

Note S1 Data set

(i) Merging data from seven sources

We merged seven sets of samples genotyped on Illumina SNP arrays. The number of samples we started with from each population (prior to the final data curation detailed below) is summarized in Table S1.1. Datasets other than the one obtained for this study were prefiltered by other researchers or in previous rounds of data curation carried out by the authors.

Name of dataset	N^*	Comments
"This study" (American and Siberian)	343	Genotyping was performed on Illumina 610-Quad arrays using a combination of genomic and whole genome amplified DNA. The genotyping was performed at the Broad Institute, with the exception of 10 of the 15 Chipewyan samples genotyped at McGill. The initial dataset was pre-filtered to eliminate samples that were genotyped twice, where genotypes were inconsistent with a DNA fingerprint, or where the call rate was <90% (later filters raised this to <95%). We restricted to autosomal SNPs, and removed SNPs with call rate <95% or no physical position.
"Kidd" (American and Siberian)	154	Genotyping was performed on Illumina 650Y arrays.
"MGDP" (Mexican ¹)	83	Genotyping was performed on Illumina HumanHap550 V3.0 arrays. We restricted to individuals inferred to be unrelated up to $2nd$ degree relatives.
"DiRienzo" (Siberian)	63	Genotyping was performed on either Illumina 610-Quad arrays (Nganasan and Yukaghir) or Illumina 650Y arrays (Naukan and Chukchi) ² .
"Willerslev" (Arctic)	142	Genotyping was performed on Illumina 650Y arrays ³ . We included all samples from ref. 3 except the Na-Dene which did not have permissions appropriate for this study. We then excluded the Yukaghir and Naukan where so many were lost in initial data curation that we removed the whole sample.
"HapMap3" (Worldwide)	799	Genotyping was performed on Illumina 1M and Affymetrix 6.0 arrays ⁴ . (The Illumina 1M contains essentially all the SNPs in the Illumina 610-Quad array so we are effectively using the Illumina 1M data from the HapMap3 genotyping.) We removed the Masai (MKK) which had a PCA pattern showing high within-population relatedness.
"CEPH-HGDP" (Worldwide)	907	Genotyping was performed on Illumina 650Y arrays ⁵ . We restricted to individuals inferred to be unrelated up to second degree relatives prior to carrying out the additional data curation steps reported below ⁶ .

Table S1.1: Illumina genotyping data sets that we merged for this analysis

* The sample size quoted here is what we analyzed prior to the final data curation steps reported below.

(ii) Curation of Native American samples

Our curation excluded samples that genotyped poorly or that had an unusual genetic background relative to other samples from the same population. We first ran the HAPMIX local ancestry inference software (Note S4) to identify segments of the genome in Native Americans and Siberians that may harbor West Eurasian or African ancestry. We then treated the genotypes in these segments as if they were missing data. This "masking" allowed us to better analyze the samples that had some recent European or African ancestry. The estimates of European and African ancestry, and proportion of the genome that was masked, are presented by population in Table S1 for Native Americans and Table S2 for Siberians. The individual ancestry estimates for the Native American samples are presented in Table S3.

We applied the following filters to remove 114 Native Americans samples from the dataset:

- *(1) 18 samples were removed due to a high missing genotype rate* We required that every sample had a genotyping missing data rate of <5%.
- *(2) 32 samples were removed due to a high proportion of West Eurasian or African mixture* We removed samples with <22% of their genomes inferred to have both alleles of entirely Native American ancestry based on the masking analysis of Note S4. The only exception was in Aleutian Islanders where this would have removed all of the samples.
- *(3) 44 samples were removed due to excess or deficiency of heterozygotes vs. expectation* All the Karitiana from the Kidd genotyping had a significant excess of heterozygous genotypes compared with the allele frequency computed in the same samples (violation of Hardy-Weinberg equilibrium). We removed these samples. We also removed a handful of additional samples due to heterozygote excess or deficiency.
- *(4) 10 samples were removed due to evidence of being at least a 2nd degree relative to others* It has already been reported that the Surui sample contained relatives⁶. For all pairs of individuals in all populations that had evidence for $>22\%$ of their genome being shared, we removed one of the pair (in general we chose to remove the one with more missing data). For this purpose, we used SMARTREL, part of the EIGENSOFT package⁷.
- *(5) 5 samples were removed due to a noisy local ancestry analysis* A total of 5 samples showed a strong mismatch between the ADMIXTURE-based estimate of European and African ancestry proportion (Note S2), and the proportion of the genome that was masked based on HAPMIX local ancestry analysis (Note S4). Visual inspection of the HAPMIX-based local ancestry inference for these 5 showed a noisy baseline ancestry inference compared with other individuals from the same populations, with narrow spikes of potential (but non-confident) non-Native American ancestry, which we interpreted as evidence for poor genotyping. We removed these samples.
- *(6) 5 samples were removed as PCA outliers relative to others from the same population* To identify samples that had unusual genotyping properties relative to other from their own populations we used Principal Component Analysis (PCA) as implemented in EIGENSOFT⁷. The outlier removal was based on the masked data (Note S4). To ensure that we were not removing samples simply because they had high proportions of their genome masked, we filled in missing data for each SNP based on the mean allele frequency of other samples in the same population (the filled-in data was only used in outlier removal; not for analyses of history). We performed outlier removal restricting to populations with at least 3 samples (outlier removal is impossible with fewer samples), and divided the populations into four groupings to make visual inspection tractable: northern North Americans, Meso-Americans, northern South Americans, and southern South Americans. We iteratively removed samples that were outliers relative to others from the same population on significant eigenvectors, until the samples appeared homogeneous. Aleuts were not included in outlier removal, as masking left almost none of their genome; however, we did remove one Aleut who from local ancestry analysis, appeared to have one chromosome from unadmixed, non-Aleut Native Americans.

After data curation, the number of Native Americans in the merged dataset was 493 (Table S1.2 reports the number of samples removed by population). Importantly, the data curation procedure was based on searching for individuals that were outliers with respect to their own population. Thus, if our curation introduces bias, it would be to make populations more homogeneous; we do not expect it to bias inferences of relationships among populations.

Population	Study	Before	After	Population	Study	Before	After	Population	Study	Before	After
Aleutian	Willersley	9	8	Guarani	This	9	6	Piapoco	HGDP	7	7
Algonquin	This	5	5	Guaymi	This	5	5	Pima	HGDP/Kidd	46	33
Arara	This	\overline{c}	1	Huetar	This	\overline{c}	1	Purepecha	This	1	
Arhuaco	This	6	5	Hulliche	This	4	$\overline{4}$	Quechua	This	41	40
Aymara	This	24	23	Inga	This	13	9	Surui	HGDP/Kidd	30	24
Bribri	This	$\overline{4}$	$\overline{4}$	Jamamadi	This	$\overline{2}$	1	Tepehuano	MGDP	27	25
Cabecar	This	32	31	Kaingang	This	$\overline{2}$	2	Teribe	This	3	3
Chane	This	\overline{c}	$\overline{2}$	Kagchikel	This	18	13	Ticuna	This	6	6
Chilote	This	10	8	Karitiana	HGDP/Kidd	34	13	Toba	This	5	4
Chipewyan	This	15	15	Kogi	This	6	4	Waunana	This	5	3
Chono	This	$\overline{4}$	$\overline{4}$	Maleku	This	4	3	Wayuu	This	17	11
Chorotega	This	1	$\mathbf{1}$	Maxal&2	HGDP/MGDP	56	49	WGInuit	Willerslev	8	8
Cree	This	5	4	Mixe	This	20	17	Wichi	This	5	5
Diaguita	This	5	5	Mixtec	This	5	5	Yaghan	This	4	4
EGInuit	Willerslev	7	7	Ojibwa	This	5	5	Yaqui	This	1	1
Embera	This	6	5	Palikur	This	3	3	Zapotec $1&82$	This/MGDP	59	43
Guahibo	This	13	6	Parakana	This	$\overline{4}$	1				

Table S1.2: Record of Native American data curation: filtering from 607 to 493 samples

* The Maya and Zapotec are broken into two subgroups for our analyses in the paper (e.g. Maya1 and Maya2).

Table S1.3: Record of Siberian data curation: filtering from 264 to 245 samples

Population	Study	Before	After	Population	Study	Before	After
Altaian	Willersley	13	12	Mongolian	Willerslev	9	8
Buryat	Willersley	18	17	Naukan	DiRienzo	16	16
Chukchi	DiRienzo/Willerslev	30	30	Nganasan $1&2$	DiRienzo/Willerslev	24	22
Dolgan	Willersley	6	$\overline{4}$	Selkup	Willerslev	9	9
Evenki	Willersley	15	15	Tundra Nentsi	This	4	3
Ket	Willersley	2	$\overline{2}$	Tuvinians	Willerslev	16	15
Khanty	Kidd	39	35	Yakut	HGDP/Kidd	40	34
Koryak	Willerslev	10	10	Yukaghir	Di Rienzo	13	13

* The Nganasan are broken into two subgroups for our analyses in the paper (Nganasan1 and Nganasan2).

(iv) Curation of Siberian data

We performed a similar analysis in the Siberian populations. This resulted in 17 Siberian populations, after splitting the Nganasan into two based on the two sources of the samples (Willerslev and DiRienzo; the structure was correlated to the sample source, suggesting that these two studies may have sampled different subgroups of the same population). We do not report on the Naukan and Yukaghir populations from the Willerslev dataset in Table S1.3 because so few samples were left from each after outlier removal; we thus removed these populations entirely from the analysis. Table S1.3 summarizes the filtering by population:

• 2 samples were removed due to evidence of being at least a 2nd degree relative to others.

• 17 samples were removed due to being outliers in PCA relative to their own population.

(v) Curation of non-Native American, non-Siberian data

We also performed PCA to remove outlier samples from non-Native American and non-Siberian populations. We removed the entire MKK population⁴ (Masai from Kenya from HapMap3) because of many statistically significant eigenvectors that were difficult to interpret. We also removed 6 other outlier samples. We started from previously filtered

datasets, and hence the number of samples prior to filtering reported in Table S1.1 is sometimes less than that in the papers that originally reported the data.

(vi) Merging and splitting of populations

Four populations were genotyped both by the Kidd and CEPH-HGDP studies but were known to be from the same original sample collection: Yakut, Karitiana, Surui and Pima. We removed the Kidd Karitiana data because of evidence for heterozygote excess (see above). The two Surui, two Pima, and two Yakut samples were indistinguishable based on PCA, and hence we merged them. The labels we used for the merged data from these populations are:

We also merged data from the Chukchi and Quechua because the data we had available from different sources were indistinguishable in PCA:

There were 4 populations for which data were available from two different sources, and for which we kept populations separate based on the source of the samples. We kept the samples separate either because these population samples have been traditionally analyzed separately (for example HapMap3 YRI and HGDP Yoruba), or because we observed differences between the two sources of samples from these populations in PCA (which could reflect genuine population substructure, so we did not want to merge the samples):

Finally, PCA showed population substructure in the Maya that did not neatly break down according to the sample source (HGDP or MGDP). This may reflect real substructure: the Maya in MGDP were sampled at multiple sites. We therefore repartitioned as follows: Maya ("Maya1" from HGDP and MGDP; "Maya2" from MGDP)

(vii) Removal of SNPs with inconsistent or potentially problematic genotyping After merging data for all populations, we curated SNPs as follows:

- *(1) 16 SNPs were removed due to an excess or deficiency of heterozygous genotypes* 6 SNPs in the data collected specifically for this study, 6 in the Kidd data, 3 in the Willerslev data, and 1 in the CEPH-HGDP data, showed an extreme excess or deficiency of heterozygotes compared with expectation given the frequency in their populations (their chi-square statistics were visual outliers from the tail).
- *(2) 16 SNPs were removed due to inconsistency in frequency across data sets* For all SNPs, we compared the frequency across populations of similar ancestry. We found 9 SNPs from the genotyping for this study, 6 from HapMap3, and 1 in MGDP, which were consistently more differentiated from the other data sets than expected from the tail of the chi-square distribution, suggesting genotyping error. We removed them.

(viii) Final datasets

After curation, we had 2,351 samples and 364,470 autosomal SNPs from 52 Native American, 17 Siberian, and 57 other populations. The average genotyping completeness was 99.88% per sample. The final datasets are listed in Table S1.4. The "unmasked" dataset reflects only the data curation steps described above. The "masked" dataset was obtained based on the results of running HAPMIX to define segments of potential African or West Eurasian ancestry due to admixture in the last few hundred years; SNPs in such segments were then treated as missing (Note S4). All datasets are available on request.

Table S1.4: Six datasets generated for this study

Note: All files are in the EIGENSOFT "packedancestrymap" format.

* These files are merged with genotypes that were previously published based on whole-genome sequencing data from a Saqqaq Paleo-Eskimo individual from Greenland³.

References for Note S1

¹ Silva-Zolezzi I, Hidalgo-Miranda A, Estrada-Gil J, Fernandez-Lopez JC, Uribe-Figueroa L, Contreras A, Balam-Ortiz E, del Bosque-Plata L, Velazquez-Fernandez D, Lara C, Goya R, Hernandez-Lemus E, Davila C, Barrientos E, March S, Jimenez-Sanchez G (2009) Analysis of genomic diversity in Mexican Mestizo

populations to develop genomic medicine in Mexico. Proc Natl Acad Sci USA 106, 8611-8616. 2 Hancock AM, Witonsky DB, Alkorta-Aranburu G, Beall CM, Gebremedhin A, Sukernik R, Utermann G, Pritchard JK, Coop G, Di Rienzo A (2011) Adaptations to climate-mediated selective pressures in humans. *PLoS Genet.* **7,** e1001375. 3

populations. Nature **467,** 52-58 (2010).

 Li J.Z. *et al.* Worldwide human relationships inferred from genome-wide patterns of variation. *Science* **319,** 1100-1104 (2008). 6

 6 Rosenberg NA. Standardized subsets of the HGDP-CEPH Human Genome Diversity Cell Line Panel, accounting for atypical and duplicated samples and pairs of close relatives. *Ann Hum Genet.* **70,** 841-847 (2006). 7 Patterson N, Price AL, Reich D (2006) Population structure and eigenanalysis. *PLoS Genet.* **2,** e190.

Rasmussen M. *et al.* Ancient human genome sequence of an extinct Palaeo-Eskimo. *Nature* **463,** 757-762 (2010). 4 International HapMap 3 Consortium. Integrating common and rare genetic variation in diverse human

Note S2 Ancestry estimates

Many of the Native American samples in this study have inherited some European and African genes since 1492. We used the ADMIXTURE clustering software to estimate the proportion of European and African ancestry in each individual¹. Following the recommendations of the user manual, prior to running the software we thinned the data until there were no pairs of polymorphisms that had allelic association of $r^2 > 0.1$, resulting in 88,079 SNPs.

We ran ADMIXTURE on the thinned dataset searching for $k=2, 3, 4, 5$ and 6 clusters. We restricted the analysis to populations that we judged were particularly relevant to learning about Native American population history:

- All Native American populations from this study.
- 5 Siberian populations chosen to be geographically relatively close to the Bering Strait or to the Arctic and to cluster in PCA with little evidence of recent mixture (Naukan, Chukchi, Koryak, Nganasan1 and Nganasan2)
- 6 European ancestry populations (French, Italian, Sardinian, Russian, CEU and TSI)
- 3 Niger-Kordofanian speaking, sub-Saharan African populations (Yoruba, YRI and LWK)

For each cluster number ($k=2, 3, 4, 5$ and 6), we identified the cluster most correlated to African and European population membership. The assignment to European and African clusters was extremely highly correlated for $k=4$ and $k=5$ (Figure S2.1). The only discrepancies between the k=4 and k=5 ancestry estimates are for European ancestry in Nganasan1 and Nganasan2, and thus we did not use the Nganasan in analyses that relied on ADMIXTURE ancestry estimates (in these analyses, we represented Siberians by the Naukan, Chukchi and Koryak only). In contrast, the estimates for k=3 were more weakly correlated to higher cluster numbers (Figure S2.1).

Figure S2.1: ADMIXTURE European and African ancestry estimates compared across k=3-5 clusters. We ran ADMIXTURE on samples from all Native American, 5 Siberian, 6 European and 3 sub-Saharan African populations. We plot the components most strongly correlated with European and African ancestry for k=3, 4 and 5. The inferences for $k=4$ and $k=5$ are strongly correlated for both African and European ancestry. The only exceptions are for the Nganasan1 and Nganasan2 (which the $k=5$ analysis identify as a separate cluster). The validation study in Note $S3$ suggests the $k=4$ estimates are strongly correlated to the truth. We focus on the k=4 estimates when we require European and African ancestry estimates.

Based on the high correlation between the $k=4$ and $k=5$ ancestry estimates, we hypothesized that the k=4 clustering provides estimates that are highly correlated to European and African ancestry proportion. To test this, we developed a new methodology for estimating a number proportional to an individual's European ancestry, which we report in Note S3. This analysis confirms that the k=4 ADMIXTURE runs are directly correlated to true ancestry proportion.

Based on the k=4 ADMIXTURE runs, we identified an "unadmixed" list of individuals in which the sum of the African and European ancestry estimates for the Native American and Siberian samples is never >0.00025. An advantage of performing analyses on this "unadmixed" dataset is that we do not need to deal with the confounder of recent European and African admixture, and we take advantage of this to establish the robustness of our results. The breakdown of the k=4 ADMIXTURE estimates by sample is given in Table S3 and by population is given in Table S1. The "unadmixed" samples include:

- 163 Native American samples from 34 populations (reduced from 493 from 52 populations)
- 56 Siberian samples from 3 populations (all the Naukan, Koryak and Chukchi samples)
- 333 samples from 7 outgroups (San, Yoruba, YRI, French, CEU, Sardinian and Han)

References for Note S2

¹ Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* **19,** 1655-1664.

Note S3 Ancestry Subtraction to address European and African admixture

(i) Motivation

There were a number of populations for which we did not have access to unadmixed samples. To learn about the history of such populations, we needed to adjust for the presence of non-Native ancestry. We used three complementary approaches to do this. The concordance of results from all these approaches increases our confidence in the key findings of this study.

- (1) *Restricting to unadmixed samples*: We restricted some analyses to 163 Native American samples (34 populations) without any evidence of recent European or African admixture (Note S2). A limitation, however, is that we could not analyze 16 populations in which all individuals were inferred to have some degree of recent admixture.
- (2) *Local ancestry masking:* We identified genomic segments in each individual that had an appreciable probability of harboring non-Native American or Siberian ancestry. We then created a "masked" dataset that treated data in these sections as missing (Note S4).
- (3) *Ancestry Subtraction:* We explicitly corrected for the effect of the estimated proportion of European and African in each sample by adjusting the value of *f4*-statistics by the amount that is expected from this admixture. This is discussed in what follows.

(ii) Details of Ancestry Subtraction

Assume that we have an accurate estimate of African and European ancestry for each sample (whether it is an individual or a pool of individuals). In practice, we used the ADMIXTURE k=4 estimates, because as described below, they appear to be accurate for Native American populations (with the possible exception of Aleuts as we discuss below). We can then define:

 $a = \frac{9}{6}$ African ancestry in a test sample $e = %$ European ancestry in a test sample 1-a-e = $\%$ Native ancestry

For many of our analyses, we compute f_4 statistics, whose values are affected in a known way by European and African admixture. We thus algebraically correct for the effect of recent European or African admixture on the test statistics, obtaining an "Ancestry Subtracted" statistic that is expected for the sample if it had no recent European or African ancestry.

The main context in which we compute *f4* statistics is in our implementation of the *4 Population Test*, to evaluate whether the allele frequency correlation patterns in the data are consistent with the proposed tree *((Unadmixed, Test),(Outgroup1, Outgroup2)),* where the *Unadmixed* population is a set of Native American samples assumed to derive all of their ancestry from the initial population that peopled America, the *Test* population is another Native American population, and the two outgroups are Asian populations. An *f4* statistic consistent with zero suggests that the *Unadmixed* and *Test* populations form a clade with no evidence of ancestry from more recent streams of gene flow from Asia. If the *Test* population harbors recent European or African ancestry, however, a significant deviation of this statistic from zero would be expected, making it difficult to interpret the results. We thus compute a linear combination of f_4 statistics that is expected to equal what we would obtain if we had

access to the Native American ancestors of the *Test* population without recent European or African admixture:

$$
S_1 = \frac{f_4(Unadmixed, Test; Out1, Out2) - (a) f_4(Unadmixed, Voruba; Out1, Out2) - (e) f_4(Unadmixed, French; Out1, Out2)}{1-a-e} \quad \left(S3.1 \right)
$$

Intuitively, this statistic is subtracting the contribution to the f_4 statistic that is expected from their proportion *a* of West African-like ancestry (Yoruba), and their proportion *e* of West Eurasian-like ancestry (French). We then renormalize by $1/(1-a-e)$ to obtain the statistic that would be expected if the sample was unadmixed.

A potential concern is that the African and European ancestry in any real Native American test sample is not likely to be from Yoruba and French exactly; instead, it will be from related populations. However, S_l is still expected to have the value we wish to compute if we choose the outgroups to be East Asians or Siberians. The reason is that genetic differences between Yoruba and the true African ancestors, and French and the true European ancestors, are not expected to be correlated to the frequency differences between two East Asian or Siberian outgroups. Specifically, the allele frequency differences are due to history within Africa or Europe, which is not expected to be correlated to allele frequency differences within East Asia and within Siberia.

(iii) Ancestry Subtraction gives results concordant with those on unadmixed samples

To compare the performance of our three approaches to address the confounder of recent European and African admixture, we computed $48 = 8 \times 6$ statistics of the form $f_4(Undmixed,$ *Test*; *Han, San*). We choose "*Unadmixed*" to be one of 8 Native American groups from Meso-America southward that have sample sizes of at least two and for which all samples are inferred to be unadmixed by ADMIXTURE k=4 (Chane, Embera, Guahibo, Guaymi, Karitiana, Kogi, Surui and Waunana). We choose "*Test*" to be one of 8 Native American populations from Meso-America southward with at least two samples that are entirely unadmixed, and that also have at least two samples that have >5% non-Native admixture according to the ADMIXTURE k=4 analysis (Aymara, Cabecar, Pima, Tepehuano, Wayuu and Zapotec1). This allows us to compare results on admixed and unadmixed samples from the same population.

If the *Test* population harbors European or West African admixture that we have not corrected, we expect to see a significant deviation of the statistic from zero. For example, *f4*(*Karitiana, French; Han, San*), corresponding to the statistic expected for an entirely European-admixed Native American population, is significant at $Z = 45$ standard errors from zero, and $f_4(Karitiana, Yoruba; Han, San)$, which gives the f_4 -value we would expect for an entirely West African-admixed Native American population, is significant at $Z = 101$.

Figure S3.1 shows the scatterplots of *Z*-scores we obtain without Ancestry Subtraction, with Ancestry Subtraction, and with local ancestry masking (Note S4). The *x-*axis shows data for the unadmixed samples from each *Test* population, while the *y-*axis shows the results for the >5% admixed samples from the same populations. We find that:

- Without Ancestry Subtraction there are significant deviations from zero $(|Z|>3)$ (Fig. S3.1A)
- With Ancestry Subtraction, there are no residual $|Z|$ -scores >3 (Figure S3.1B)
- With local ancestry masking (Note S4), there are again no residual |Z|-scores > 3 (Figure S3.1C), showing that this method also is appropriately correcting for the admixture.

Figure S3.1: Ancestry Subtraction and local ancestry masking both correct for old world admixture. We computed *f4(Unadmixed, Test; Han, San),* which is sensitive to European or West African ancestry in the *Test* population, for 8 "*Unadmixed"* populations from Meso-American southward with all samples inferred to be completely unadmixed and with a sample size of at least 2, and for 6 "Test" populations from Meso-America southward with at least two unadmixed samples and at least two samples with >5% non-Native American ancestry. (A) Without ancestry correction, the $f₄$ statistics in the admixed samples are $Z > 3$ standard errors from zero. (B) With Ancestry Subtraction, and (C) local ancestry masking, the test statistics are always within $|Z| < 3$.

(iv) Robustness of ADMIXTURE ancestry estimates used in Ancestry Subtraction

We were concerned that Ancestry Subtraction might lead to erroneous inferences about history, if the ADMIXTURE k=4 ancestry estimates were inaccurate. To assess the robustness of the ADMIXTURE $k=4$ estimates, we used the fact that f_4 statistics can infer quantities proportional to ancestry, even without accurate surrogates for the ancestral groups¹. Specifically, to estimate a number proportional to European ancestry here, we can compute:

f4(San, West Eurasian; Unadmixed, Test) (S3.2)

If the *Unadmixed* and *Test* populations are sister group that diverged from a homogeneous ancestral population since both from West Eurasians, this statistic has an expected value of zero. However, if the test sample has some recent European ancestry that is not corrected for, its frequency will be correlated to the West Eurasian outgroup, resulting in an f_4 statistic that has a value proportional to its European ancestry.

A complication in computing this statistic is that Native American, Siberian, and East Asian populations are not all equally genetically related to West Eurasian populations, as we can see empirically from *4 Population Tests* of the proposed tree *(Yoruba, (French, (East Asian, Native American)))* failing dramatically whether the *East Asian* population is Han, Chukchi, Naukan and Koryak. The explanation for this is outside the scope of this study (it has to do with admixture events in Europe, as we explain in another paper in submission). In practice, however, it means that we cannot simply use a European population like French to represent West Eurasians in Equation S3.2, since if we do this, Equation S3.2 may have a non-zero value for a Native American population, even without recent European admixture.

To address this complication, we took advantage of the fact that east/central Asian admixture has affected northern Europeans to a greater extent than Sardinians (in our separate manuscript in submission, we show that this is a result of the different amounts of central/east Asian-related gene flow into these groups). To quantify this, we computed the statistic *f4(San, West Eurasian; Pop1, Pop2)* for *West Eurasian = Sardinian* and *West Eurasian = French,* and for 24 Siberian and Native American populations *(Pop1 and Pop2)* (Figure S3.2). Figure S3.2 shows a scatterplot for all 190=20×19/2 possible pairs of these populations*.* Within non-Arctic Native populations, and within Arctic populations (East Greenland Inuit, Chukchi, Naukan and Koryak), the statistics are close to zero, consistent with their being (approximate) clades relative to West Eurasians. In contrast, there are deviations from zero when the

comparisons are between non-Arctic Native and Arctic populations, with non-Arctic Native populations showing consistent evidence of being genetically closer to West Eurasians.

Figure S3.2: French and Sardinians have different proportions of Asian admixture, letting us learn a correction factor. We compute *f4(San, West Eurasian; Pop1, Pop2)* for 20 Native American and Siberian populations with at least 3 samples inferred to be unadmixed. The statistics are divided into comparisons of two non-Arctic Native populations or two arctic populations (where the statistics are usually close to zero), and comparisons of non-arctic and arctic populations where they often deviate strongly. The statistics are highly correlated, with the magnitude for West Eurasian = Sardinian 0.75 of West Eurasian = French.

The observation of non-zero statistics when one of the Native populations is Arctic and the other is a more southern Native American population is a complication, since we would like Ancestry Subtraction to work not just for southern Native American populations, but also for northern North Americans who have inherited genetic material from multiple streams of Asian migration. However, the fact that Sardinian statistics are smaller than the French statistics by a constant factor (0.75), allows us to adjust for this difference by regression. Specifically, we can compute a linear combination S_2 of the *French* and *Sardinian* statistics that subtracts out the effect of central/east Asian gene flow into West Eurasians and has an expected value of zero. This can be viewed as the expected value of $f_4(San, West Eurasian;$ *Pop1, Pop2)*, for a hypothetical West Eurasian population that does not have any history of admixture from Asians (because we have subtracted away that ancestry):

$$
S_2 = \frac{1}{1 - 0.75} [f_4(San, Sardinian; Pop1, Pop2) - 0.75 f_4(San, French; Pop1, Pop2)] \tag{S3.3}
$$

In practice, we had to also deal with a further complication of African admixture in some populations, so we computed a slightly more complicated statistic that subtracts out the expected effect of this ancestry:

$$
S_3 = \frac{1}{1 - 0.75} \begin{pmatrix} [f_4(San, Sardinian; Pop1, Pop2) - af_4(San, Sardinian; Pop1, Pop2)] \\ -0.75[f_4(San, French; Pop1, Pop2) - af_4(San, French; Pop1, Yoruba)] \end{pmatrix} (S3.4)
$$

We compared S_3 to the ADMIXTURE $k=4$ estimate for diverse Native American populations. For the reference "Pop1" in *S3*, we used a pool of unadmixed Native American, Koryak, Naukan and Chukchi samples (Note S2), deleting any individuals that overlapped the *Test* sample. For the *Test*, we analyzed populations with at least three samples).

Figure S3.3 shows that the inferences are highly correlated $(r^2=0.97)$, providing confidence in the ADMIXTURE k=4 estimates. However, two populations are notable in that their ADMIXTURE estimates are $|Z| > 3$ standard errors from the fitted regression line:

- The Chilote have an ADMIXTURE k=4 estimate of 37.7% ancestry vs. an extrapolated 44.0 \pm 1.6% from our method (nominally Z=3.9 standard errors different).
- The Aleuts have an ADMIXTURE k=4 estimate of 64.8% European ancestry vs. an extrapolated $56.7 \pm 2.2\%$ from our method (nominally Z=3.7 standard errors). We

hypothesize that this difference is due to over- or under-correcting for European ancestry in the Aleuts due to inaccurate ADMIXTURE k=4 ancestry estimates. Thus, we cannot confirm our finding that the Aleuts share ancestry with the Inuit based on Ancestry Subtraction. However, the robustness of the inferences from local ancestry masking in other cases makes us think the inference based on masking is likely to be correct in this case too.

Figure S3.3: Correlation between k=4 ADMIXTURE estimates of European ancestry and the S_3 **statistic.** We restrict to populations with at least 3 samples to reduce noise in the visualization. The high correlation suggests that both methodologies are producing meaningful inferences about ancestry. The strongest discrepancies are seen in the Chilote and Aleuts; we are cautious about using Ancestry Subtraction for these populations.

References for Note S3

 1 Reich, D., Thangaraj, K., Patterson, N., Price, A.L. & Singh, L. Reconstructing Indian population history. *Nature* **461,** 489-494 (2009).

Note S4 Masking segments of non-Native ancestry

(i) Strategy

One of our main methods for dealing with the confounding factor of recent European and African admixture is "local ancestry masking". In this strategy, we identify subsets of the genome with an appreciable probability of non-Native American ancestry, and flag or "mask" them. We can then restrict analyses to unmasked parts of the genome.

The success of such an approach relies on three ingredients: (i) admixture has occurred recently enough that there are multi-megabase genomic segments where it is possible to infer ancestry with confidence; (ii) we have dense enough genotyping data to perform local ancestry inference, and (iii) the analysis of the resulting "masked data" provides unbiased inferences about history.

To implement local ancestry inference, we used HAPMIX, which employs a haplotype Hidden Markov Model to model each segment of the genome as a mixture of two ancestral panels of haplotypes provided by the user¹. HAPMIX was developed and tested for the case of African Americans, in which the samples being studied are a mixture of two ancestral populations for which there is access to panels of samples that are reasonably good surrogates for the ancestral populations. For this situation, HAPMIX has been shown to make inferences about ancestry that are about 99% correlated to the true ancestry¹.

For Native Americans, there are greater challenges in local ancestry inference than for African Americans, because of three-way admixture (European, African and Native American ancestry), and because for Native Americans, unadmixed surrogates for the ancestral populations are often not available. However, we hypothesized that for our purpose it may be adequate to apply a local ancestry inference engine as a "black box", using ancestral panels that include collections of haplotypes that are drawn from diverse populations some of which may even be admixed themselves, and mask out segments of the genome that are identified as having even a small probability of being of non-Native ancestry. By using a stringent threshold—masking segments inferred to contain non-Native ancestry with even a small probability—we hypothesized that the subset of the genome that remained would be effectively unadmixed. We verified this hypothesis empirically by comparing results on masked data to results on unadmixed samples with no masking (Note S2), and to results from Ancestry Subtraction (Note S3).

HAPMIX requires that the samples from the ancestral panels are phased, and for this purpose we pooled all the samples in the parental panels and ran the BEAGLE software². To run on the Native American and Siberian samples, we treated each sample as a putative mixture of two ancestral haplotype panels obtained from the phased data:

- (i) *Non-Native Panel:* 538 samples representing both West Eurasian and African ancestry: 24 Basque, 45 Bedouin, 108 CEU, 28 French, 12 Italian, 42 Palestinian, 25 Russian, 28 Sardinian, 88 TSI, 8 Tuscan, 109 YRI and 21 Yoruba.
- (ii) *Native Panel*: All Native American + Siberian populations.

We ran HAPMIX on each of the Native American and Siberian samples in turn, using the remaining samples (all but the one being analyzed) as one parental panel and all the European and African samples as the other. For each individual, we used software settings corresponding to a prior hypothesis of a non-Native proportion of 5%, and a number of generations since mixture of 10. These priors are chosen to be sensitive to even small

proportions of non-Native admixture. However, previous simulations have shown that HAPMIX priors have minimal effect on ancestry inference for admixture in the last handful of generations¹, the scenario relevant here.

At each locus, HAPMIX infers the probability that an individual has $0 (p_0)$, $1 (p_1)$ and $2 (p_2)$ alleles of non-Native ancestry. Figure S2 shows examples. We defined the expected number of non-Native alleles at any locus is $E = p_1 + 2p_2$. We then masked any section of the genome with E>0.01, choosing a stringent threshold because we wished to remove any segment that had even a small chance of harboring non-Native ancestry.

Figure S4.1: Comparison of the estimated proportion of non-Native ancestry to the proportion of the genome masked. On the *x*axis we plot the ADMIXTURE k=4 estimate of the percent European and African ancestry, and on the *y*axis the percent of the genome masked. If masking is perfect, we expect $y = 2x - x^2 = 1 - (1 - x)^2$ of the genome to be masked, and this expectation is shown by the smooth curve. There is a strong correlation between expected and observed. In practice, we mask 2.1% more of the genome than expected on average, reflecting our aggressive masking.

To assess if masking is performing as expected, Figure S4.1 plots the ADMIXTURE k=4 estimate for each sample against the masked proportion of the genome³. The expected proportion *y* of the genome that is masked given that a fraction *x* of their alleles are of non-Native American ancestry is $y = 2x-x^2 = 1-(1-x)^2$ (assuming Hardy-Weinberg equilibrium), and is a good match to the observed data with the exception that the masked portion of the genome is on average 2.1% in excess of the theoretical expectation overall. Some excess is expected given the aggressive threshold we use to remove segments that are of potentially non-Native American ancestry, so we do not find this excess to be surprising.

There are also a few outlier samples in Figure S4.1 for which more of the genome is masked than would be expected from the genome-wide ancestry estimate. Detailed examination of the strongest outliers, who have $>11\%$ more of the genome masked than expected from theory, shows that they were almost all cases where the method never inferred more than one non-Native chromosome (Figure S4.1). This is the pattern expected for a first generation mixture of an admixed individual and an unadmixed individual—an individual who is not in Hardy-Weinberg equilibrium—leading to a larger proportion of their genome being masked than would be expected from an individual whose parents are both equally admixed. The fact that these individuals are outliers is expected, and restricting to subsets of the genome that are unmasked for these individuals should not result in any bias in historical inferences, since the segments that remain after the masking are expected to be entirely Native American in origin.

(ii) No evidence that masking biases our inferences about history

A concern is that there are biases in the segments of Native American genomes that we are masking. Because we are running HAPMIX in a "black box" mode for a scenario in which it has never been rigorously tested (three way mixture and using complex mixtures of

populations some of which are poor surrogates for the true ancestral populations as haplotype panels), it may be producing inaccurate estimates of ancestry probability at some segments of the genome, causing us to include in our dataset genuine segments of non-Native American ancestry despite the stringent thresholds that we apply to retain only the most confidently inferred segments.

We explored whether the thresholds we used for local ancestry masking are substantially affecting our inferences. The main analyses in the paper are based on E<0.01 and using all Siberian and Native American samples as the ancestral Native panel. However, we also explored the effect of masking using more permissive thresholds $(E<0.1)$, and performing the local ancestry inference using a Native panel that consisted only of Native Americans. The percentage of the genome masked was similar in all four analyses that we performed (15.6%- 17.0%; Table S4.1). For the analyses reported in the main paper, we decided to use the threshold of E<0.01, and also to use both Native Americans and Siberians for the Native ancestral panel, because: (a) we wished to be as confident as possible that we are analyzing Native American segments for studying history; (b) we only lose a small amount of data by discarding segments with even a small probability of non-Native ancestry; and (c) visually, ancestry inferences in Arctic populations were crisper when we included Siberians in the ancestral panel (presumably because we used a more comprehensive ancestral haplotype panel).

Masking threshold	Ancestral Native panel	% genome masked in Native Americans
E<0.1	Native Americans only	15.6%
E < 0.01	Native Americans only	17.0%
E<0.1	Native Americans and Siberians	15.3%
E < 0.01	Native Americans and Siberians	16.6%

Table S4.1: Percentage of the genome masked when using different masking strategies

As a second approach to testing whether our inferences are robust to the masking procedure, throughout the paper we also compared key results obtained with the masked data to those from either (a) removing samples that are inferred to have any admixture at all (Note S2), or (b) explicitly correcting for non-Native American ancestry (Ancestry Subtraction; Note S3). The consistency of inferences made from masked data with those made from the other approaches is also evident when we build Neighbor Joining trees (Figure S3) and when we build an Admixture Graph relating populations (Figure S4).

References for Note S4

¹ Price AL, Tandon A, Patterson N, Barnes KC, Rafaels N, Ruczinski I, Beaty TH, Mathias R, Reich D, Myers S (2009) Sensitive detection of chromosomal segments of distinct ancestry in admixed populations. *PLoS Genet.* **5,** e1000519. 2

² Browning SR, Browning BL (2007) Rapid and accurate haplotype phasing and missing data inference for whole genome association studies using localized haplotype clustering. *Am J Hum Genet* 81, 1084-1097.

 3 Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* **19,** 1655-1664

Note S5 Correlation of genetic diversity with distance from the Bering Strait

We estimated heterozygosity by using the masked data (so as to eliminate the confounder of recent European and African admixture). We restricted analysis to populations with at least five samples to reduce sampling variation. To obtain a heterozygosity estimate for each population, we used the masked dataset. The heterozygosity estimate was obtained by dividing the number of heterozygous genotypes over all individuals from the population, by the number of genotypes that were present in the dataset (Table S1).

Geographic distance from the Bering Strait was computed using great arc routes from an Anadyr start point at 64.8N 177.8E, with the location of each population specified by the coordinates in Table S1 (where more than one sample was available for a population, we used a position mid-way between the two sampling locations and averaged population heterozygosity over the samples). We computed a Pearson correlation coefficient between the mean observed population heterozygosity and the distance from Beringia, for all 32 Native American populations with a sample size of at least 5. We evaluated statistical significance with a t-distribution transformation (using the R-package¹). The data we used for these analyses are reported in Table S5.1.

To evaluate the effects of coasts as facilitators of migration, we also computed "effective", or "least-cost path" distances². Compared to the geographic great arc distances, effective distances incorporate the effects of one or several landscape components. They are computed as least-cost paths on the basis of a spatial cost map that incorporates these components. The effective distance is computed as the sum of costs ("cost distance") along the paths. Because the relative cost of landscape components is arbitrary, we tested a range of combinations. For example, a ratio of 1:10 coastline/land means that it is ten times more costly to go through land than through coastline. In addition to simple great arc distances, we used the following coastline/inland cost combinations: 1:2, 1:5, 1:10, 1:20, 1:30, 1:40, 1:50, 1:100, 1:200, 1:300, 1:400 and 1:500.

When all populations are considered, we observe a negative correlation between heterozygosity and distance from the Bering Strait (Figure S5, $r=0.48$, P=0.007). This correlation increases when considering effective distances, reaching a maximum at a coastline/inland cost combination of 1:5 (Figure S5, $r = -0.51$, P= 0.004).

When we exclude from this analysis the four northern North American populations (Aleuts, East Greenland Inuit, West Greenland Inuit and Chipewyan) that have unambiguous evidence of additional streams of genetic input from Asia (Note S6) the negative correlation remains (*r*=-0.34, P=0.091) becoming stronger when effective distances are considered, again with a maximum at a coastline/inland ratio of 1:5 (Figure S5 $r = -0.39$, P= 0.049).

An exception to the pattern of decreasing heterozygosity with distance from Beringia is populations from the Isthmo-Colombian area which mostly have low diversity relative to the expectation based on their distance from the Bering Strait. As we document in the main text, these populations derived most of their ancestry from eastern South America and the current geographic location of some of them (north of the Panama isthmus) reflects complex population movements and admixtures in the region. Further exclusion from this analysis of the Isthmo-Colombian populations results in a correlation of heterozygosity with distance

from the Bering Strait of $r=0.50$ (P=0.022). This increases with effective distances reaching a maximum at a coastline/inland ratio of 1:10 (Figure S5: *r*= -0.70, P= 0.0004).

Population	N	Heterozygosity	Great Arc Distance (m)
Aleutian [*]	8	0.260	1,788,963
Chipewyan [*]	15	0.251	2,998,535
Ojibwa	5	0.249	5,184,797
West Greenland Inuit*	8	0.247	5,408,260
Pima	33	0.259	5,432,128
Algonquin	5	0.237	5,619,796
East Greenland Inuit*	7	0.237	5,786,292
Tepehuano	25	0.246	6,205,875
Mixtec	5	0.247	7,105,459
Maya	37	0.250	7,138,397
Mixe	17	0.242	7,140,781
Zapotec	22	0.248	7,181,122
Kaqchikel	13	0.250	7,538,473
Cabecar [*]	31	0.221	8,397,297
Guaymi*	5	0.214	8,588,582
Arhuaco [*]	5	0.208	8,746,097
Wayuu	11	0.234	8,788,814
Embera [*]	5	0.221	9,025,514
Guahibo	6	0.230	9,481,686
Inga	9	0.230	9,576,373
Piapoco	7	0.235	9,833,731
Ticuna1	6	0.225	10,391,952
Karitiana	13	0.221	11,346,772
Quechua1	40	0.244	11,484,968
Surui	24	0.206	11,493,384
Aymara	23	0.244	11,941,135
Wichi	5	0.220	12,486,648
Guarani	6	0.246	12,739,695
Diaguita	5	0.243	12,960,201
Chilote	8	0.238	13,914,216

Table S5.1: Heterozygosity and distance from the Bering Strait

* These populations were removed in sub-analyses.

References for Note S5

¹ R Development Core Team. R: A language and environment for statistical computing. (Vienna, Austria, 2010).
² Ray N (2005) PATHMATRIX: a geographical information system tool to compute effective distances among

samples. *Molecular Ecology Notes* **5,** 177-180.

Note S6 Documentation of at least three streams of Asian gene flow into America

(i) Motivation

A key question is whether Native Americans today descend from a single ancient gene flow event from Asia, or alternatively harbor ancestry from multiple streams of Asian gene flow. To address this, we began by performing 4 Population Tests¹ using the statistic f_4 (*Southern*) *Native American*, *Test Population; Outgroup1*, *Outgroup2*) where the statistic is defined as:

$$
f_4(A, B; C, D) = \frac{1}{n} \sum_{i=1}^{n} (a_i - b_i)(c_i - d_i)
$$
 (S6.1)

Here, a_i , b_i , c_i and d_i are the variant allele frequencies at SNP *i* in populations A, B, C and D respectively. The statistic is proportional to the correlation in allele frequencies differences *(Southern Native American - Test Population*) and *(Outgroup1 - Outgroup2)* over all SNPs. It has an expected value of zero if the *Southern Native American* and *Test Population* are sister groups that descend from a homogeneous ancestral population. By using a Block Jackknife standard error, we obtain an approximately normally distributed Z-score that serves a formal test for whether the 4 populations are consistent with the unrooted tree.

(ii) Most Native Americans descend from a homogeneous Asian ancestral population We computed the *f4* statistic using all 52 Native American populations in turn as the *Test Population*. For the pair of Asian outgroups, we used all possible pairs of 10 populations: Han and 9 Siberian populations, restricting to Siberian populations with at least ten samples that are not known to have any history of back-migration from Arctic Native Americans (this criterion excluded the Naukan Eskimo who are culturally related to the Greenland Inuit, and the Chukchi some of whom are known to have admixed with the Yupik-speaking Naukan).

For each of 52 Native American populations, we computed up to $135 = 45 \times 3 f_4$ statistics. There were $45=9\times8/2$ possible pairs of Asian outgroups and we tested all. We also tested three *Southern Native American* reference samples: (i) 13 Karitiana (unadmixed South Americans), (ii) 5 Guaymi (unadmixed Meso-Americans), or (iii) 158 individuals from an "Unadmixed Pool". The "Unadmixed Pool" was obtained by pooling all individuals from Mexico southward inferred by ADMIXTURE k=4 to be unadmixed (Note S2). The *f4* statistics obtained using the "Unadmixed Pool" were highly correlated to those in the Karitiana and Guaymi, but with smaller standard errors owing to larger sample size and reduction of population-specific genetic drift.

There were two minor complications:

- (i) When the Karitiana and Guaymi were the *Test Population*, we could not use them as the *Southern Native American population.* We thus only computed $90 = 45 \times 2 f_4$ test statistics for testing these two populations for compatibility with a simple tree.
- (ii) When the Unadmixed Pool contained individuals that were also in the *Test Population*, we removed the samples in the *Test Population* from the pool so as not to use the same samples twice. This slightly reduced the sample size of the Unadmixed Pool.

Table S1 presents the maximum |Z| score for a deviation from zero obtained for these *f4* statistics, for each of 52 Native American *Test Populations*. We used a threshold for significance of $|Z| > 4.5$, corresponding to P<0.05 after correcting for 7,020 hypotheses tested ((52 populations) \times (45 statistics) \times (3 *Southern Native American* populations)). There were 4 populations that crossed this threshold, all from northern North America (nearly identical

results are obtained for the Ancestry Subtracted data and unadmixed samples; Table S1). Their maximum |Z| scores are given in Table S6.1. The same test for the Saqqaq Greenland sample—which we performed for the approximately one sixth of SNPs for which we have data for the Saqqaq individual—shows that they too must have ancestry from later Asian gene flow (maximum $|Z|=5.9$; Table S6.1).

			Max. Z for different Southern Nat. Am.	P-value from Bonferroni	P-value for the
Population	Karitiana (36 tests)	Guaymi (36 tests)	Unadmixed Pool (36 tests)	correction for 135 tests in max. Z -score analysis	Hotelling T- test
W.G. Inuit	16.5	14.6	14.2	10^{-9}	10^{-9}
E.G. Inuit	16.4	14.6	14.6	10^{-9}	10^{-9}
Chipewyan	6.0	4.6	4.8	2×10^{-7}	10^{-9}
Saqqaq	5.9	5.2	5.3	5×10^{-7}	2×10^{-9}
Aleutian	4.9	4.6	4.9	3×10^{-4}	9×10^{-5}

Table S6.1: Populations with different relationship to Asians vs. southern Native Americans

Note: This table lists all populations with both a Hotelling *T*-test P<0.005 and maximum |Z|-score of ≥4.5. All analyses are performed on masked data, but results are consistent in the unmasked data and in unadmixed samples (Table S1).

evidence of 3 different patterns of relatedness to Asians among Native Americans. We plot *f4* statistics for all possible pairs of northern Native American populations with the strongest evidence of a distinct relationship to Asians compared with more southern Native Americans. Two groupings of populations harbor significantly different historical relationships with a panel of 10 Asian outgroups. Within groups, *f4* statistics are highly correlated, whereas across groups they are significantly different: the P-value in each panel is from Table 1: the Hotelling *T-*test for whether the vectors of f_4 statistics are consistent with being scalar multiples of each other. The dashed line is added to highlight the difference in within-group and across-group comparison.

(iii) New method for distinguishing the number of distinct migrations into America For each of the *Test Populations* from North America with a significantly different pattern of relatedness to the panel of 10 Asian populations from what is seen in Southern Native Americans, we plotted the values of 45 *f4* statistics of the form *f4*(*Southern Native American*, *Test Population; Outgroup1*, *Outgroup2*), using the Unadmixed Pool as *Southern Native Americans*. Figure S6.1 plots the 45 *f4* statistics for all possible pairs of five populations (the four Native American groups and the Saqqaq Greenland sample) in the masked data. We observe two groupings of populations that differ qualitatively from each other as well as from more southern Native Americans. One grouping includes Greenland Inuits (and possibly Aleution Sample of the Test Populations (and possibly the Saqqaqa).

Aleutian E. Green. Inuit W. Green. Inuit Aleutian and across-group consisted line is added by the difference in the difference in the Sales of each of t

To place these qualitative observations on a solid statistical footing, we generalized the analysis of *f4* statistics by developing new methodology. If two Native American populations derive all their non-First American ancestry from the same ancestral stream of Asian gene flow, their vectors of f_4 statistics are expected to be scalar multiples of each other, and we developed a formal statistical test for whether this is the case. Given a phylogeny and a set of four populations *P, Q, R, S* we define F_4 as the expected value of the f_4 statistic, that is:

$$
F_4(P,Q,R,S) = \mathbb{E}[f_4(P,Q,R,S)] = \mathbb{E}[(p-q)(r-s)] \tag{S6.2}
$$

Here, *p, q, r, s* are alleles of *P, Q, R, S*. We code an allele as 1 if it is a variant allele and 0 if it is a reference allele (the opposite convention gives the same *f4*-statistics).

We now choose a *Southern Native American* group *P* (say Karitiana or a pool of populations from Meso-American southward) and an *Outgroup1* population *R* (say Han) in Asia. Thus, we can write a two-dimensional matrix of F_4 values with rows corresponding to the number of Native American populations (*Q*) that are tested, and columns corresponding to the number of Asian populations (*S*) that are tested:

$$
X(Q, S) = F4(Karitiana, Q; Han, S)
$$
\n(S6.3)

Theorem

Let r be the rank of X, and n the number of independent gene flows into the Americas. Then

 $r + 1 \le n$ (S6.4)

The proof is straightforward. We use induction on *n*. If $n = 1$, then $r = 0$, or equivalently $X =$ 0. This is our familiar *f4-*based *4 Population Test*. A single gene flow from Asia initially can only increase the rank by 1. Subsequent drift in the Americas does not change *X* except to add noise (since allele frequency changes due to drift are uncorrelated to the allele frequency differences between the Asian groups *R* and *S*). Admixture of populations within the Americas only has the effect of adding new rows to the matrix that are linear combinations of the pre-existing rows, and thus does not increase the rank. This completes the proof.

To apply this result, we fix populations *P* and *R*, choose *m* Native American populations to represent *Q*, and choose *n* Asian populations to represent S. We then have an $m \times n$ matrix *Y*:

$$
Y(Q, S) = f_4(P, Q; R, S) \tag{S6.5}
$$

Our matrix *X* is the expected value of our data matrix *Y*, given a specific demographic history relating the analyzed populations. Using a weighted Block Jackknife², we can estimate a covariance matrix *V* for *Y*. *V* has dimension $mn \times mn$. To score for rank *k* we can fit $Y(B,D)$ = $A \times B$ where *A* is $m \times k$, and *B* is $k \times n$. The log-likelihood is:

$$
L(A,B) = -\frac{1}{2} \sum_{q,s,q',s'} C(q,s) V^{-1}(q,s,q',s') C(q',s')
$$
 (S6.6)

where

$$
C = Y - AB \tag{S6.7}
$$

This likelihood function allows us to apply a Likelihood Ratio Test (LRT). We can postmultiply *A* by any non-singular $k \times k$ matrix *M*, and pre-multiply B by *M*. It follows that our rank *k* model for *Y* has *k(m+n−k)* degrees of freedom. Testing rank *k+1* versus rank *k* is then a standard LRT, leading to a χ^2 statistic under the null hypothesis that the F_4 matrix has rank *k*. For the important case $m = 1$, in which we wish to test if *X* is 0 (or equivalently that the rank of *X* is 0), our statistic is a Hotelling T^2 statistic³. We refine our test, not using the χ^2 distribution, but an *F*-statistic.

In our weighted Block Jackknife we use 5 centimorgan blocks, with the weight ω_i for block *i* being the number of SNPs in block *i*. The *F*-test requires as a parameter the number of 'independent' blocks *d*. We use an ansatz:

$$
d = \frac{\left(\sum_{i} \omega_{i}\right)^{2}}{\sum_{i} \omega_{i}^{2}}\tag{S6.8}
$$

and take T^2 to be $F_{n,d-n}$ distributed under the null. Fortunately *d* is large (several hundred) and *F* is approximately $\chi_{[n]}^2$, so the exact value of *d* is not very important here. To test larger values of *k* we simply use the asymptotic χ^2 distribution.

(iv) At least three streams of gene flow from Asia are needed to explain the data

We used the new statistical method described in section (iii) to identify 43 southern Native American populations that are consistent with deriving all their ancestry from the same initial migration within the limits of our resolution. To identify this set of populations, we began by using the Brazilian Karitiana as a "seed" *Southern Native American* population (*P* in Equation S6.4), and considered each of the other 52 Native American populations in turn as the *Test Population* (*Q* in Equation S6.4). A total of 41 populations gave non-significant test statistics for deriving from the same ancestral gene flow event as the Karitiana ($P > 0.1$), and we pooled them. We then retested the remaining 14 populations now using the pool to represent *Southern Native Americans*, and identified 2 additional populations with nonsignificant statistics (including Karitiana). The final pool of 43 is a subset of the 48 identified by maximum |Z|-score analysis.

Table S1 gives the P-values from a formal test for whether each of the 52 Native American populations in the dataset has evidence for deriving ancestry from a distinct stream of Asian gene flow from the 43 *Southern Native American* populations (when the *Test Population* is one of the 43 *Southern Native Americans*, we construct a new pool of 42 populations excluding the *Test Population*)*.* This identifies four populations with Hotelling *T-*test Pvalues <0.00009, which are the same as the four populations that emerge as significant form the maximum $|Z|$ -score analysis (Table S6.1). The only other formally significant signal is in the Maya2 ($P=0.0007$), but the maximum Z-score analysis is not significant here ($P=0.22$), and the closely related Maya1 show no signal $(P=0.50)$. Given that after correcting for testing 52 hypotheses this observation is only weakly significant ($P=0.04$) we view this result as uncompelling. In what follows, we therefore focus on studying the four populations that give consistent signals of ancestry from later streams of Asian gene flow in both the maximum $|Z|$ score and Hotelling *T-*test analyses. The same analysis applied to the Saqqaq Greenland sample shows that it, too, derives ancestry from a later stream of Asian gene flow ($P=2\times10^{-9}$), consistent with the original analysis of data from this sample⁴, and so we add this population as a fifth group in what follows.

For all $10 = 5 \times 4/2$ possible pairs of the 5 populations in Table S6.1, we evaluated whether the data are consistent with the hypothesis that the later Asian genetic material in both of the populations derives from the same source; that is, we tested the null hypothesis that the rank of the matrix is *k=1*. To represent *Southern Native American* populations in this analysis, we

used the pool of 43 Native American populations from Meso-America southward consistent with having ancestry entirely from First Americans (Table S1). The P-values are given both in Table 1 and in the bottom right of the panels in Figure S6.1. This identifies two groupings of populations consistent with the qualitative patterns in Figure S6.1: Eskimo-Aleut speakers (East / West Greenland Inuit and probably Aleuts), and the Chipewyan and possibly the Saqqaq. Within groupings, P-values are always non-significant or marginally significant, whereas across groupings, they are significant.

Based on these results, we pooled populations for further testing: Southern (43 populations; 406 samples), Eskimo-Aleut (East Greenland Inuit, West Greenland Inuit, and Aleuts; 23 samples), and Chipewyan (15 samples). We also considered the Saqqaq as a potentially fourth group. Application of the methods of (iii) to our data result in three main findings: (1) At least three streams of gene flow from Asia to America occurred. Specifically, when we

- simultaneously analyze Southern Native Americans, Eskimo-Aleut speakers, and Chipewyan, we reject a single stream of later Asian gene flow (P=0.011; Table S6.2).
- (2) The Chipewyan have a different pattern of relatedness to Asians than the Eskimo-Aleut.
- (3) The Saqqaq are consistent with having their Asian ancestry from the same stream of later gene flow as the Chipewyan (P=0.29; Table S6.2).

Table S6.2: Analysis of the masked data indicate at least 3 streams of gene flow with Asia

Note: For analyses involving the Saqqaq, we have about six-fold fewer SNPs. This table is somewhat redundant to Table 1.

Table S6.3: Analysis restricted to unadmixed samples confirms ≥3 streams of gene flow

Note: This is the same as Table S6.2 except we restrict to unadmixed samples: a pool of 30 "Southern" populations identified by the same iterative process as described for the masked dataset, 3 East Greenland Inuit, 2 Chipewyan and 1 Saqqaq.

To assess if our inference about the minimum number of streams of gene flow between Asia and America is robust to local ancestry masking, we repeated the analyses using the subset of samples that we inferred in Note S2 are unadmixed. Results are consistent, although due to the smaller sample sizes for the Chipewyan and Eskimo-Aleut speakers, we no longer have

power to distinguish the Asian ancestries in these groups (P=0.49; Table S6.3). Our data continue to be consistent with no more than 3 streams of gene flow when we include the Saqqaq (Table S6.3).

(v) A genetic link between the Saqqaq and Na-Dene speakers

In the Saqqaq genome paper, the authors co-analyzed the data they collected with data from diverse present-day populations from Siberia and the America. Based on the patterns that they observed in Principal Component Analysis, they argued that the Saqqaq have ancestry from a different stream of gene flow into America than Eskimo-Aleut speakers, Na-Dene speakers, and Southern Native Americans⁴. However, this is not a formal test: the failure to cluster together in the first few principal components does not necessarily imply that populations are unrelated; just that they do not share much genetic drift on their common ancestral lineage.

Our conclusions differ:

- We confirm that the Saqqaq derive from a different stream of Asian gene from southern Native Americans ($P=2\times10^{-9}$ rejecting 1 stream), and also that they derive from a different stream of Asian gene flow from Eskimo-Aleut speakers ($P=2\times10^{-6}$ rejecting the hypothesis that Southern Native Americans, Eskimo-Aleut and Saqqaq derive from 2 migrations) (Table S6).
- We cannot confirm that Saqqaq derive from a stream of Asian gene flow distinct from that which led to Na-Dene speakers like the Chipewyan. When we test the hypothesis that the Saqqaq and Chipewyan descend from the same second stream, we cannot reject it $(P=0.29)$, and we also cannot reject the hypothesis that Southern Native Americans, Eskimo-Aleut, Chipewyan and Saqqaq derive from just 3 streams of Asian gene flow $(P=0.27)$. In Note S7, we confirm these inferences by presenting a formal model showing that the Saqqaq and Chipewyan can be fit as deriving from the same stream of Asian ancestry.

Our finding that the Saqqaq harbor ancestry that is deeply shared with the Chipewyan raises the possibility of a historical link between the ancestors of the Saqqaq and Na-Dene speakers, that is, that they descend from a common stream of Asian gene flow into America. However, an alternative that we cannot rule out is that there were two streams of Asian gene flow into America from related Asian populations whose ancestries we cannot distinguish.

An important direction for future research will be to study additional Na-Dene speaking populations and Siberian populations. This will provide more power to test if the Asian ancestry in these two groups is different. It will also allow more general statements about whether the ancestry in the Chipewyan (related to the Saqqaq) is shared across Na-Dene speakers. We note that the authors of the Saqqaq genome paper were unable to share with us the data from the Na-Dene speaking population they studied, and so we could not evaluate whether that sample harbors the same signal of Saqqaq-related ancestry as the Chipewyan.

References for Note S6

¹ Reich, D., Thangaraj, K., Patterson, N., Price, A.L. & Singh, L. Reconstructing Indian population history. *Nature* **461,** 489-494 (2009). 2

Busing, F.M.T.A., Meijer, E. & van der Leeden, R. Delete-m jackknife for unequal m. *Statistics and Computing* 9, 3-8 (1999).

³ Mardia K.V., Kent J.T. & Bibby, J.M. *Multivariate Analysis*. Academic Press (1979).

Rasmussen M. *et al.* Ancient human genome sequence of an extinct Palaeo-Eskimo. *Nature* **463,** 757-762 (2010).

Note S7 Modeling the peopling of America

(i) Admixture Graphs

Trees present an oversimplified view of population relationships, as they assume all groups descend from a common ancestor by a series of bifurcations without subsequent admixture.

As a tool for learning about population mixture events, we used Admixture Graphs $(AGs)^{1}$. AGs are generalizations of trees that accommodate the possibility of unidirectional admixture between branches (edges) of the tree. Thus, AGs allow us to propose models of population relationships that are more complex than a simple tree, and to test whether they are consistent with the data. While many AGs in principle might be consistent with a dataset, our goal is to identify a parsimonious AG that does not predict any pattern of allele frequency correlation across populations (as measured by *f-*statistics) that is grossly inconsistent with the data. This distinguishes an AG from the Neighbor Joining tree of Figure 1C, which is grossly inconsistent in that it predicts patterns of allele frequency correlations among populations that are discrepant with the data as reflected in highly significant *4 Population Tests.*

By itself, an AG just specifies the topology of population relationships (like a tree). However, once a topology is specified, one can infer the mixture proportions for each admixture event, as well as the amount of genetic drift that occurred historically on each lineage (variation in allele frequencies due to sampling variation in the context of limited effective population size) that best fit the data. An AG in which these quantities are specified determines the values of all possible "*f*-statistics" measuring the correlation in allele frequencies among two (f_2) , three (f_3) , and four (f_4) populations1. We can then compare these to the observed values (which have a standard error from a Block Jackknife) to assess whether there is any evidence for a poor fit. (The *4 Population Test* is a special case of Admixture Graph model testing, since it tests whether particular f_4 statistics have the value of zero expected if those four populations are related by a simple tree.) A valuable feature of AGs is that they are robust to ascertainment bias of SNPs (how the SNPs were chosen for inclusion in the study), making them useful for inferring tree topologies even using data from SNP microarrays¹.

To assess whether a proposed AG is consistent with a dataset, we have implemented software (ADMIXTUREGRAPH) that begins with a proposed topology, and finds the combination of branch lengths and admixture proportions that best fit the data. We measure the fit to data by testing the match between all possible *f-*statistics predicted by the model and the data: for a given set of *N* populations, this is $(N(N-1)/2 f_2$ statistics, $3N(N-1)(N-2)/6 f_3$ statistics, and $3N(N-1)(N-2)(N-3)/24 f₄$ statistics. A complication is that the *f*-statistics are correlated (for example, all the f_3 and f_4 statistics can be written as linear combinations of f_2 statistics), and thus it is difficult to know how many hypotheses we are effectively testing. To deal with this, we compute a χ^2 statistic measuring the difference between all observed and predicted fstatistics taking into account the covariance structure (and using an error covariance from a Block Jackknife). This serves as a score that allows us to climb to a best fitting model.

At present, we do not know how to calculate how many degrees of freedom are effectively being used in the model (given the correlation among all the *f*-statistics), and hence we do not believe that our AG technology supports a formal *P*-value for a goodness-of-fit test. At best, we have a nominal P-value. This is similar to problems that affect almost all model-fitting in population genetics, where "Composite Likelihoods" are used that do not support formal

goodness-of-fit tests because of correlations among the statistics used to constrain the model. However, we can nevertheless make statements about consistency of the data and model:

- (a) For a fixed complexity of Admixture Graph (fixed number of populations and admixture events), we have a formal test for which graph topologies are most likely. We use this in Figure 3 to produce the coloring of the edges, which shows all the AGs that are consistent with the proposed topology within a χ^2 differential of 3.84 (P<0.05 by a χ^2 test with one degree of freedom). The idea is that while we do know how many degrees of freedom are relevant to the computation of our χ^2 statistic, for a fixed number of parameters, we can compare the fits of AGs to the data, because they all have the same number of degrees of freedom. By taking the difference between the AG with the smallest (best fitting) χ^2 value, and other AGs with the same number of parameters, we have a formal test of the relative goodness of fit given a fixed number of parameters.
- (b) Another way to make meaningful statements about whether a fitted AG is consistent with data is to test whether it predicts *f-*statistics among populations that are never too extreme. In practice, we view any AG that produces an f -statistic more than $|Z| > 4$ standard errors from expectation as a graph that we wish to avoid. (For AGs with a sufficient number of populations, $|Z| > 4$ is expected by chance even if the graph is a correct representation of history, and so this may be too stringent a criterion.) We also count the number of *f*statistics that are $|Z| > 3$ standard errors from expectation, and minimize this too.

(ii) The relationship of First Americans to speakers of Eskimo-Aleut languages

We first used the Admixture Graph methodology to search for models that could relate Eskimo-Aleut speakers to the group of Native American populations stretching from Canada all the way to southern Chile who are consistent with deriving from the same ancestral population, a group whose ancestry we call "First American".

Figure S7.1 shows how each Eskimo-Aleut speaking populations in turn can be fit into an AG with populations of entirely First American ancestry (we used Algonquin to represent Canadians, Zapotec1 to represent Meso-Americans, and Karitiana to represent South Americans). All the AGs are consistent with the data in the sense that none of the predicted *f*statistics are $|Z| > 3$ standard errors from expectation. The P-values from our approximate χ^2 statistic are shown in the upper left corner of each panel, and are always non-significant.

Eskimo-Aleut speakers have >50% First American ancestry

Inuit and Aleutian islanders can be fit to allele frequency correlation patterns if we treat them as admixtures of a First American lineage that branched off within the radiation of Native Americans (below Algonquin), and an East Asian lineage that is a sister group to Han (Figure S7.1). AGs without an admixture event in the history of these populations are poor fits (see the *4 Population Tests* in the previous section). Strikingly, the inferred First American ancestral proportions are always at least 50%. Thus, the data suggest that after arriving in the Americas, the Asian ancestors of the Inuits and the Aleuts admixed with resident Native Americans. The mixed populations then gave rise to groups with these ancestries today.

To assess whether alternative topologies are equally good fits to the data as the one shown in Figure S7.1, we used the East Greenland Inuit as a representative Eskimo-Aleut speaking population, and tried all possible AGs with 0 or 1 admixture events relating the populations in the top left panel of Figure S7.1. Only two AGs are consistent with our data, both specifying admixture in the history of the East Greenland Inuit. The best fitting AG is shown. The χ^2 statistic increases by 1.8 for the next-best fitting AG (the Algonquin branching off more recently than the First American admixture into the Inuit, an AG we discuss further below). All other AGs have a χ^2 for the fit at least 32 higher and so are not plausible fits.

Figure S7.1: Inuits, Aleuts, Chukchi and Naukan are well modeled as harboring First American ancestry. All these populations can be modeled as mixtures of First Americans and an Asian lineage related to Han. The AGs show drifts in parts per 1000 in units proportional to F_{ST} , and mixture proportions on dotted lines. The AGs are consistent with the data in that none produces |Z|-scores more than 3 standard errors from 0 whether. We report P-values in the top left (masked data).

Speakers of Algonquin languages carry the deepest First American lineages

The AGs that are fit in Figure S7.1 hypothesize that the deepest First American leading is that leading to the Algonquin, not the one admixing into Eskimo-Aleut speakers. This is counterintuitive, as one might expect the deepest Native American branches to be in the most northern parts of America (found in admixed form in Eskimo-Aleut speakers). The fit of this AG to the data is only slightly poorer than the model shown in Figure S7.1, as reflecting in the fact that the two models have a χ^2 statistic difference of only 1.8, which is not significant.

To explore this further, we refit the AG in the top left of Figure S7.1, now replacing the Algonquin with another population speaking a related language, the Ojibwa. The χ^2 statistic for the model shown is 6.3 less the alternative model in which the Ojibwa are not the deepest First American lineage (P=0.012). This suggests that the Figure S7.1 topology is a better fit to the data, and that Algonquin-speakers do in fact carry a deeper First American lineage.

Movement of First American genes into Asia, mediated by Eskimo-Aleut speakers

We next built a larger AG that analyzes multiple Eskimo-Aleut speaking populations together. Figure S7.2 shows that we obtain an excellent fit for a model in which the Naukan (Siberian Eskimo), both Greenland Inuit populations, and the Aleuts are in the same AG (to

help in visualization, blue is used to indicate First American ancestry, and red to indicate Eskimo-Aleut related ancestry). A history that fits this model is:

- (1) First American ancestors migrated to America >15,000 years ago
- (2) Eskimo-Aleut ancestors migrated more recently, mixing with First Americans they encountered. This mixed population was ancestral to present-day Eskimo-Aleut speakers.
- (3) Movement of Eskimo-Aleut speakers to Asia accounts for the First American ancestry in the Naukan. The First American ancestry in the Chukchi could reflect contacts between the Naukan and Chukchi² (a subset of the samples are from Aion Island, who have some ancestry from Yupik-speaking Naukan). Alternatively, it could reflect a separate history of gene exchange between Chukchi and Native Americans. Circum-Arctic gene flow in both directions between Siberia and America has been supported by various data, including single locus studies, and our study confirms this.

Figure S7.2: An AG that fits all Eskimo-Aleut speaking Native American populations along with the Naukan Siberian Eskimo. There are no f -statistics $|Z| > 3$ standard errors from expectation, and the nominal P-value is 0.77. The model specifies that Eskimo-Aleut speakers (red) descend from a**n** admixture event of First American (blue) and Asian lineages. This occurred after the initial migration of Eskimo-Aleut Asian ancestors to the Americas, after which the ancestors of Aleuts separated from the Inuit. A back-migration of Eskimo-Aleuts would have then led to the Naukan, who admixed with Asians they encountered (\sim) % of their ancestry). We tried all alternative AGs, and found that the only others consistent with the data corresponded to the change of the recent Asian admixture in Naukan coming off in a slightly different place in relation to Han (lineages labeled as drifts "9" and "15").

(iii) A model of history that relates the Chipewyan and Saqqaq to First Americans

We next identified a model of history that fits the data for the Saqqaq and Chipewyan, who were suggested by Note S6 to have some related ancestry. Following the analyses involving Eskimo-Aleut speakers, we identified a simple AG for each of the populations separately, and then fit them along with Zapotec1, Karitiana, Algonquin, and both Han and Yoruba as outgroups. We then evaluated the robustness of the fits and explored more complex AGs.

The ancestors of both Chipewyan and Saqqaq admixed with First Americans

We first tried fitting both Chipewyan and Saqqaq in a simple way into the AG, without admixture. In both cases, there is no good fit. For the Saqqaq, the nominal significance of the fit is P=0.006 and there is an outlier *f-*statistic at Z=3.1. For the Chipewyan, the nominal significance of the best fitting AG without an admixture event is $P<10^{-9}$.

We next tried fitting both populations with a single admixture event, and found good fits for both Chipewyan (P=0.33) and Saqqaq (P=0.44) (for both, there are no *f*-statistics with values more than 3 standard errors from expectation). For the Chipewyan, there are several AGs that are topologically similar to the one in the left panel of Figure S7.3 that are equally good fits to within the limits of our resolution. For the Saqqaq, the AG shown in Figure S7.3 is the only one that fits the data at all (the next-best fitting AG has a χ^2 statistic that is 6.9 higher).

These analyses suggest that after arriving in America, the Asian ancestors of both populations admixed with the First American populations already there (an estimated 89% for the Chipewyan speakers, and 14% for the Saqqaq). The finding of admixture with First Americans is parallel to what we infer occurred in the history of Eskimo-Aleut speakers.

Figure S7.3: Admixture Graphs that fit Chipewyan and Saqqaq. We show the best fitting graphs for Chipewyan and Saqqaq (nominal P-values at top left, and in parentheses when computed on unadmixed samples). The coloring is added to highlight the streams of ancestry leading to First Americans (blue), and Saqqaq and Chipewyan (green)

Chipewyan and Saqqaq admixed with a deeper First American lineage than Eskimo-Aleuts A notable difference between the Eskimo-Aleuts on the one hand, and the Chipewyan and Saqqaq on the other, is the source of their First American ancestry. Comparing Figure S7.1-3, the First American ancestry in Eskimo-Aleut speakers is inferred to come from a more derived lineage than that seen as Algonquin speakers, whereas the Chipewyan and Saqqaq are consistent with having their First American ancestry from the deep Algonquin lineage. This supports the view, suggested also by Note S6, that the stream of later Asian gene flow that contributed ancestry to Eskimo-Aleut speakers, followed a different historical course than the one(s) that contributed to the Chipewyan and Saqqaq. It also is consistent with the suggestion in Note S6 that the Chipewyan and Saqqaq may have shared Asian ancestry.

A model of shared Asian ancestry in the Chipewyan and Saqqaq that fits the data

We finally constructed an AG that fit the data for both the Chipewyan and Saqqaq as deriving from the same Asian lineage, with subsequent admixture in different proportions from a deep First American lineage related to that in present-day Algonquin speakers (rightmost panel of Figure S7.3). There is no evidence for a poor fit ($P=0.40$, with no outlier *f*-statistics $|Z| > 3$ standard errors from expectation). The next best fitting AG with the same complexity is a significantly worse fit (the χ^2 statistic is 9.2 higher). Thus, our data continue to be consistent with the model that the Chipewyan and Saqqaq harbor a distinct and possibly shared streams of later Asian ancestry compared with First Americans and Eskimo-Aleut speakers.

(iv)A model that fits data from First Americans, Eskimo-Aleut speakers, & Chipewyan

We identified an AG that fits the data for selected populations from representatives of all the groupings of populations simultaneously, which we show in Figure 2. This AG is a fit to the

data in that it gives a nominal P-value of 0.68 and produces no outlier *f-*statistics more than 3 standard errors from expectation. We also determined that no AGs with fewer admixture events are fits to the data. The inferred topology and mixture proportions are consistent with the AGs in Figures S7.1-3, suggesting that the inferences of those earlier analyses are robust to the addition of extra populations. It is possible (and even likely) that the true history is more complicated than that shown in Figure 2, but we view the AG as a null hypothesis about the relationships among the ancestral populations that contributed genes to Native Americans, which is the most parsimonious model that we could identify that is consistent with the *f*statistics. A goal of future work should be to identify features of genetic data in these populations that cannot be explained by this model.

Figure S7.4: *f4 Ratio Estimation* **allows estimation of the proportion of First American ancestry.** The expected value of f_4 can be inferred by tracing drift paths between the first two and last two populations in the statistic; the expected value is the genetic drift on the overlapping section (*Y*), multiplied by the proportion of ancestry going through those lineages (*p*) with a sign determined by whether the genetic drift paths are in the same or opposite directions. By computing a ratio of two *f4* statistics for populations whose relationships are accurately described by the AG, we can obtain an estimate of the First American admixture proportion *p*: how much of a *Test Population*'s ancestry is of First American origin.

(v)Alternative inferences of mixture proportions from *f4 Ratio Estimation*

As a complement to the AG analysis, we also used f_4 Ratio Estimation¹, to infer First American ancestry proportions for northern North American populations in whom we have detected evidence for admixture between First American ancestry and more recent gene flow events from Asia. If the AGs are accurate descriptions of population relationships, we can estimate the proportion of First American ancestry for a *Test Population* based on a ratio of *f4* statistics, as shown in Figure S7.4. A feature of *f4 Ratio Estimation* is that is it based on fewer populations simultaneously (so there are fewer errors that one can make in the modeling), and it also produces a standard error from a Block Jackknife (not available from our AG fitting software). A caveat, however, is that the precision of the estimates from f_4 Ratio Estimation is only as good as the model. If the model is wrong (not modeling some admixture events that actually occurred), then we expect there to be systematic errors in the estimates

Table S7.1: Estimates of First American ancestry proportions

Population	Estimates from AGs of Figs. S7.1 & S7.3	f ₄ Ratio Estimation
Chipewyan	89%	$89.8 \pm 1.6\%$
East Greenland Inuit	59%	$59.0 \pm 1.6\%$
West Greenland Inuit	58%	$57.5 \pm 1.7\%$
Aleutian	56%	$56.4 \pm 3.5\%$
Naukan	52%	$52.6 \pm 1.4\%$
Chukchi	40%	$40.5 \pm 1.2\%$
Saqqaq	14%	$11.3 \pm 2.8\%$

* The f4 Ratio Estimates use *FirstAmerican1* = Algonquin and *FirstAmerican2* = Zapotec1 for the Greenland Inuit, Aleuts, Naukan and Chukchi (Figure S7.1) and *FirstAmerican1* = Zapotec1 and *FirstAmerican2* = Algonquin for Chipewyan and Saqqaq (Figure S7.3).

(vi) A model that fits the data for 16 First American and 2 Outgroup populations

To build an AG fitting data for 16 Native American populations (Figure 3), we restricted to populations that had samples sizes of at least 2 and are consistent with descending from a homogeneous ancestral population without subsequent gene flow from Asians (that is, they are of entirely First American ancestry). We included all populations with entirely First American ancestry among the populations we attempted to fit into the AG. We used Yoruba (West Africans) and Han (Chinese) as outgroups.

Our process of building the AG was *ad hoc,* involving manually adding populations until we were no longer able to add additional ones without producing many *f-*statistics |Z|>3 standard errors from expectation, or without producing a large incremental increase of the difference between the χ^2 statistic and estimated number of degrees of freedom. An important area for future research will be to develop more principled algorithms for building AGs. Nevertheless, we believe that the AG in Figure 3 is useful in providing a hypothesis for how these populations are related that is not grossly inconsistent with the data. The allele frequency correlation patterns in a dataset with this number of SNPs provide strong constraints on possible relationships, and finding *any* model that relates such a large number of populations provides a useful starting point for further research into population relationships.

Of the 16 Native American populations in the AG, 13 can be fit to a simple tree with no evidence of admixture. An additional 3 can only be included in the AG through admixture (Cabecar, Inga and Guarani). The resulting AG (Figure 3) provides a reasonable fit in the sense that there is only one *f*-statistic $|Z| > 3$ standard errors from zero $(|Z| = 3.2$, not surprising given that we evaluated 11,781 statistics), and the nominal significance of the entire fit is P=0.53. The AG fitting also produces estimates of genetic drift on each lineage (in units scaled to be comparable to $1000\times F_{ST}$), and mixture proportions, which are shown in Figure 3. Standard errors in *f*-statistic values are \sim 0.001. Thus, short branches (e.g. of length $1 =$ 1000×0.001) are not reliably inferred; the data are consistent with trifurcations at such nodes.

To assess the robustness of our inferences to local ancestry masking, we repeated the AG analysis on a subset of 10 Native American populations that contained at least one sample without any European or African ancestry (this excluded Algonquin, Hulliche, Chilote, Inga, Kaingang and Mixtec). Figure S4A shows the fitted AG on the masked data and Figure S4B for the subset of samples from these populations that are completely unadmixed. For the analysis on the masked data, only two of 2,211 *f-*statistics are |Z|>3 from expectation (both $|Z| < 3.2$), and the overall P-value for the fit is P=0.37. For the analysis that restricts to unadmixed samples, there are no *f-*statistics |Z|>3 standard errors from expectation, and the overall fit is P=0.42. The consistency with the topology of Figure 3 provides confidence that our inferences of population relationships from masked data are not artifactual.

The Admixture Graph analysis in Figure 3 suggests that the Inga, Guarani, and Cabecar can be modeled as resulting from simple admixture events. We discuss each case in turn.

Inga

In the tree of Figure 1C, the Inga cluster with their geographic neighbors rather than with their linguistic neighbors, suggesting *a priori* that a mixture event is plausible. To test if the Inga can be fit into the tree without admixture, we created a new AG in which the Inga were removed, and then reinserted them at each edge of the AG without admixture. The best fitting AG in which the Inga are not admixed is one in which they are in a clade with the Guahibo (consistent with the AG in which the inferred largest mixture component is from a group

related to the Guahibo; Figure 3). However, this AG is clearly inconsistent with the genetic data: there are 35 f -statistics that are $|Z|$ >3 greater than expectation, with the largest being $|Z|=4.8$. When we model the Inga as an admixed population, we do obtain a reasonable fit as shown in Figure 3. We also explored all other AGs that model the Inga as deriving from a single admixture event, and found only one that is statistically consistent (the χ^2 statistic is 2.5 larger) and this AG has a similar topology. We conclude that the Inga descend from a mixture of lineages related to western South American Andean-speaking groups (like Quechua and Aymara) and Amazonian South American Equatorial-Tucanoan-speaking groups (like Guahibo), consistent with their speaking and Andean language but living on the eastern side of the Andes close to Amazonian populations.

Guarani

The Guarani (like the Inga) cluster in Figure 1C with their geographic neighbors rather than with their linguistic neighbors, suggesting *a priori* that they might be admixed. Consistent with this, the Guarani can be well-fit into the AG is as an admixture of their immediate geographic neighbors and an Equatorial-Tucanoan speaking group (whose language group they share) (Figure 3). We also tried fitting the Guarani without admixture. The single best fitting AG places the Guarani as a clade with the ancestral population of the Wichi. For this AG, there are 9 f -statistics that are more than $|Z| > 3$ standard errors from expectation with the largest at $|Z|=3.8$; moreover, the χ^2 statistic for the best model without admixture is 22.8 higher than the best model with admixture. Thus, a history of admixture is strongly supported by the data. We tried all possible insertion points of the two admixing lineages leading to the Guarani, and found only two AGs with χ^2 statistics within 3.8 of the best model. In all of these AGs, one admixing lineage is a clade with the Wichi. The other lineage is always in the Guahibo-Surui-Palikur clade, but its exact placement within the clade is uncertain.

Chibchan-speaking populations

The third inferred admixture event is in the Cabecar, a Chibchan-speaking population from north of the Panama isthmus. Figure 3 shows red and blue coloring to indicate the edges that are consistent with being the insertion points of the two lineages ancestral to the Cabecar and that have χ^2 statistics within 3.84 of the best fitting AG. When we instead fit the data for the Cabecar without admixture, the fits are poorer (Table S7.2). The difference between the nominal χ^2 statistic and the number of degrees of freedom jumps from -2 (AG of Figure 3) to 22, and the number of $|Z|$ -scores greater than 3 jumps from 1 to 14. Thus, the data appear to strongly support admixture in the history of the Cabecar.

To assess the generality of the inference of admixture of North and South American lineages in the history of Chibchan-speakers, we measured the fit of the AG model to the genetic data for the 10 Chibchan-Paezan speaking populations in the largely Chibchan-Paezan speaking clade of Figure 1C (the only populations that do not speak Chibchan-Paezan languages in this clade are the Wayuu and Chorotega and we excluded them). We proceeded as follows:

- (a) We removed the Cabecar from the AG of Figure 3, and then reinserted each of the Chibchan-clade populations as a mixture of two lineages from the resulting AG. (Usually the admixing lineages are similar in their inferred insertion points to the Cabecar.)
- (b) Each of the 10 populations in turn is treated as unadmixed and we find the best fit model.

Table S7.2 shows the results. All of the 10 populations fit the data far better with a model of an admixture of North and South American lineages than with a model of no admixture: the difference between the nominal χ^2 statistic and the estimated number of degrees of freedom always jumps by at least 14 (Huetar) to as high as 25 (Waunana) between the best fitting

model without admixture and the one with admixture. Without admixture, 9 of the 10 populations have at least nine $|Z|$ -scores >3 ; in contrast, with admixture, there are at most 5 $|Z|$ -scores $>$ 3. (The worst fit is the Huetar.)

Motivated by these results, we attempted to fit a substantial proportion of Chibchan-speaking populations into an AG in which there was a single admixture event ancestral to South and North American Chibchan speakers. We were able to obtain a reasonable fit for an AG with three populations (Cabecar, Maleku and Kogi). However, we had difficulty in fitting a larger number. We hypothesize that this reflects additional admixture events or isolation-bydistance processes involving populations related to other groups in the AG of Figure 3.

These analyses produce two key inferences:

- (1) A model of admixture of North and South American lineages is needed to fit the data for almost all Chibchan-speaking populations.
- (2) All Chibchan-speakers today likely inherit most of their genetic material from ancestors in South America, as the lineage that the AG fits as contributing most of the ancestry of Chibchan speakers falls within the radiation of South Americans (after the branching of Andean-speaking South Americans like Quechua; indicated by red shading in Figure 3).

One hypothesis that could explain these observations is that the North American Chibchan speakers inherited all their North American-related ancestry through admixture with populations their ancestors encountered during back-migration to North America. If this is the case, then a second scenario is required to explain the evidence of admixture in South American Chibchan-speakers. An alternative hypothesis that could explain these observations is that the North American-related lineages detected in Chibchan speakers reflect earlier admixture events between North and South American lineages, which are shared in the history of all Chibchan-speakers. An important direction for future research will be to distinguish these hypotheses.

References for Note S7

¹ Reich, D., Thangaraj, K., Patterson, N., Price, A.L. & Singh, L. Reconstructing Indian population history. *Nature* **461,** 489-494 (2009). 2

 $²$ Reuse, W. Siberian Yupik Eskimo: The Language and Its Contacts with Chukchi. Studies in indigenous</sup> languages of the Americas. Salt Lake City: University of Utah Press, 1994.

Figure S1. Sampling locations of 17 Siberian populations

Color codes refer to linguistic family affiliation.

Figure S2. Examples of masking of segments of non-Native ancestry.

Estimates of the number of European or African alleles (*y* axis) at each position across chromosome 2 (*x*-axis), as examples of the inferences we use for local ancestry masking. Results are shown for selected Native American samples, with the population, sample ID, and proportion of the genome masked shown in the top right. Our masking restricts to loci where the expected number of European or African alleles is <0.01, corresponding to 16.6% of genotypes averaged over the 493 Native American samples.

Figure S3. Trees are consistent for masked and unadmixed samples

To test whether the masking procedure biases the inference of tree topologies, we restricted to 21 Native American populations (out of the 52 in Figure 1C) that according to ADMIXTURE had at least one sample that was inferred to be substantially admixed (>2.5% non-Native American ancestry) and at least one inferred to be unadmixed (0%). We built Neighbor Joining trees of these populations along with African and Asian outgroups based on (A) the masked data restricting to samples that were inferred to be admixed (n=175, average of 15.6% non-Native American ancestry), and (B) unmasked data restricting to unadmixed samples $(n=94)$. The tree topologies are very similar even though the samples in the two panels are completely different, indicating that masking is not substantially biasing inferences about tree topology. The most notable difference is the placement of the Yaghan. Exploratory analysis (not shown) suggests that this might reflect a true pre-Colombian admixture event rather than an artifact (the Yaghan may be an admixture of Andean lineages and deep South American lineages, and hence both A and B may be reflecting features of a true history of admixture that cannot be accommodated by a simple tree).

Figure S4.

Admixture Graphs are consistent for masked and unadmixed samples

To assess if the inferences of the Admixture Graphs are biased by local ancestry masking, we fit the same AG topology as in Figure 3 to the data, restricting to the subset of 10 Native American populations that included at least one entirely unadmixed sample (this removed the Algonquin, Inga, Chilote, Hulliche, Mixtec and Kaingang). (A) We show the AG obtained using masked data on all samples including ones with recent European or African admixture. The AG gives no evidence for being a poor fit to the data, in the sense that there are only two *f-*statistics that are more than $|Z| > 3$ standard errors from expectation (both $|Z| < 3.2$; which is not too striking given that we computed 2,211 statistics). The nominal χ^2 statistic also suggests a reasonable fit (P=0.37). (B) We also repeated the analysis on the subset of samples from the same populations that are entirely unadmixed based on the ADMIXTURE k=4 analysis of Note S2. While the inferred mixture proportions and branch lengths change slightly, there are no f -statistics $|Z| > 3$ standard errors from expectation, and the nominal significance of the test gives no evidence for a poor fit $(P=0.42)$.

Figure S5. Heterozygosity and distance from the Bering Strait.

At the top we show the square of the correlation (R^2) between mean population heterozygosity and distance from the Bering Strait, restricting to populations with at least 5 individuals genotyped. In addition to Great Arc distances, we used the following coastline/inland cost combinations as "effective distances": 1:2, 1:5, 1:10, 1:20, 1:30, 1:40, 1:50, 1:100, 1:200, 1:300, 1:400 and 1:500. Correlations with P-values <0.05 are shown in red. At the bottom we show scatter-plots of heterozygosity and effective distance from the Bering Strait at the coastal/inland cost ratio maximizing the heterozygosity-distance correlation. The x-axis in the scatterplots is in units of effective distance, with no specific meaning for the absolute values. (A) Shows results for all populations across the Americas. (B) Excludes the North American populations with evidence of ancestry from later streams of gene flow from Asia. (C) Shows results when Chibchan populations from the Isthmo-Colombian area are also excluded (Chibchan speakers show evidence of back-migration from South America into Central America).

Table S1. Summary data for 52 Native American populations

1 For "language family", we use Greenberg's classification of the "superfamily" Amerind into 7 subfamilies.

 2 Estimate of European and African ancestry from ADMIXTURE k=4 (Note S2).

 3 The proportion of the genome masked is based on HAPMIX local ancestry inference (Note S4).

³ The 135 = 45 x 3 possible f₄(Unadmixed, Test; Outgroup1, Outroup2;) statistics are based on 45 = 10x9/2 possible pairs of outgroups (9 Siberian with ≥10 samples and Han), and 3 Unadmixed populations (Karitiana, Guay maximum statistic is |Zmax|>4.5 corresponding to P<0.005 after correcting for multiple hypothesis testing, or where the Hotelling *T‐*test P<0.005.[

Table S2. Summary data for 17 Siberian populations

Yukaghir 13 Uralic-Yukaghir Russia 68 150 Di Rienzo 0.100 0.273
¹ Language classification follows Ruhlen 1991.
² The proportion of the genome masked (only done in the masked dataset) is based on removing segments where Native chromosomes is >0.01. The masking of the Siberian samples is performed simultaneously with the masking of the Native American samples.

Table S3. Individual data for 493 Native American samples

