Native Americans derive from a small number of Asian founders who likely arrived to the Americas via Beringia. However, additional details about the initial colonization of the Americas remain unclear. To investigate the pioneering phase in the Americas we analyzed a total of 623 complete mtDNAs from the Americas and Asia, including 20 new complete mtDNAs from the Americas and seven from Asia. This sequence data was used to direct high-resolution genotyping from 20 American and 26 Asian populations. Here we describe more genetic diversity within the founder population than was previously reported. The newly resolved phylogenetic structure suggests that ancestors of Native Americans paused when they reached Beringia, during which time New World founder lineages differentiated from their Asian sister-clades. This pause in movement was followed by a swift migration southward that distributed the founder types all the way to South America. The data also suggest more recent bi-directional gene flow between Siberia and the North American Arctic.
Introduction

The mitochondrial DNA haplogroup nomenclature that is widely used today in population and medical genetics, forensic science, and in other interdisciplinary studies, traces back to the analysis of Native American populations by Torroni et al. [1] [2]. The first four letters of the phylogenetic alphabet for mtDNA haplogroups - A-D - were coined to refer to just four founding haplogroups that exhibit virtually all North and South American mtDNA diversity.

Genetic studies demonstrate that Native Americans inherited their mitochondrial DNA (mtDNA) from a handful of founders who arrived from Asia via Beringia [1] [2]. No more than four major pan American and three minor North American founding mtDNA haplotypes (A2, B2, C1, D1 and X2a, D2, D3, respectively) have been convincingly established in previous studies of control region sequence, RFLP markers and 30 complete mtDNA genomes (Table 1) [11–14]. The paucity of established founding mtDNAs suggests that the number of migrants that initially peopled the Americas was relatively low. However, determining the full range of diversity surviving to the present day in the founding population requires high-resolution mtDNA sequence data. Previous estimates of mtDNA diversity are predominantly based on control region sequences representing only a minor fraction of the mtDNA genome. In addition, control region sequences experience a high frequency of recurrent mutations, potentially obscuring the identification of additional founding mtDNAs [14–19].

Even though some additional minor founder types have been later identified in North America, such as X, the hypothesis of just four major founder types in the initial colonization of the New World remains uncontested. However, the timing of their entry remains debated. Previous studies of mtDNA data place estimates for the peopling of the New World in a broad range from 11,000 to over 40,000 years before present (ybp) [reviewed by 20], although more recent estimates range from 20,000–15,000 ybp [21]. Recent archaeological evidence places Homo sapiens in northeastern Siberia at the Yana Rhinoceros Horn Site as early as 30,000 ybp [22] about twice the 15,000 ybp [23] date for humans at the southern end of South America. These archaeological dates suggest two likely scenarios. First, the ancestors of Native Americans peopled Beringia before the Last Glacial Maximum, but remained locally isolated (likely due to ecological barriers) until entering the Americas at 15,000 ybp (Beringian incubation model, BIM) [24]. Second, the ancestors of Native Americans did not reach Beringia until just before 15,000 ybp, and then moved continuously on into the Americas, being recently derived from a larger parent Asian population (direct colonization model, DCM).

The DCM model hypothesizes the presence of founding mtDNA haplotypes that include members from both Northeast Asia and the Americas. It presumes a continuous movement of recently derived migrants across Beringia. In contrast, the BIM model predicts widespread, derived founding haplotypes specific to the Americas that are not found in Asia. This implies that migrants were isolated for an extended period of time before entering the Americas and that the founder haplotypes arose in situ in Beringia. Once in the Americas, these immigrants spread southward. Therefore, the phylogeographic distribution of this diversity can provide insights into the mode of the initial phase of the peopling of the Americas. A nested hierarchy of diversity from north to south in Native American founding haplogroups would reflect a gradual peopling, whereas a uniform distribution of Native American founding haplotypes both in North and South America implies a rapid occupation.

Results

Previous studies of mtDNA variation recognized no major subclade structure within each New
World haplogroup [13][14][24]. A few studies of mtDNA variation suggested subclade structure [12], but lacked the power to convincingly demonstrate it. In this study we identified three sub-clades - C1b, C1c and C1d - that incorporate nearly all of Native American haplogroup C mtDNAs. All three are widely distributed in the New World. They are absent in Asia, and show similar coalescence times of approximately 13,900±2,700 years ago (Figure 1). Similar coalescence times were estimated for the other major founder haplogroups - A2, B2 and D1 - suggesting the simultaneous divergence of all founder clades across North and South America. A different C1 sub-clade in Asia - C1a [26] - likely derives from the same ancestral population as the three Native American sub-clades. Thus C1b, C1c and C1d are likely independent New World founders. In addition to C1 sub-clades, we defined two additional founders – D4h3 and C4c. These differ by several mutations from the Asian-derived ancestral clades, D4h and C4, respectively (Figure S1d,c). Haplogroup D4h3 ranges from Alaska to Tierra del Fuego and has recently been identified in Alaskan skeletal remains (10,300 ybp) [27]. We identified haplogroup C4c in two Ijka-speakers from Colombia, but its distribution in the Americas remains poorly characterized.

Discussion

Our phylogeographic analysis of a new mitochondrial genome dataset allows us to draw several conclusions. First, before spreading across the Americas, the ancestral population paused in Beringia long enough for specific mutations to accumulate that separate the New World founder lineages from their Asian sister-clades (Figure 2) [4][24], [28–29]. Second, founding haplotypes are uniformly distributed across North and South America instead of exhibiting a nested structure from north to south (Figure 1). Thus, after the Beringian standstill, the initial North to South migration was likely a swift pioneering process, not a gradual diffusion. This scenario matches the pattern of distribution of the first archaeological sites in Northeast Asia and the Americas [22],[23]. Third, the largely autochthonous pattern of variation seen in Native American mtDNAs suggests that the swift migration was followed by long-term isolation of local populations accompanied with the development of regional haplotypes within continental founder haplogroups [1].

In addition to illuminating the peopling process during the pioneering phase, the new dataset allows identification of more-recent genetic exchanges around and across Beringia (Figure 2). Specifically, haplogroup D2 consists of two sister clades, one found only in Siberia (D2b) and the other found in northernmost Eskimos, Chukchi, Aleut, and Athapaskans (D2a). While sub-haplogroup D2a is shared between ethno-historically close related Beringian Aleuts and Eskimos, (Figure S1) its sister clade D2b is spread among populations from distantly related linguistic groups (Tungusic, Turkic, Mongolic) (Table S2). A close relationship of matrilineal
Materials and Methods

The sample-set comprises 601 Native American individuals from 20 populations distributed throughout the Americas (23 Dogrib from Subarctic Canada; 20 Apaches, 20 Northern Paiutes, 11 Zunis from Southwest US; 77 Ngöbes, 34 Kunas from Panama; 39 Emberas, 57 Waunanas from Panama and Colombia; 47 Arsarios, 48 Koguis, 29 Ilkas, 42 Wayuus, 27 Coreguajes, 22 Vaupes from Colombia; 12 Secoyas-Sionas, 32 Cayapas from Ecuador; 9 Tucuman, 18 Salta, 25 Catamarca, 5 Mocovi from Argentina and 3764 samples from 26 Asian populations (51 Eskimos; 155 Chukchi; 120 Selkups; 66 Kets; 70 Tundra Nanets; 275 Tuvas; 185 Khakas; 339 Altaians; 170 Shors; 71 Koryaks; 85 Nanais; 122 Uyghurs; 406 Kazakhs; 58 Gilyaks; 61 Oroks; 105 Kirghiz; 48 Uzbek; 38 Tajiks; 201 Buriats; 324 Evenks; 105 Evens; 22 Yukaghirs; 423 Yakuts; 157 Dolgans; 107 Nganasans). A subset of these sequences were reported elsewhere [34–42].

First, haplogroup affiliations of the individual samples were determined through RFLP analysis and DNA sequencing of the HVS I region, if not known earlier [34–42]. Samples that could not be assigned to haplogroups A–D or X were investigated for evidence of recent admixture, particularly among populations with well established historical accounts of co-existence of Native American and either European or African populations. Samples of European or African origin were excluded from the current study. Further, 20 Native American and 7 Asian samples were selected for complete sequencing of mtDNA genomes. Using these 27 novel and 113 published Native American and relevant Asian complete or coding region sequences [4–26]. Preparation of sequencing templates was carried out following standard protocols, employing FIREPol polymerase (Solis BioDyne). Purified products were sequenced with the DYEnamic™ ET terminator cycle sequencing kit (Amersham Pharmacia Biotech) and analyzed on MegaBace1000 or ABI 3730xl sequencers. Sequences were aligned and analyzed with the Wisconsin Package (GCG) or ChromasPro 1.34.

DNA was extracted using conventional methods [34–42]. Coalescence-age calculations and SDs were estimated based on the phylogenies of complete sequences [3–6]. Given the global propensity of young mtDNA clades showing a significant excess of non-synonymous mutations, application of the raw molecular clock [49] in intra-species data sets is problematic [46]. Therefore, for dating the coalescent times of founder haplogroups we employed only synonymous transitions between the np 577-16023, assuming the rate of 3.5×10-8 (SD 0.1×10-8)/year/position [46]. The complete mtDNA genome data can be found in Genbank.

Supporting Information
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Author Contributions

Conceived and designed the experiments: TK RM. Performed the experiments: ET MR. Analyzed the data: CB MM TK RM ET MR. Contributed reagents/materials/analysis tools: CB CM RV TK RM DS OR CM EK SF MG VS MG SZ LO LD MV JD. Wrote the paper: CM RV MM TK RM ET DS.

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Native Americans derive from a small number of Asian founders who likely arrived to the Americas via Beringia. However, additional details about the initial colonization of the Americas remain unclear. To investigate the pioneering phase in the Americas we analyzed a total of 623 complete mtDNAs from the Americas and Asia, including 20 new complete mtDNAs from the Americas and seven from Asia. This sequence data was used to direct high-resolution genotyping from 20 American and 26 Asian populations. Here we describe more genetic diversity within the founder population than was previously reported. Information about the open-access article ‘Beringian standstill and spread of Native American founders.’ in DOAJ. DOAJ is an online directory that indexes and provides access to quality open access, peer-reviewed journals. The newly resolved phylogenetic structure suggests that ancestors of Native Americans paused when they reached...
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