, genetic studies carried out on Native American populations have revealed their close affinity with Asian populations, which appears to disprove the Solutrean hypothesis (Schurr *et al.*, 1990; Torroni *et al.*, 1993; Karafet *et al.*, 1999).

Skeletons of first Americans

Human skeletal remains dating from the Late Pleistocene and the Early Holocene – thus closely related to the earliest inhabitants of the Americas – have so far only been found in sites located in a limited number of regions on the continent, most of which lie in South America (Jantz & Owsley, 2001). These sites are: i) Kennewick (Washington), dated to 9 ka (Taylor *et al.*, 1999), and Warm Mineral Spring (Florida), dated to 10 ka (Powell *et al.*, 1999), in the USA; ii) the Mexico basin, dated to 10 ka (Gonzales-Jose *et al.*, 2005), and Baja California, dated to 6 ka (Gonzales-Jose *et al.*, 2003), in Mexico; iii) Sabana de Bogotá, dated to between 10 and 6 ka (Neves & Pucciarelli, 1991), in Colombia; iv) Toca de Oncas, dated to 8 ka (Hubbe *et al.*, 2004), Lagoa Santa, dated to between 11 and 7.5 ka (Neves & Hubbe, 2005), and Capelinha, dated to 8.5 ka (Neves *et al.*, 2005), in Brazil; v) Arroyo Seco 2, dated to between 9.8 and 8.3 ka (Scabuzzo & Politis, 2007), in Argentina; and vi) Palli Aike, dated to between 8 and 7 ka (Neves *et al.*, 1999), in Chile.

When researchers analysed craniometric variation in both ancient and recent Native Americans, using traditional methods at first and geometric morphometrics more recently, they found morphological differences that led to the overall sample being divided into two groups. Assuming that cranial morphology reflects the history of human populations (Neves & Pucciarelli, 1991), this difference has been interpreted by some as evidence of at least two separate migratory events in the American continent over time.

The crania of the earliest Americans (earlier than around 10 ka) have been referred to as the “Paleoamericans”. These tend to be similar to those of extant Australians, Melanesians and Sub-Saharan Africans, like the Late Pleistocene specimen from Zhoukoudian Upper Cave (skull 101; Neves & Pucciarelli, 1998). Indeed, they exhibit a dolichocephalic morphology, prognathic and low faces, with relatively low, broad orbits and noses. By contrast, late prehistoric and recent Native Americans, also known as “Amerindians”, tend to exhibit a cranial morphology similar to later and modern East Asian populations (“Mongoloids”), with a brachymorphic cranial vault, orthognathic high, broad faces, and relatively high and narrow orbits and noses (Lahr *et al.*, 1995; Van Vark *et al.*, 2003; Neves & Hubbe, 2005).

According to this two-wave model, referred to as the Two main biological components/stock hypothesis (Neves & Pucciarelli, 1991; Pucciarelli *et al.*, 2003), the Paleoamericans derived from South-East Asian populations of the Late Pleistocene, which spread throughout America around 16 ka, whereas the Amerindians derived from the East Asian populations of the Early Holocene, which spread through the American continent around 10 ka, replacing, or assimilating, the Paleoamericans (Lahr *et al.*, 1995; Van Vark *et al.*, 2003; Neves & Hubbe, 2005; Hubbe *et al.*, 2010; Hubbe *et al.*, 2011).

Many scholars have criticized this scenario: for example, Gonzales-Jose and colleagues (2008), after analysing a group of 576 Late Pleistocene/Early Holocene and modern crania, suggested that it would be more appropriate to

view the groups usually labelled Paleoamericans, Amerindians and Mongoloids as variants along a morphological continuum. In another recent study, based on both cranial morphology and molecular data, Perez and colleagues (2009) analysed skeletal samples from central-eastern Argentina (Arroyo Seco 2), where a diachronic sequence ranging from Early to Late Holocene occurs. Although the morphology of the diachronic samples – i.e. those from i) the Middle and Late Holocene sample and ii) from the other, more recent Late Holocene sample – is clearly different, they found that both groups belong to the same mtDNA haplogroups. It has consequently been suggested that the morphological variations between ancient and recent Native Americans is more likely the result of random (genetic drift) or non-random (natural selection or plasticity) micro-evolutionary factors than of distinct waves of immigrants.

Genetic evidence

Autosomal genes, Y chromosome, mitochondrial DNA

Many Native American populations have been genetically characterized since the second half of the 20th century. The characterization of these populations was initially based on classic genetic markers [i.e. on the expression of autosomal markers at the blood level (red cell, protein and serum systems) (Cavalli-Sforza *et al.*, 1994; Crawford, 1998; O’Rourke, 2006)], whereas in the late 1990s both maternal (mitochondrial DNA) and paternal (non-recombining portion of Y chromosome, or NRY) markers were included in the analyses.

As regards the autosomal genes, Wang and colleagues (2007) recently studied 24 Native American populations: not only did they show that the average level of heterozygosity of Native American autosomal genes is 6.5% lower than the average of our species, but they also found a decline in genetic variability that was directly correlated with the population’s distance from the Bering Strait. When Schroeder and colleagues

(2009) studied a D9S1120 allele with a high frequency in Native American populations, they argued that it was more likely to have resulted from common descent within a small founder population than from natural selection.

Studies on the NRY, including the analysis of both the Single Nucleotide Polymorphisms (SNPs) and Short Tandem Repeats (STRs), have shown that the two most common haplogroups in Native American populations are Q and C, while the most common lineages are Q1a*-MEH2, Q1a3*-M346, Q1a3a1-M3 and C3b-P39 (Karafet *et al.*, 2006; Karafet *et al.*, 2008; Zegura *et al.*, 2004; Blanco-Vetea *et al.*, 2010; Jota *et al.*, 2011). Mulligan and colleagues (2004) and Zegura and colleagues (2004) have estimated that the coalescence for both these haplogroups occurred between 12 and 15 ka. Moreover, a high-resolution analysis of the haplogroup Q, partially reshaped the phylogeny of this branch and showed that Native Americans and Southern Altaian populations share a common ancestor (Dulik *et al.*, 2012), thereby providing important information concerning the possible origin of the populations that colonized the American continent.

Early research on mtDNA, using both Restriction Fragment Length Polymorphisms (RFLPs) and sequencing of the hypervariable segment I (HVR-I), indicated that all Native Americans belonged to five haplogroups (A, B, C, D and X) (Torroni *et al.*, 1993; Forster *et al.*, 1996; Brown *et al.*, 1998). A greater degree of molecular resolution, achieved in recent years by sequencing entire mtDNA (Bandelt *et al.*, 2003; Achilli *et al.*, 2008), led to the identification of 15 sub-haplogroup lineages considered to be founders (A2*, A2a, A2b, B2, C1b, C1c, C1d*, C1d1, C4c, D1, D2a, D3, D4h3a, X2a and X2g) (Perego *et al.*, 2010). Although there are some discrepancies in the coalescence age estimates of the mtDNA haplogroups between studies, they commonly range from 15 to 20 ka (O'Rourke & Raff, 2010). This date is a rough approximation of the drastic genetic bottleneck that forms the basis of one hypothesis regarding the earliest colonization of the Americas.

Ancient DNA

The analysis of samples of genetic material taken directly from ancient biological remains (ancient DNA analysis), despite certain limitations (i.e. limited availability of samples, preservation state of organic material, possible contamination), may be considered the only means of directly investigating the genetic diversity of extinct populations.

The earliest hard evidence (i.e. not merely archaeological) of the presence of humans on the American continent came to light in 2008 at the Paisley 5 Mile Point Caves in Oregon, where 14 human coprolites, dated to 14.27-14 ka, were discovered among the remains of an encampment. The DNA obtained from 6 of these coprolites yielded Native American SNPs diagnostic for the mtDNA founding haplotypes A2 and B2 (Gilbert *et al.*, 2008a).

A biological sample (teeth) from the On-Your-Knees Cave site on the Prince of Wales Island in Alaska, dated to 10.3 ka, proved to belong to an individual characterized by the mtDNA lineage D4h3a (Kemp *et al.*, 2007): a rare lineage that has been found in some extant individuals living on the Pacific coast of South America (Perego *et al.*, 2007).

Genetic material was also recently obtained from a frozen hair belonging to a Paleo-Eskimo individual excavated from an early settlement in Greenland (Saqquaq) and dated to 4.5-3.4 ka. The sequencing of 79% of the nuclear genome showed this individual possessed thick, dark hair, brown eyes and probably shovel-shaped front teeth, a characteristic that is common among both Asian and Native American populations (Rasmussen *et al.*, 2010). The Saqquaq Paleo-Eskimo was also found to belong to the mtDNA lineage D2a1, a lineage that had not previously been observed among modern Native American and Neo-Eskimo populations. This indicates that the first humans to colonize the far north of the American continent were not either the Native Americans or the ancestors of the Neo-Eskimo expansion (Thule culture), which instead started in Alaska around 1 ka (Gilbert *et al.*, 2008b).

Moreover, the genetic characterization of samples belonging to two individuals from the

China Lake site in British Columbia, dated to 4.95 ka, showed that these individuals belonged to the mtDNA haplogroup M: an Asian haplogroup no longer present in Native American populations (Malhi *et al.*, 2007). This is the first, and so far only, reported case of genetic discontinuity between ancient and modern Americans.

As very few biological remains with good nucleic acid preservation have been found in archaeological sites, the vast majority of ancient DNA studies on ancient American populations were conducted on samples younger than 5 ka. More recent samples show that haplogroup frequencies for the populations of the entire American continent have not changed substantially over the last 4 ka, with similar haplogroups being obtained for both the ancient and modern populations, though some changes in regional haplogroup patterns do emerge (reviewed in Raff *et al.*, 2011).

Models for the earliest Americans

In the last couple of decades, genetic analyses, often combined with paleoecological and archaeological data, have allowed researchers to develop population models designed to interpret the origin, dynamics and migratory routes followed by the first American settlers.

The first attempt to use uniparental genetic data to develop a model for the earliest Americans was made by Bonatto & Salzano (1997); they analysed the mtDNA region of 544 extant Native American individuals by testing the model developed by E. Szathmáry, who had used classical genetic markers (Szathmáry, 1981, 1984). It is known as the Beringian incubation model or Beringian source population (Foster, 1996; Bonatto & Salzano, 1997; Bodner 2012). According to this model, Beringia played a central role as the place in which the ancestral population of the Americas differentiated genetically before colonizing the continent. This model was subsequently further elaborated by Tamm and colleagues (2007), who analysed the complete mtDNA sequence of 623 Native Americans

and Asian individuals. They hypothesised, on the basis of the phylogenetic structure of the mtDNA haplogroups of Native Americans, that this ancestral population, upon being prevented from heading further South by the last glaciation, was forced to settle in Beringia for such a long time (Beringian standstill) that the New World founder lineages had sufficient time to differentiate from their Asian sister clades (Fig. 2b).

Kitchen and colleagues (2008) and Mulligan and colleagues (2008) instead used the Bayesian skyline plot analysis (Drummond *et al.*, 2005) to develop a model used to explain the dynamics of the colonization of the Americas, which they called Three-stage colonization model. According to this model, the first phase in this process was the genetic divergence between peoples of central-eastern Asia and the ancestors of Native Americans, which took place more than 30 ka; this phase was followed by a period of isolation, spanning 7-15 ka, during which a genetic drift process led to variants that later became characteristic of Native American populations; the third and final phase was the colonization of the whole continent, which started about 16 ka and coincided with the end of the last Ice Age.

Some scholars have used the analysis of uniparental genetic markers to trace the possible migratory routes followed by the first American settlers. For instance, according to Fagundes and colleagues (2008), the colonization of Beringia during the LGM was characterized by a marked drop in the number of people migrating from Asia. By contrast, the end of the last Ice Age witnessed an increase in the number of people arriving from Asia, which coincided with the colonization of the continent and is hypothesized to have followed a trajectory along the Pacific coast between 18 and 15 ka, according to the so-called Single migration hypothesis (Fig. 2b).

Other authors, such as Schurr and Sherry (2004), instead support the scenario proposed by Torroni and colleagues on the basis of genetic data (1992), according to which the populations known at that time as the Amerindians and Na-denes derived from independent waves that colonized the Americas via two migratory routes.

The first route was along the Pacific coast and was followed around 14.7 ka by populations characterized by high frequencies of the A, B, C and D mtDNA haplogroups as well as by some peculiar NRY haplogroups. The other route was through the ice-free corridor between the Laurentide and Cordilleran ice sheets (cf. Figs 1, 2b), which was taken by populations characterized by high frequencies of the X mtDNA haplogroup as well as other peculiar NRY haplogroups around 12.5 ka.

Perego and colleagues (2009), who studied the geographical distribution of two rare mtDNA lineages (D4h3 and X2a), came to the same conclusion. They argue that the Americas were first colonised about 15-16 ka by two human groups that followed these different routes: the Pacific coast and the ice-free corridor in the North. This hypothesis is supported by the recent identification of 14 living individuals belonging to the extremely rare Native American mtDNA lineage C4c, who display the same age and geographical distribution as the mtDNA lineage X2a and appear to belong to the human group that entered North America from Beringia through the ice-free corridor (Kashani *et al.*, 2012), as well as by the identification of 43 subjects belonging to the rare mtDNA clades D1g and D1j (Bodner *et al.*, 2012) and 46 belonging to the clades B2i2 (former B2l) and C1b13 (de Saint Pierre *et al.*, 2012a; de Saint Pierre *et al.*, 2012b). These haplogroups characterize populations from the Southern Cone of South America (Chile and Argentina), whose geographical distribution and inferred origin are consistent with the earliest archaeological sites in South America, who may have taken the migratory route that ran along the Pacific coast.

By contrast, Ray and colleagues (2009), who used Approximate Bayesian computation (ABC) methods (Beaumont *et al.*, 2002) to analyse 401 autosomal microsatellite loci belonging to 29 Asiatic and Native American populations, claim that neither a single nor a dual wave of migration from Asia can explain the observed level of genetic diversity of the Amerindian populations. They believe, instead, that the data available may be better explained by assuming that

the initial settlement of the American continent after the LGM was followed by an extended gene flow between Asian and American populations inhabiting the Arctic landscapes. They called this scenario the Recurrent gene flow model. De Azevedo and colleagues (2011) support this model and believe that it could explain not only the extant genetic diversity, but also the variation in craniofacial shape observed between Native American skeletons dating from different periods in the past.

Final remarks

As we have seen, many hypotheses have been advanced over time to explain the human colonization of the American continent. Very recently, in the most comprehensive survey of genetic diversity in Native American populations conducted so far, a large group of scholars analysed data from 52 Native American and Eurasian groups, genotyped using single nucleotide polymorphisms. Their data indicate that the vast majority of Native American populations descend from a homogeneous group, which they called First American, that reached the Americas from Asia presumably by crossing the Bering Strait more than 15 ka; however, the populations of the Arctic regions (speakers of the Eskimo-Aleut languages) inherited almost half their ancestry from a second stream of Asian genes, while the Chipewyan group from Canada (speakers of the Na-Dene language) inherited roughly one-tenth of their ancestry from a third stream (Reich *et al.*, 2012) (Fig. 3).

This scenario interestingly resembles the Three-Wave migration/tripartite model proposed in the late 1980s which combined linguistic, dental morphology and low resolution genetic data (Greenberg *et al.*, 1986). It however partially differs from the 1980s model insofar as Greenberg and colleagues did not explicitly predict the possible admixture between the First Americans and the subsequent streams of Asian migrants.

Whether or not this scenario is confirmed by future studies, it seems reasonable to presume

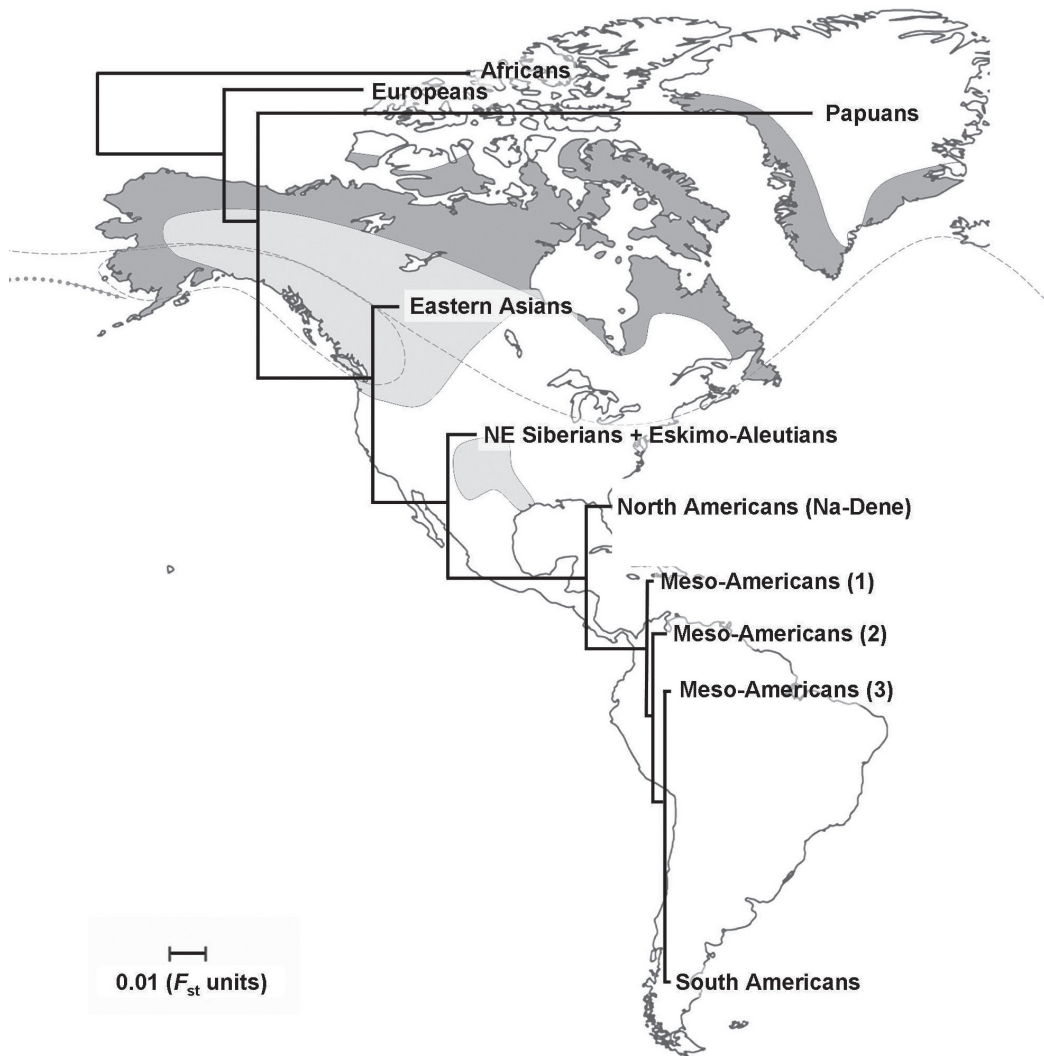


Fig. 3 - Simplified reproduction of a neighbour-joining tree relating Native American to selected non-American populations, based on F_{st} distances (Reich et al., 2012) and consistent with the Three-wave migration/tripartite model introduced by Greenberg, Turner II & Zegura (1987). Modified from Reich and co-workers (2012).

that further insights on the puzzling anthropological issue of the colonization of the New World will most likely come from a multidisciplinary approach (based on skeletal/dental morphology,

archaeology, palaeoecology and linguistic data) combined with large scale genetic analyses (using both recent and ancient DNA data) made possible by recent technological advances.

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