The history and evolution of the Denisovan-EPAS1 haplotype in Tibetans

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Recent studies suggest that admixture with archaic hominins played an important role in facilitating biological adaptations to new environments. For example, interbreeding with Denisovans facilitated the adaptation to high-altitude environments on the Tibetan Plateau. Specifically, the EPAS1 gene, a transcription factor that regulates the response to hypoxia, exhibits strong signatures of both positive selection and introgression from Denisovans in Tibetan individuals. Interestingly, despite being geographically closer to the Denisova Cave, East Asian populations do not harbor as much Denisovan ancestry as populations from Melanesia. Recently, two studies have suggested two independent waves of Denisovan admixture into East Asians, one of which is shared with South Asians and Oceanians. Here, we leverage data from EPAS1 in 78 Tibetan individuals to interrogate which of these two introgression events introduced the EPAS1 beneficial sequence into the ancestral population of Tibetans, and we use the distribution of introgressed segment lengths at this locus to infer the timing of the introgression and selection event. We find that the introgression event unique to East Asians most likely introduced the beneficial haplotype into the ancestral population of Tibetans around 48,800 (16,000–59,500) y ago, and selection started around 9,000 (2,500–42,000) y ago. Our estimates suggest that one of the most convincing examples of adaptive introgression is in fact selection acting on standing archaic variation.

Significance

The discovery of the archaic Denisovan hominins is one of the most significant findings in human evolutionary biology in the last decade. However, as of today, we have more questions than answers regarding this mysterious hominin group. This study leverages the information from the well-known example of adaptive introgression on the EPAS1 gene in Tibetans, to gain insight on the history of our species’ interaction with Denisovans. We show that the Tibetan-EPAS1 haplotype came from the East Asian-specific Denisovan introgression event, and it remained selectively neutral for a long time in the population before positive selection occurred, which may be concurrent with the permanent inhabitation of the Tibetan Plateau after the Last Glacial Maximum (LGM).

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The authors declare no competing interest.

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humans. One common acclimatization to the hypoxic environment is an increase in hemoglobin concentration (25), which increases blood viscosity and is associated with increased risk of pregnancy complications and cardiovascular disease (26, 27). Remarkably, Tibetans have a severely blunted acclimatization response compared to lowlanders at high altitudes and tend not to suffer from clinically elevated hemoglobin concentration (28). This presumed adaptive response is directly associated with variants in the EPAS1 gene, which encodes a transcription factor in the hypoxia response pathway.

The remarkable Denisovan connection to Tibetans' high-altitude adaptation has led to more questions regarding this already mysterious hominin group. For example, why are populations with Denisovan ancestry, including the Tibetans and Oceanians, located far away from the Denisova Cave in Siberia? One explanation for these seemingly puzzling findings is a large Denisovan geographical range. Multiple introgression events or a higher initial proportion of introgression may explain why some human populations exhibit higher levels of Denisovan introgression despite being located far away from the Altai Mountains in Siberia. Indeed, Browning et al. (7) proposed two Denisovan introgressions into modern East Asians, one of which is shared with Papuans and South Asians. More recently, Jacobs et al. (29) proposed an additional introgression event into the ancestral population of Papuans, making a total of three Denisovan introgression pulses in Asia. Their estimates of split times between the Denisovan groups that admixed with modern humans are large enough (280–360 ka) to suggest that there were multiple Denisovan-like hominin groups inhabiting diverse locations in Asia.

In this study, we investigate the surviving Denisovan introgressed segments in Tibetans to address the following questions: Do Tibetans exhibit signatures of more than one Denisovan introgression? If so, which introgression event introduced the beneficial EPAS1 haplotype, and when? Did selection act immediately after introgression, or plausibly later when modern humans began inhabiting the Tibetan Plateau? To address these questions, we examined the EPAS1 gene sequences from a combined dataset of 78 Tibetan individuals from two previously published studies (23, 30), among which 38 are high-coverage whole-genome sequences (30). We leveraged information from the introgressed tracts in Tibetans to infer the key time points related to the Denisovan introgression, as well as the onset of selection. We also employed the whole genomes in the combined dataset to demonstrate that the ancestors of modern Tibetans, similar to other East Asian populations (7), experienced two Denisovan introgression events. Our results provide resolution to the East Asian-specific Denisovan admixture event that led to one of the most fascinating stories of human adaptation, and shed light on the effects of different evolutionary processes that shape patterns of adaptive introgression in humans.

Results

Evidence of Three Distinct Archaic Introgression Episodes with Ancestral Tibetans: One from Neanderthals and Two from Denisovans.

To characterize the genomic landscape of archaic introgression in Tibetans and to determine the number of introgression pulses, we applied the method developed in Browning et al. (7), SPrime. This is a reference-free method that detects sets of diagnostic single-nucleotide polymorphisms (SNPs) that tag putatively archaic-introggressed segments in different regions of the genome. Applying SPrime to the autosomes of 38 Tibetan genomes (30), we inferred 1,426 regions, each containing a set of diagnostic, putatively archaic-introggressed SNPs using Africans (YRI) (31) as an outgroup. The remnants of archaic introgression in Tibetans are spread widely across the genome, illustrated by the presence of SPrime-inferred segments on all 22 autosomes (SI Appendix, Fig. S1). Following Browning et al. (7), for each segment, we computed the match rate of Tibetans against the Altai Neanderthal and Denisovan genomes at the different sets of diagnostic SNPs. The match rate distribution is visualized as a contour plot in Fig. 1. Most regions detected show high affinity to Neanderthal (~80% matching) and low affinity to Denisovan representing the highest peak (colored in red) in the plot. This is consistent with the observation of higher rate of Neanderthal introgression compared to Denisovan introgression in all Eurasian populations (13). We also observe two additional peaks representing segments of the genome that have low (~10%) affinity with Neanderthals and higher (~50% and ~80%) affinity with Denisovans. These two peaks represent putative Denisovan introgressed segments, and the bimodal distribution of Denisovan match rates is concordant with the hypothesis of two pulses of admixture with Denisovan-like archaic humans in East Asia (7), as only one peak in the match rate (with the Altai Denisovan) distribution is expected under a single pulse of introgression.

Fig. 1. Introgressed segments in EPAS1 and the genome-wide match rate with archaic individuals. This figure shows the density distribution of match rate to archaic individuals (Altai Denisovan or Neanderthal) in putatively archaic introgressed segments in 38 Tibetans, inferred by the SPrime program using Africans (YRI) as the outgroup. The match rate is defined as the proportion of alleles at the SPrime diagnostic polymorphic sites in a putatively introgressed segment that are present in the genome of archaic individuals at those positions (7). For a given segment, a match rate of 0 denotes that at a given set of diagnostic sites inferred by SPrime, none of the alleles at those sites match the corresponding alleles in the sequenced archaic human. The color range denotes the density of the contours, with red indicating high density and yellow indicating low density. The red star represents the matching coordinates for the introgressed segment within the EPAS1 gene.
**Table 1. Archaic introgression segments within 200 kb of the EPAS1 gene region inferred by SPrime**

| Segment length | Upstream region | Core region | Downstream region |
|----------------|----------------|-------------|-------------------|
| 140.9 kb       | 50.5 kb        | 150.9 kb    |
| Positions (hg19) | chr2:46,317,587–46,458,516 | chr2:46,550,132–46,600,661 | chr2:46,657,114–46,808,047 |
| No. of archaic-specific alleles | 63 | 29 | 81 |
| Match rate with Altai Neanderthal | 13.6% | 28.57% | 22.53% |
| Match rate with Altai Denisovan | 72.73% | 82.14% | 46.48% |
| Reference outgroup | YRI, CEU | CEU | YRI, CEU |
| Input data | 38 Tibetan whole genomes |

The SPrime program infers three introgressed segments at or within 200-kb range of the EPAS1 gene in Tibetans. One segment is within the gene (core region), another segment is upstream of EPAS1, and the third segment is downstream of EPAS1. Table shows the match rates to the Altai Denisovan and Neanderthal, the length of each segment, the chromosome and position range, number of diagnostic SNPs detected by SPrime, and the outgroup used by SPrime. The match rate is defined as the proportion of alleles at the SPrime diagnostic SNPs that are present in the sequenced archaic individual.

**EPAS1 haplotype, and 3) allele sharing between African and Denisovans is similar to other non-Tibetan populations (SI Appendix, Fig. S3 and Table S4). Therefore, the most parsimonious explanation is shared ancestry with archaic humans, but the presence of a few archaic alleles in this region hinders the detection of introgressed segments using algorithms such as SPrime. In fact, repeating the SPrime analysis using modern Europeans (CEU, who do not harbor the Denisovan variants at EPAS1) as an outgroup population does detect the putatively adaptive archaic segment in the core region of EPAS1 (chr2:46,550,132–46,600,661, hg19) that matches with high affinity to the Altai Denisovan (82.14%) but not the Altai Neanderthal (28.57%; Table 1). In this region, the SPrime-inferred variants in Tibetans are more similar to the Denisovan (SI Appendix, Fig. S3), and as expected, exhibit high genetic differentiation between Tibetans and Han Chinese (as measured by \( F_{ST} \); see SI Appendix, Fig. S4). Using Europeans (CEU) as an outgroup for detecting introgression in Tibetans results in a similar genome-wide distribution of match rates as observed earlier, but with fewer inferred segments in general, indicating that these are primarily a subset of the ones we obtained when using the YRI as an outgroup (SI Appendix, Fig. S2B).

The East Asian-Specific Denisovan Introgression Event Introduced the Beneficial EPAS1 Haplotype to Ancestral Tibetans. We showed in the previous section that, similar to other East Asians (7, 29), Tibetans also display evidence of two Denisovan introgression events, with one being unique to East Asians. Next, we tried to determine which of these two admixture events introduced the beneficial haplotype in EPAS1. To do so, we compared the introgressed segments in the 38 Tibetans at EPAS1 to the SPrime-inferred regions that exhibit the highest (>60%) Denisovan match rate and a low (<40%) Neanderthal match rate (peak from Fig. 1 and SI Appendix, Fig. S5). These segments were likely introduced via an East Asian-specific introgression event with a Denisovan population more closely related to the Altai Denisovan; other populations (e.g., South Asians, Oceanians) lack introgressed segments with this level of affinity to the Altai Denisovan (7).

Since SPrime does not infer the introgressed segments for each individual chromosome, we applied a hidden Markov model (HMM) (13, 17, 38) to infer the Denisovan-introgressed tracts in each Tibetan haplotype (Methods). We show that the introgressed segments in EPAS1 exhibit high Denisovan affinity, as shown in Fig. 1, and are of similar length as other segments with the highest Denisovan affinity (SI Appendix, Fig. S6 A and B). Segments in EPAS1 are only outliers in terms of the tract frequency (Fig. 24), which is in concordance with the expectation of positive selection acting on this region. Based on these observations, we propose that the EPAS1 haplotype in Tibetans was introduced through the pulse of East Asian-specific Denisovan introgression.

High affinity to a single Denisovan genome alone, however, does not necessarily mean the introgressed segment originated from Denisovans. To examine the possibility of the beneficial haplotype in EPAS1 instead originating from Neanderthals, we obtained the distribution of Neanderthal–Neanderthal divergence captured by computing the \( D \) statistic (39) in the form of \( D(\text{Denisovan}, \text{Altai Neanderthal}, \text{Vindija Neanderthal}, \text{Chimp}) \)

![Fig. 2. Introgressed tract length and frequency and \( D \) statistics.](image-url) A shows the lengths (x axis) and frequencies (y axis) of introgressed tracts inferred by an HMM applied to the high Denisovan affinity regions detected with SPrime in 38 Tibetans. These regions have a match rate <40% to Neanderthals and >60% to Denisovans (Fig. 1 and Methods). The red triangles represent the introgressed tracts at EPAS1, including a long and a short segment (80 and 40 kb, respectively). The tract frequency is the number of haplotypes harboring the tract of a specific length divided by the total number of haplotypes. B shows the distribution of divergence between two Neanderthals captured by the \( ABBA-BABA(\text{D}) \) statistic in the form of (Denisovan, Altai Neanderthal, Vindija Neanderthal, Chimp) in nonoverlapping 32.7-kb windows (black solid curve). The shaded gray area is defined by the lower 5% percentile of the distribution (to the Left of \( D \) = 0.125). The red arrow points to the value of \( D(\text{Denisovan}, \text{Neanderthal}, \text{Tibetan}, \text{Chimp}) \) at the 32.7-kb window within \( EPAS1 \) identified in Huerta-Sánchez et al. (23). The value of \( D(\text{Denisovan}, \text{Neanderthal}, \text{Tibetan}, \text{Chimp}) \) in the adaptive 32.7-kb region in \( EPAS1 \) is statistically significant (\( P = 0.006 \)).
in nonoverlapping 32.7-kb windows (SI Appendix, Methods) to match the length of the previously identified adaptive EPAS1 haplotype in Tibetans (23). This distribution, as expected, has the highest density at 1, as the two Neanderthals are more genetically related to each other (Fig. 2B). If the Tibetan EPAS1 haplotype was introduced by Neanderthals instead of Denisovans, we would expect the value of $D$(Denisovan, Neanderthal, Tibetans, Chimpanzee) within the distribution because we would expect more sharing of derived alleles between Neanderthal and the Tibetan EPAS1 haplotype. However, instead, we found that the value of $D$(Denisovan, Neanderthal, Tibetans, Chimpanzee) at EPAS1 is significantly lower ($P = 0.006$), indicating that the Tibetan haplotype does not originate from a Neanderthal population, and that a Denisovan origin for the adaptively introgressed EPAS1 haplotype in Tibetans has the greatest support.

The Denisovan Introgression Introducing the Tibetan EPAS1 Haplotype Occurred More than 48,000 y Ago. We next sought to infer the timing of Denisovan admixture and positive selection acting on EPAS1. Since the introgressed haplotypes generally become fragmented over time due to recombination (40), the distribution of introgressed tract lengths in an admixed population can be computed across the genome, and is commonly used to infer the time of admixture (41–43). For example, simulations show that, as expected, a more recent admixture time leads to higher mean introgressed tract length (42, 44). However, some studies have suggested that selection also affects the mean introgressed tract length differently depending on whether one conditions on the present-day allele frequency (42). Here, with simulations we confirm that the mean introgressed tract length increases with stronger positive selection when not conditioning on the current allele frequency (Fig. 3 and SI Appendix, Fig. S10). This is because, under positive selection, the tract reaches high frequencies sooner while it is still long and has not broken up by recombination. As the process of recombination then continues to break up the haplotype into more fragments, the probability that recombination results in the merger of two introgression tracts increases. In other words, the effect of selection is mediated by the allele frequency increase, which elevates the probability of back-recombination between introgression fragments. Since selection acted on EPAS1 variants, we need to account for both positive selection and archaic admixture in the modeling of the system.

We used an approximate Bayesian computation (ABC)-based inference framework (45–47) and a set of summary statistics to

![Fig. 3](https://doi.org/10.1073/pnas.2020803118)
infer three parameters: selection coefficient (s); the timing of selection (Tsel) and admixture (Tadm). We used the program SLiM 3.2.0 (48) to simulate forward in time the evolution of a 100-kb genomic segment representing the EPASI gene under a human demographic model that considers three populations (Denisovans, Tibetans and an outgroup population; see model A in SI Appendix, Fig. S7). At a given admixture time (Tadm), a single pulse of admixture is introduced from Denisovans to the ancestral population of Tibetans at a fixed proportion of 0.1%. We chose 0.1% as previous genome-wide estimates of Denisovan ancestry in modern-day East Asians range from 0.06% (6) to 0.5% (14). Subsequently, the adaptive mutation that arose in the Denisovan population remains neutral in the Tibetan population until the selection onset time (Tsel). Additionally, the Tibetan population experienced two bottlenecks: one representing the out-of-Africa bottleneck (Ne = 1,860), and a second bottleneck (Ne = 1,000) around the time of the European–Asian split. After the second bottleneck, the population size recovers to a size of Ne = 7,000 (see SI Appendix, Methods and model A in SI Appendix, Fig. S7). We chose these sizes based on estimates of the ancestral population of East Asians and Europeans (49, 50) and based on pairwise sequentially Markovian coalescent (PSMC) results for the Tibetans studied here (30). In the simulations, the admixture time and selection coefficient were drawn from a uniform prior. The onset of selection is bounded above by the drawn admixture time, resulting in a prior that is uniform when conditioned on the admixture time.

We computed six summary statistics in both the simulations and the observed data that summarize the distribution of introgressed tract lengths. These statistics include the mean, the SD, the max, and the number of tracts with length within the following three intervals: [0, 30 kb], [30 kb, 60 kb], [>60 kb]. Tract lengths were inferred by an HMM for each simulated chromosome and for each chromosome in the observed data at the EPASI sequences from the combined dataset of 78 Tibetans (SI Appendix, Figs. S8 and S9; Methods). We first confirmed that the summary statistics chosen were informative about the parameters, especially under the demographic model we simulated. By directly tracking the introgressed segments in SLiM, we see a correlation between the statistics describing the distribution of tract length and the selection coefficient (s), the time of admixture (Tadm), as well as the standing variation. Even further, if we introduced admixture to selection (Tadm − Tsel) (Fig. 3 and SI Appendix, Fig. S10).

We obtained a total of 400,000 simulation replicates using parameters drawn from the prior distributions and their summary statistics for the ABC inference. We used the program ABC-ToolBox (51) with a rejection algorithm to retain the best-fitting 1,000 simulations for the posterior distributions. We estimated the admixture time (Tadm) at 1,950 generations ago (48,760 y ago, assuming 25 y/generation; mode of posterior density: 95% credible interval [15,987–59,500 y ago]). The selection time estimate (Tsel) is 357 generations ago (8,930 y ago; mode of posterior density: 95% credible interval [2,500–42,563 y ago]; SI Appendix, Fig. S11, model A; Table 2). Furthermore, by comparing a model of selection on standing archaic variation with selection on newly introduced archaic variants, we find more support for selection acting on standing archaic variation (Bayes factor = 5.04; SI Appendix, Table S1 and Methods), indicating that selection did not act immediately after introgression. The selection coefficient of the EPASI haplotype (s) was estimated to be 0.018.

The bias of our ABC-based estimation method was assessed by computing the distribution of relative errors (Methods) using 1,000 randomly sampled simulation replicates. We found that our method had highest accuracy estimating the gap period between admixture and selection start time (Tadm − Tsel). The summary statistics also show high agreement between the observed data and the retained simulations (SI Appendix, Fig. S13). To evaluate the goodness-of-fit of our inference, we performed posterior predictive simulations (SI Appendix, Fig. S14), which showed that the observed statistics are within the range of newly simulated summary statistics using parameters drawn from the posterior distribution.

While our primary demographic model described above has two bottlenecks, prior demographic inference on Tibetans alone has not indicated a bottleneck in Tibetan populations around the Eurasian–East Asian split time (22, 52), unlike for example Han Chinese. As there is no clear consensus on the demographic history of Tibetan samples, and the number of plausible models is large, we considered three other models to investigate how distinct demographic scenarios change our conclusions. Specifically, we consider a model with a second bottleneck happening more recently in the past (model B in SI Appendix, Fig. S7), a model with a single bottleneck (model C), and a model with a single bottleneck and higher introgression proportion (model D). When the introgression proportion is 0.1% (models A–C) our point estimates range from [43.5–48.7 ka] for the time of introgression and [7.5–12.3 ka] for the time of selection. In model D where the introgression proportion is 1.0%, our point estimates are 52.7 ka for the time of introgression and 9.2 ka for the time of selection. All these estimates are consistent with selection of EPASI occurring on standing archaic variation.

To contextualize our estimates from our primary model (model A), we created a figure similar to figure 4 in Jacobs et al. (29) describing evolutionary events and relationships between Deniso- van populations. Our point estimates suggest that the East Asian-specific Denisovan introgression occurred at an earlier time (~48 ka) than the Papuan-specific Denisovan introgression (~30 ka) that they report.

**Archaic Introgression Affects Multiple Genes in Other Biological Pathways.** Last, we investigated whether other genomic regions that were influenced by archaic introgression show signals of positive selection in Tibetans. We first asked whether the SPrime-inferred segments overlap with other high-altitude adaptation candidate genes (22, 28, 53, 54) (SI Appendix, Table S3). We found a total of 11 unique regions harboring archaic segments that overlap with either a candidate gene core region, or within 200 kb of the gene’s flanking region (SI Appendix, Table S5 and Fig. S2 A and B). However, most of these segments do not show signals of positive selection—for example, most SPrime alleles except those associated with EPASI and FANCA were not significantly differentiated between Tibetans and Han Chinese compared to their genome-wide mean FST of 0.02 (22) (SI Appendix, Fig. S16). This is also true for another well-known gene associated with high-altitude adaptation, the EGLN1 (22) gene on chromosome 1, which shows elevated FST across the gene region, and harbors archaic alleles from Neanderthals, but shows no evidence that these archaic variants are under positive selection (low FST on the archaic alleles). Given the evidence so far, in terms of high-altitude adaptation, only the EPASI gene region shows a clear adaptive introgression signal.

| Parameter | Estimate | Credible interval, 95% |
|-----------|----------|------------------------|
| Tadm, generations, ka | 1,950.303 (48.76 ka) | 639.500–2,380.000 |
| Tsel, generations, ka | 357.032 (8.93 ka) | 100.000–1,702.500 |
| Selection coefficient | 0.018 | 0.005–0.099 |

We used an approximate Bayesian computation (ABC) approach to estimate three parameters related to the evolutionary history of EPASI in Tibetans, including the admixture (Tadm) and positive selection start time (Tsel), and the selection coefficient. We show the point estimates of parameters using the mode of the posterior distributions, and 95% credible intervals. We convert times to year units by assuming that one generation is 25 y. These estimates assume demographic model A (SI Appendix, Fig. S7).

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Next, we examined whether other biological pathways received contributions from archaic introgression that facilitated positive selection. We considered all diagnostic SNPs identified from SPrime using YRI as outgroup, among which most presumably originated from Neanderthal, Denisovan, or other unknown archaic populations. The introgressed segment in the EPAS1 region is not included in this analysis due to the concern that its exceptionally strong selection signal may dampen the weaker signals in other pathways.

Since archaic introgressed alleles are preserved in mosaic patterns on the genome, we looked for subtle signals of positive selection in subsets of a pathway by detecting enrichment of high-frequency archaic alleles in genes contained in each pathway. Using the R package signet (55), we identified five pathways from the National Cancer Institute/Nature Pathway Interaction Database (NCI) (56) where the archaic alleles are enriched and potentially under positive selection ($P < 0.05$; SI Appendix, Table S6), among which two are insulin-related pathways that both contain the gene RHOQ. Interestingly, this gene is downstream (155 kb) of EPAS1.

Discussion

Previous studies have shown that archaic introgression contributed to a range of phenotypic variation in modern humans (5, 57), and that a number of introgressed genes were plausibly subject to positive selection (17, 18). Here, we use sequencing data of the most convincing example of adaptive introgression, EPAS1 in Tibetans, to address a series of questions regarding the origin and the timing of Denisovan introgression in East Asia. Our work supports the two-pulse Denisovan admixture model proposed by Browning et al., and our analysis suggests that the beneficial haplotype of EPAS1 in Tibetans originated from the East Asian-specific Denisovan introgression, involving a Denisovan group that is more closely related to the Altai Denisovan individual from the Denisova Cave. Besides EPAS1, archaic introgression has left segments in various genes across the genome, and affected multiple biological pathways including hypoxia.

This work provides a timing estimate of the East Asian-specific Denisovan introgression, which we inferred at around 48 ka, and is consistent with archaeological evidence showing Denisovan ancestry in modern human individuals from 34 to 40 ka (59, 60). Our point estimate suggests that the East Asian-specific admixture event was more ancient (48 ka) than the Papuan-specific pulse (30 ka), and closer to the first Denisovan introgression that is shared by Asian and Oceanian lineages (45 ka, Fig. 4) (29). Interestingly, the low mismatch observed in Jacobs et al. between Altai Denisovan and East Asian-specific Denisovan introgressed segments suggests that the Denisovan population that introgressed uniquely into East Asians (D0 in Fig. 4) was more closely related to the Altai population (that the sequenced Altai Denisovan belonged to) compared to the other two Denisovan populations (D1 and D2 in Fig. 4) that introgressed into humans.

The timing of human settlement in the Tibetan Plateau, including archaic hominins, remains under investigation. The discovery of a partial mandible from the Middle Pleistocene (Xiahe Denisovan) in Baishiya Karst Cave (BKC), located at 3,280-m altitude in the Tibetan Plateau suggests that Denisovan-like archaic hominins may have been present at high altitude at least 160 ka (61). More recently, analysis of mtDNA recovered from sediment excavated in this cave (inferred to be from ~100 to ~60 ka and maybe as recently as 45 ka) revealed that it grouped most closely with Denisovan mtDNA (62), suggesting that Denisovan-like populations may have inhabited this region for a long period of time. In contrast, evidence for modern human activity has been found on the interior of the Tibetan plateau as early as 40 ka from the Nwwa Devu site (63), although long-term human settlements on the high-altitude plateau are believed to be rare at that time. Currently, existing archaeological evidence generally supports two settlement scenarios. The archaeological sites from middle to late Holocene (62–65) indicate that year-round large-scale settlements of people on the plateau started after 3.6 ka facilitated by the advent of agriculture, while analyses on the mobility of hunter-gatherers (66–68) suggest that permanent inhabitation (most likely on a smaller scale) may have occurred more distantly in the past. Our estimate of the Denisovan East Asian-specific admixture time (48 ka) from tract lengths surrounding the EPAS1 gene is larger than most estimates of when modern humans permanently settled in the Tibetan Plateau, suggesting that the admixture most likely occurred outside of this region.

Furthermore, our estimate of the onset time of positive selection on EPAS1 (~8.9 ka) suggests that selection did not target the Denisovan introgressed alleles immediately after introgression, and possibly coincides with the time of permanent hunter-gatherer Tibetan settlements of populations from lowland East Asia during the Late Pleistocene or early Holocene (64). While evidence of even earlier arrivals exists (e.g., Denisovan from BKC and modern humans at the Nwwa Devu site, 40 ka or earlier), it is unclear how long they survived in the Tibetan Plateau, whether they were genetically adapted to the hypoxic environment, or if modern Tibetans are their direct descendants. Only one study has reported ancient DNA from the Himalayas (the Nepalese side) where the oldest samples date to 3.15 ka (65). Interestingly, low mismatch observed in Jacobs et al. between Altai Denisovan and East Asian-specific Denisovan introgressed segments suggests that the fragments are the remnants of archaic DNA that may have been present in the population, which is consistent with our estimation of the Denisovan admixture in Asia (1, 6). Those studies used all of the surviving Denisovan segments (in Papuans or Tibetans), and it is unclear whether one estimate is an average of the two Denisovan introgression events (7, 29) into East Asians, or if the estimate is closer to one of the introgression events. By contrast, we are using the data of a single gene that clearly has been the target of selection, having the advantage that, because it is a small local region of the genome, it is highly likely that the fragments are the remnants of archaic DNA introduced by a single admixture event. The second difference is that we account for positive selection in our inference since we assume neutrality, and it is unclear whether adaptively introgressed loci could change or bias estimates from genome-wide summary statistics of introgression (e.g., the distribution of introgressed tract lengths, linkage disequilibrium decay pattern). Finally, we use an ABC framework for parameter estimation, while the estimation methods used by others (30, 32, 52) could also lead to some differences in the inferences.

We acknowledge that we have made several assumptions and choices in our work. First, we rely on the sequencing data of only a single gene, which is reflected in our large credible intervals. One way to reduce uncertainty might be to use all the putative introgressed segments introduced via the East Asian Denisovan introgression event, but doing so would require making a different set of assumptions regarding how selection is acting on each of those regions. Second, we have assumed a demographic model for Tibetans from estimates of population size changes from PSMC curves. Our conclusion that selection of EPAS1 acted on standing archaic variation also stands true under all scenarios. We also do not know what the real distribution of tract lengths looks like in Tibetans, and we have inferred that using an HMM. How accurately the HMM infers the true tract lengths in Tibetans is unknown, but other methods [e.g., ArchaicSeeker 2.0 (30)] yield similar results (SI Appendix, Methods and Fig. S15). Even if the HMM does not capture the true Tibetan tract lengths, by applying the HMM to both the real data and the simulated data, we hope
that the same bias occurs in both, reducing the likelihood of distorting the parameter estimates.

During the last decade, we have begun to appreciate that gene flow between archaic and modern humans played a major role in shaping human evolution as well as our genetic diversity. The introduction of archaic variants evidently facilitated adaptations to local environments in multiple populations. Our results for EPAS1 demonstrate the importance of selection on standing archaic variation, which other studies suggest is widespread (69, 70). However, recent work infers that selection immediately after introgression explains most examples of adaptive introgression from Neanderthals in Europeans (71). More analysis of other adaptively introgressed loci in multiple populations will further elucidate whether and under what conditions selection on standing archaic variation is the primary mode for adaptation. As we continue to sequence the remains of other archaic and modern humans, a high-resolution picture of archaic introgression in modern humans is expected to be revealed.

Materials and Methods

Genomic Data from Tibetan Population. For the whole-genome analyses in this study, we used 38 Tibetan samples from Lu et al. (30). We phased the data with Beagle 5.0 (72, 73), with the 1,000 genomes worldwide populations as imputation reference. SNPs that were very rare (<5% frequencies) or very common (>95% frequencies) were removed from the phased variant call format files (VCFs) that are used for downstream analyses, including the SPrime analysis. For inferring the timing of admixture and selection as well as the selection strength we combined the sequences from 40 Tibetan individuals from Huerta-Sánchez et al. (23) covering a 120-kb region at the EPAS1 gene, and the 38 Tibetan sequences at the EPAS1 locus from Lu et al. (30).

Genomic Data from Worldwide Population. This study utilized the following data collections as reference: 1) modern human individuals from 1000 Genomes Project (31); 2) archaic human genomes: Altai Neanderthal (13), Vindija Neanderthal (8), and Altai Denisovan (1).
that were private in one of the two datasets [the 40 Tibetans from Huerta-Sánchez et al. (23) and the 38 Tibetans from Lu et al. (30)] before joining them for the analysis. We plotted the inferred archaic-introgred segments from 78 Tibetans as a heat map in the R environment. Each row represented a haplotype from Tibetan individuals, and each column represented a genomic position in EPAS1 core region. The sites that were inferred to be archaic-introgressed were highlighted in yellow, in contrast to blue that denoted nonintrogressed sites.

We further compared the results of the HMM inference on the tract length per haplotype with the inference on the 38 Tibetan individuals published by another method, ArchaiSeeker 2.0 (AS) (30). ArchaiSeeker and the HMM showed high level of agreement (SI Appendix, Fig. S15; both methods inferred an mode in intermediate-length tracts (~40 kb), while HMM inferred a few more larger tracts (~80 kb) and AS inferred more shorter tracts (~10 kb).

ABC Inference. To infer the parameters, we simulated the evolution of the EPAS1 region under the demographic model described in Results (model A in SI Appendix, Fig. S7). We allow the admixture time (tadm) to vary between [500, 2,400] generations ago, selection time (tsel) to vary between [100, 2,400] generations ago, and selection coefficient (s) between (0, 0.1). The simulated segment has length L of 100 kb, which was the approximate size of the EPAS1 region. The mutation rate μ was set to 1.0e-8 as estimated from Huerta-Sánchez et al. (23), and the recombination rate r is uniform across the segment at 2.3e-8 (23).

We applied the same HMM framework that we used for the observed data to each simulation replicate, to infer the introgressed tracts on simulated haplotypes. Given the location of HMM-inferred introgressed tract(s) in each simulation, we computed the tract length from each simulated chromosome, and recorded six summary statistics that describe the tract length distribution. The mean (μ), the SD (σ), the maximum length (max), and (4–6) the number of tracts with length within the following three intervals: [0, 30 kb), [30 kb, 60 kb) and [>60 kb]. The six summaries (K) were computed for the EPAS1 100-kb region in 78 Tibetan individuals. For a given parameter θ, the posterior probability is therefore Pr(θ | K).

We applied the program ABCtoolbox (SI) to infer parameters by retaining the same summary data that best matched the observed data (as in six summary statistics). The program compares the simulated data with the observed data, and implements a rejection algorithm adjustment on retained simulations. We chose the closest 1,000 retained simulations to generate the posterior, and subsequently plotted the marginal posterior distribution of admixture time and selection start time in an R program, and obtained the mode and the 95% credible intervals (Table 2 and SI Appendix, Fig. S11) from the marginal posterior distribution.

We computed the relative errors [Res, (true-estimate)/true] by randomly sampling 1,000 simulations from the 400,000 set. For each of the 1,000 simulations, we used ABCtoolbox to infer the parameters using the rest of the simulations (a total of 399,000). We compared the difference between the inferred parameters and the true parameters (that generated the simulation replicate) for each of the 1,000 randomly drawn simulations. We plotted the distribution of differences as histograms in SI Appendix, Fig. S12. To evaluate the goodness-of-fit in our inferred scenario, we performed posterior predictive checking by randomly sampling sets of parameter values that generated 500 of the 1,000 retained simulations, and used each parameter combination to generate a single simulation, and computed the summary statistics. We plotted the relationship of pairwise summary statistics between the observed data, the sampled 500 retained simulation, and the generated 500 simulation replicates under the 500 parameter combinations, and show that the summary statistics from the observed data are within the range of both retained posterior and the newly simulated data (SI Appendix, Fig. S14).

Biological Pathway Network Analysis. We looked for subtle signals that a subset of genes within a pathway network may be selected, by searching for enrichment of archaic alleles in gene networks in biological pathway databases and finding the highest scoring subnetwork (S3). First, we searched for overlaps between the putatively introgressed segments inferred by SPrime and protein-coding genes in modern humans using the ENSEMBL database (75, 76), which resulted in 3,292 genes by combining the searches using either the YRI or CEU as outgroup populations. At the overlapping SPrime diagnostic SNPs, we calculated their frequencies in the Tibetan population. We used the maximum archaic allele frequency in each gene as input score for the enrichment test. Alternatively, if no archaic allele was found in a gene, the gene received a score of 0. We then computed the subnetwork scoring using the National Cancer Institute/Nature Pathway Interaction Database (NCI) (56) as reference for signaling and metabolic pathways, using the HSS algorithm provided under R package signet (55). The final score of each subnetwork was normalized using the mean and SD of 10,000 simulated random networks of the same size. We reported only the significant subnetworks with P values less than 0.05 as candidate biological pathways that undergo positive selection because of archaic allele enrichment (SI Appendix, Table S6).

Data Availability. All scripts necessary to reproduce the ABC and simulation results from this work can be found on Github, https://github.com/xzhang-popper/EPAS1Project. The use of 38 Tibetan whole genomes by this work is permitted by The Ministry of Science and Technology of the People’s Republic of China (permission no. 2020BATA0143) at the National Genomics Data Center (https://bigd.big.ac.cn/search?tbody=gs&aq=PJRC2000246). The EPAS1 sequences of the 40 Tibetans used here are available at the Sequence Read Archive (accession no. SRX1265938).
