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Inland-coastal bifurcation of southern East Asians revealed by Hmong-Mien genomic history

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ABSTRACT

The early history of the Hmong-Mien language family and its speakers is elusive. A good variety of Hmong-Mien-speaking groups distribute in Central China. Here, we report 903 high-resolution Y-chromosomal, 624 full-sequencing mitochondrial, and 415 autosomal samples from 20 populations in Central China, mainly Húnán Province. We identify an autosomal component which is commonly seen in all the Hmong-Mien-speaking populations, with nearly unmixed composition in Pahng. In contrast, Hmong and Mien respectively demonstrate additional genomic affinity to Tibeto-Burman and Kra-Dai speakers. We also discover two prevalent uniparental lineages of Hmong-Mien speakers. Y-chromosomal haplogroup O2a2a1b1a1b-N5 diverged ~2,330 years before present (BP), approximately coinciding with the estimated time of Proto-Hmong-Mien (~2,500 BP), whereas mitochondrial haplogroup B5a1c1a significantly correlates with Pahng and Mien. All the evidence indicates a founding population substantially contributing to present-day Hmong-Mien speakers. Consistent with the two distinct routes of agricultural expansion from southern China, this Hmong-Mien founding ancestry is phylogenetically closer to the founding ancestry of Neolithic Mainland Southeast Asians and present-day isolated Austroasiatic-speaking populations than Austronesians. The spatial and temporal distribution of the southern East Asian lineage is also compatible with the scenario of out-of-southern-China farming dispersal. Thus, our finding reveals an inland-coastal genetic discrepancy related to the farming pioneers in southern China and supports an inland southern China origin of an ancestral meta-population contributing to both Hmong-Mien and Austroasiatic speakers.

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INTRODUCTION

Starting ~9,000 years before present (BP), China is the second earliest birthplace of agriculture, following the Near East[1,2]. Subsequently, farming dispersed into adjacent areas of East Asia, especially Southeast Asia, the Korean Peninsula, and the Japanese archipelago. However, it is still not fully settled whether and to what extent human migration propelled the dispersal of agriculture. Particularly for Southeast Asia, recent archaeogenetic studies support that the East Asian ancestry of the first farmers in Southeast Asia can be traced to southern China[3,4], raising the further issue on the deeper demographic prehistory of both regions.

The movement of farming populations has also been presumed to facilitate the expansion of their languages[5]. Seven major language families in East Asia are involved in this hypothesis: Austroasiatic, Austronesian, Hmong-Mien, Japonic, Kra-Dai (formerly known as Tai-Kadai), Koreanic, and Sino-Tibetan[6]. Compared with the others, the Hmong-Mien language family lacked writing system until the recent centuries, hence its notably obscure history, and most of the history was conserved either by oral legends, or literature documented in Chinese, which often confused different populations. Therefore, linguistic and genetic studies are crucial to the reconstruction of the Hmong-Mien prehistory. Some linguists have attempted to connect Hmong-Mien with other language families genealogically, but none of the hypotheses are widely accepted[7]. Likewise, in contrast to previous studies based on mitochondrial[8] or Y-chromosomal[9] data, there has been no genome-wide studies concentrating on Hmong-Mien-speaking populations up to date.

Currently, Hmong-Mien speakers mainly dwell in South Central and Southwest China, with sporadic distribution in Mainland Southeast Asia (MSEA) due to the migration within the recent centuries[7]. The linguistic phylogeny of the Hmong-Mien languages remains controversial, while a common hypothesis categorizes them into two sub-branches: Hmongic and Mienic. Whereas the Hmongic languages receive more influence from Tibeto-Burman languages, the Mienic languages show more impact from Chinese. In Húnán Province of Central China, both clades distribute, including two Hmongic languages: Hmong (to be specific, Qo Xiong) in the northwest Húnán and Pahng (Hm Nai) in the southwest, as well as a Mienic language (Mien, or specifically, Iu Mien) in the southeast. Along with a high variety of other Sino-Tibetan- and Kra-Dai-speaking groups (Fig. 1B), especially Sinitic Tǔhuà and Wǎxiāng (known for its retentions of Old Chinese[10]), Tǔjiā of Tibeto-Burman, and Kam of Kam-Sui, Central China is an appropriate field to depict a fine-scale genetic landscape of Hmong-Mien speakers with neighboring populations.

RESULTS

To comprehensively represent the genetic diversity of Central China, we sampled and genotyped 903 high-resolution Y-chromosomal samples, 624 full-sequencing mitochondrial samples, and 415 autosomal samples from Central China, mainly in Húnán Province, covering all the 11 officially recognized ethnic groups of this region (Fig. 1). Given the criteria of both the officially recognized ethnicity and linguistic affiliation, we subdivided these groups into 20 populations. We then analyzed our data along with genetic data of ancient and modern populations from published studies (see Method).

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Genetic structure of Húnán Province

The pattern of principal component analysis (PCA) of East-Asia-wide populations[11] (Fig. 2B) shows that at least three meta-populations contribute to the genomic diversity of populations in Húnán: one represented by northern East Asians [Northern Hàn, Mongolic and Tungusic speakers, Japanese, Korean, Tibeto-Burmans (except Lāhù and Burmese), and the Devil's Cave sample (referred as "Devil's Cave" for short below)[12]; right], one by southeast Asians [Austronesians, Austroasiatic and Kra-Dai speakers, and a Neolithic Laotian individual (labelled as "Laos_LN_BA")[3]; left], and one by Hmong-Mien-speaking Hmong and Pahng (top). Compared with other Kra-Dai- (Dǎi and Thai) and Tibeto-Burman-speaking (Tibetan, Sherpa, Nàxī, and Yí) populations, Kam and Tǔjiā in Húnán shift towards Hmong and Pahng, suggesting an additional genomic impact on them from Hmong-Mien speakers, or/and gene flow between Kam and Tǔjiā. Including subpopulations of Hàn in Húnán (Gàn, Xiāng, Hakka, and Southwest Mandarin), Southern Hàn clusters between northern East Asians and Southeast Asians, consistent with the "Yellow/Yangtze River Ghost Population" theory and the inference that Hàn Chinese is the admixture of both proposed populations[13].

To present a finer-scale genomic structure within this region, we performed PCA[11] (Fig. 2A) and model-based clustering[14] (K = 3, Fig. 2C) exclusively for populations in Húnán. We observe that the genomic structure is highly associated with the linguistic affiliation of populations. Northern Tǔjiā, Kam, and Pahng construct three extremes in the PCA of populations in Húnán (top, bottom, and right, respectively, Fig. 2A), matching their highest proportion of ancestral components respectively related to Sino-Tibetan, Kra-Dai, and Hmong-Mien in ADMIXTURE plot (green, red, and blue, respectively, Fig. 2C). Gàn, Xiāng, and Hakka are modeled with nearly equivalent proportion of Sino-Tibetan- and Kra-Dai-related ancestral components (Fig. 2C) and cluster with each other in the PCA plot (Fig. 2A) with non-positive pairwise F_{ST} to each other (all < 0.000, Extended Data Fig. 1), indicating that intermarriage among main subpopulations of Hàn is not significantly fewer than marriage within a subpopulation. On the contrary, Wǎxiāng and Tǔhuà significantly deviate from the main subpopulations of Hàn and cluster with Tǔjiā and Kam in PCA (Fig. 2A) and TreeMix[15] (Fig. 3A) plot and harbor additional Sino-Tibetan- and Kra-Dai-related components in PCA plot (Fig. 2C), respectively, consistent with the particularity of their languages distinct from other Sinitic ones.

Focusing on the genomic relationship among Hmong-Mien speakers, we find that Pahng, Hmong, and Mien cluster together in the TreeMix plot for populations in Húnán (Fig. 3A). In ADMIXTURE plot (Fig. 2C), Pahng is modeled with a nearly unmixed form of Hmong-Mien-related component, consistent with the results of admixture f_3-statistics (Table 1) and pairwise F_{ST} (Extended Data Fig. 1). The significant positive values of all the f_3(Pahng; Hmong, Other Central Chinese)[16] (all $Z > 14.7$) support that Pahng has not received substantial gene flow from non-Hmong-Mien-speaking populations and shows that Pahng has undergone a strong and unique genetic drift. Supporting the latter inference, all the pairs of F_{ST} between Pahng and other populations (all ≥ 0.018) are higher than the ones among other population themselves (all ≤ 0.017). The analyses above confirm that Pahng represents a Hmong-Mien-sharing ancestry that is absent, or at least with fewer amounts, in non-Hmong-Mien populations. Corresponding to the intermediate position between Pahng and Tǔjiā

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in the PCA plot (Fig. 2A) and a substantial contribution of Sino-Tibetan-related component in ADMIXTURE plot (Fig. 2C), we find an additional migration to Hmong from Tibeto-Burman source modeled by TreeMix (Nàxī and Yí, Fig. 3B), supporting an additional genomic affinity of Hmong to Tibeto-Burman speakers. Meanwhile, Mien approximately clusters with Kam in the PCA plot (Fig. 2A), confirming that Mien carries both Hmong-Mien-related and Kra-Dai-related ancestries. Other than Hmong-Mien-speaking populations, Southern Tǔjiā is the only one who harbors significant Hmong-Mien-related ancestral component (Fig. 2C), which is explainable due to its geographic proximity to Hmong and possible intermarriage (Fig 1A). Key findings of admixture f_3-statistics of populations in Húnán (Table 1) are compatible with the inference above. Given that some Sinitic populations, especially Tǔhuà, also have a substantial Kra-Dai-related ancestry, we suggest that the dispersal and subsequent admixture associated with Hmong-Mien-related ancestry are highly parallel to the dissemination and language contact of different Hmong-Mien languages.

We then identify a series of motif Y-chromosomal lineages reflecting the demographic history (Fig. 4A and Extended Data Fig. 2A). Fitting their common linguistic affiliations, Hmong and Pahng share the patrilineage O2a2a1b1a1b-N5 (included in O2a2a1b-M7) with the second-highest (18/95 = 18.9%) and the highest frequency (7/21 = 33.3%), respectively. This patrilineage is also present in Shē[17], a population in southeastern China who once spoke Hmong-Mien. The highest frequency patrilineage for Hmong, O1b1a1a2a-F5506 (23/95 = 24.2%, included in O1b1a-PK4), is also exclusive for Hmong. Three dominant patrilineages for Hàn Chinese[18], Oα-F5, Oβ-F46, and Oγ-F11 (respectively included in O2a2b1a1-M117, O2a2b1*-M134(xM117), and O2a1b-IMS-JST002611), have a considerable proportion (57/463 = 12.3%, 44/463 = 9.5%, 58/463 = 12.5%, respectively) within main subpopulations of Hàn in Húnán, partly supporting a demic diffusion of Sinitic languages in this region. Compared with that, Wǎxiāng and Tǔhuà respectively have a noticeable frequency of Y-haplogroup N2a1-F1833 (6/39 = 15.4%, included in N-M231) and D1a2-F1531 (3/17 = 17.6%, included in D1a-M15), following their special linguistic and autosomal history. Intriguingly, Tǔjiā and Kam share Y-haplogroup Cα2a1-F5477 (included in C2a-F1067) with a high frequency (24/90 = 26.7% and 13/71 = 18.3%, respectively), despite their different linguistic affiliations, supporting a history of gene flow between both on the paternal side. In contrast, mitochondrial haplogroups present a uniform distribution among populations (Fig. 4B and Extended Data Fig. 2B), indicating the practice of exogamy on the maternal side. The very exceptions are Pahng and Mien, both Hmong-Mien-speaking, with the significant correlation with mitochondrial haplogroup B5 (χ^2 = 1.03×10^{-11} and 1.73×10^{-5}, respectively, whereas Bonferroni corrected $p = 2.3\times10^{-4}$).

We also performed coalescent time estimation (see Method) to calibrate the separation of some uniparentally inherited lineages (Fig. 5). Approximately synchronous with the time estimation of Proto-Hmong-Mien (~2,500 BP)[19], we estimate the most recent common ancestor (TMRCA) of O2a2a1b1a1b-N5 as ~2,330 BP (Fig. 5B), consistent with slight precedence of the divergence of patrilineage than the dispersal of language and ancestral population. By phylogenetic analysis (Fig. 5A), most of the Pahng (with one exception) and Mien (with two exceptions) samples affiliated to mitochondrial haplogroup B5 are identified as the subclade B5a1c1a (TMRCA in ~9,800 BP). Given the substantially higher frequency of Hmong-Mien speakers (63.6%, 21 out of 33) than the others in

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B5a1c1a, we propose a Hmong-Mien origin of this subclade.

In summary, we find strong evidence from genomic and uniparental analyses for a founding population who is responsible for the dispersal of Proto-Hmong-Mien and thus has a substantial genetic impact on present-day Hmong-Mien speakers, hence naming it as "Ancestral Hmong-Mien" (AHM).

Inland-coastal bifurcation of southern East Asians

One of the core issues regarding the history of the Hmong-Mien language family is its place of origin. Whereas linguistic evidence, such as the reconstructed vocabulary related to wet-rice cultivation, supports a Yangtze Basin origin of Hmong-Mien[7], archaeological record reveals a quite more complex scenario. During the Neolithic, there were two independent agricultural centers in southern China: Yangtze Delta and the middle Yangtze[1,20] (here we definite the northern boundary of "southern China" as Qín Mountains and Huái River). The former partly explains the coastal route for farming expansion as far as Taiwan and Luzon, while the latter accounts for another inland expansion of agriculture into Southwest China and MSEA[21]. In particular, there is a strong bond between the material culture of Neolithic Yangtze Delta and later Austronesian Pacific islands previously described[22]. Nevertheless, it is still ambiguous about the relationship between Proto-Hmong-Mien and these agricultural centers. Therefore, to determine the genomic relationship between AHM and other populations is pivotal to the resolution of the issue.

Besides AHM, previous genetic studies have already distinguished two other ancestral populations highly associated with the farming dispersal from southern China[3,4,23]. One of them is represented by the East Asian ancestry of Neolithic farmers in MSEA, as well as present-day isolated Austroasiatic-speaking groups (*e.g.*, Htin and Mlabri), who largely maintain genetic continuity with the former[4], hence responsible for Austroasiatic expansion and named as "Ancestral Austroasiatic" (AAA). The other is represented by Austronesian-speakers in Taiwan (*e.g.*, Amis and Atayal) in nearly unmixed form[23] and accounts for the dissemination of Austronesian languages, hence addressed as "Ancestral Austronesian" (AAN).

Given that, we computed a series of *D*-statistics[16] to address the genomic relationship between AHM and other populations (Table 2, Extended Data Table 2 & 3). Characterized by significant negative *D* (X, Mbuti; Devil's Cave, Amis) [X = Hàn, Hmong (representing AHM), Htin/Mlabri (representing AAA); $Z < -3.3$ (transversion only); Extended Data Table 3], the major genomic component of Hàn, AHM, and AAA are phylogenetically closer to AAN (represented by Amis) than Devil's Cave, hence constituting a monophyletic lineage of southern East Asians. Accordingly, we name this genomic component of Hàn as "southern East Asian ancestry of Hàn" (SEAH). Thus, Devil's Cave is supposed to symmetrically relates to AHM, AAA, AAN, and SEAH. In view of that, Hmong harbors increasing genomic affinity to most of the northern East Asians best represented by Tibetan in addition to AHM [*D* (Tibetan, Mbuti; Hmong, Amis) > 0, $Z = 7.0$, Table 2], and Hàn receives slightly more genomic influence from northern East Asians than Hmong [*D* (X, Mbuti; Hmong, Hàn) < 0, $Z = -2.9$ for Nganasan, -2.7 for Devil's Cave]. Devil's Cave inclines towards Austronesian in Taiwan than isolated Austroasiatics in *D*-statistics [*D* (Devil's Cave, Mbuti; Htin/Mlabri, Amis/Atayal) < 0, $Z < -7.9$], compatible with a deep ancestry from Australasian lineage

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harbored by isolated Austroasiatics as previously proposed[4].

Revealed by the direction of *D*-statistics, different modern and ancient populations in Southeast Asia do not equally relate to Hmong, Hàn, and Amis, and can primarily categorize into three groups (Table 2). Most of Neolithic and Bronze Age samples in MSEA and isolated Austroasiatics incline towards Hmong than Hàn but symmetrically relate to Hmong and Amis. Austronesians in Luzon and Visayas highly deviate to Amis from Hmong, but symmetrically relate to Hmong and Hàn. Present-day Kra-Dai speakers and Khmer possess increase affinity both to Hmong than Hàn and to Amis than Hmong. The results above imply an intricate internal structure within the southern East Asian lineage.

Addressing that, we modeled the admixture graph[16] to determine the phylogenetic structure of AHM, AAA, and AAN (Fig. 6). Consequently, we find the best fitting is that AAN is an outgroup to the clade constructed by AHM and AAA (Fig. 6A), supported by the lowest and feasible worst-fitting Z-score ($|Z| = 2.43$), the lowest final score of log-likelihood, and the positive shared drift exclusively for AHM and AAA ($f_2 = 0.006$). Conversely, the other two possible cases (AHM or AAA as an outgroup, Fig. 6B and C, respectively) introduce unfitting Z-scores ($|Z| = 4.54$ and 4.53, respectively) and zero-length branches, indicating unfeasible assumptions about the topology. Given the current and traceable historical distribution of relevant populations, we provisionally call the lineage constructed by AHM and AAA as an "inland lineage" and the one represented by AAN as a "coastal lineage".

To extensively model the genomic relationship of three distinct groups of Southeast Asians to the two lineages, we formally leverage the result of *D*-statistics [Supplementary Information (SI) Note 1]. Consistent with the result of admixture graph, our analysis indicates that AAA is closer to AHM than AAN (SI Note1.2). Our analysis also suggests that SEAH collectively forms a clade with AAN in contrast to AHM (SI Note 1.3) and that Second_MSEA has an increasing affinity to AAN than First_MSEA (SI Note 1.4). Considering the arrival of such an AAN-like lineage in MSEA, we find that the signal first occurred in the Bronze Age Vietnamese (dated to ~2,000 BP) who shows an increasing affinity to Amis and a decreasing affinity to Hmong (Table 3). Given the Austro-Tai hypothesis[24] that proposes a genealogical relationship between Kra-Dai and Austronesian languages, the occurrence of the coastal lineage in MSEA is appropriate to be explained as a southwestward migration wave related to Kra-Dai expansion[3] after the presence of AAA in this region. Distinct from other Austronesians, both Malay and Dusun show an increasing affinity to Hmong than Hàn (Table 2). Given the closer relationship between AHM and AAA than AAN, this is also consistent with the genetic[23], linguistic[25], and archaeological[26] evidence of an early Austroasiatic presence in Sumatra and Borneo. By contrast, the genomic affinity of SEAH to AAN is consistent with the language contact between Chinese and Kra-Dai languages, especially the Tai sub-branch[27]. All the evidence above indicates an earlier occurrence of the inland lineage than the coastal lineage in inland Southwest China and MSEA and supports an inland-coastal bifurcation of the southern East Asians, corresponding to the two distinct routes for agricultural expansion from southern China.

Apart from the autosomal evidence, populations sharing the inland lineage also closely connect on multiple levels. The shared vocabulary of Hmong-Mien with Austroasiatics seems to be more

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fundamental than one with other neighboring language families[7,28], implying an arguably common history in an earlier stage. Regarding uniparental lineages, Hmong-Mien speakers and Austroasiatics (or Neolithic farmers in MSEA) also share a series of Y-chromosomal and mitochondrial motif haplogroups, such as Y-haplogroup O1b1a1-M95 [O1b1a1a2a-F5506 for Hmong; O1b1a1b-F789 for Malaysia_LN, Laos_LN_BA[3], and various present-day Austroasiatic-speaking populations[29]] and O2a2a1b1-M209 [O2a2a1b1a1b-N5 for Hmong-Mien speakers; O2a2a1b1a1*-F2309(xM113, xN5) for Kinh[17]; compared with O2a2a1b2-F5511 for Amis[17]], and mitochondrial haplogroup B5a1 [B5a1c1a for Hmong-Mien speakers; B5a1a for Vietnam_N and Thailand_LN_IA[4]; B5a1c for Thailand_LN_IA[4]]. Especially for O2a2a1b-M7 (Fig. 5B), the divergence time of O2a2a1b-M7 [~14,710 BP, TMRCA for O2a2a1b1a1-F2309 (lineage shared by Hmong-Mien and Austroasiatic speakers) and O2a2a1b2-F5511 (Amis-related lineage)] is significantly older than the divergence time of O2a2a1b1a1-F2309 (11,460 BP), consistent with the closer relationship of AHM and AAA than AAN. Besides, the divergence time for O2a2a1b1a1-F2309 and O1b1a1-M95 (~11,660 BP) and rapid diversification [O1b1a1a-F1252, ~11,170 BP; O1b1a1a1-F2924, ~10,080 BP] for subgroups of the latter (Extended Data Fig. 4) may approximately synchronize with the dawn of Neolithic revolution in inland southern China (~9,000 BP[2,21]).

Diffusion of the southern East Asian lineage

Neolithic agriculture in Yangtze Basin is famous for its earliest domestication of sinica/japonica rice (*Oryza japonica*), which later spread into the vast tract of land ranging from Japan to Madagascar[1,30]. To test whether the agricultural diffusion from southern China is concomitant with the emigration from the same region, we used the *qpAdm*[31] to model the spatial and temporal distribution of the southern East Asian lineage, comprising both the inland (*i.e.*, AHM and AAA) and coastal (*i.e.*, AAN) lineages (Fig. 7 & Extended Data Table 4).

We started from the two-way model of Amis (representing the southern East Asian lineage) and Devil's Cave (representing the northern East Asian lineage), adding additional reference populations (Hòabìnhian, Afanasievo, Namazga, and Kolyma) when the initial model fails (see Method). In addition to MSEA populations (9.5–30.0%)[3,4], South Asia[32,33] (Kharia, 61.6 ± 2.2%), and the Jōmon individual (58.1 ± 2.7%) dated to ~2,600 BP[3], Hòabìnhian-like component also occurs in present-day Tibetans (32.3 ± 2.0%), suggesting a once wide-ranging distribution of the Australasian lineage in these regions prior to the agricultural expansion in East Asia. Regarding the East Asian lineage, there is no significant difference in fitness of Jōmon using either Devil's Cave (p =0.619) or Amis (p =0.558) as the proxy of East Asian, suggesting that the East Asian ancestry of Jōmon may phylogenetically be basal to the mainstream northern or southern East Asians. By contrast, the East Asian ancestry of Tibetan, which is probably related to the Tibeto-Burman dissemination in Tibetan Plateau, can be represented by Devil's Cave (67.7 ± 2.0%, p = 0.178) rather than Amis (p = 1.24×10^{-4}), consistent with the North China origin of the Sino-Tibetan language family[34,35].

Fitting the scenario of the expansion of Neolithic farmers in southern China, southern East Asian lineage represented by Amis predominates in Hàn (69.3 ± 2.8%), other populations in southern China (69.2–78.6%), and populations in MSEA (54.3–76.7%). Hàn and Tibeto-Burman-speaking populations (Nàxī, Yí, and Tǔjiā) tend to have more Devil's Cave ancestry (23.5–30.7%) than their

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neighbors, consistent with our previous inference. Compared with isolated Austroasiatics, Kinh and Thai/Khmer respectively harbor additional Devil's Cave (18.7 ± 7.5%) and Namazga (8.4 ± 1.0% for Thai, 3.7 ± 1.1% for Khmer) ancestry, likely reflecting immigration from Hàn Chinese and South Asians in the historical period, accordingly. Apart from MSEA, Austroasiatic-speaking Kharia in Central India is also estimated with Ami-like component (18.5 ± 3.0%), indicating a southern East Asian origin of the Muṇḍā branch.

Although the Devil's Cave ancestry is generally the predominant East Asian lineage in North Asia and adjacent areas, there is an intriguing discrepancy between the eastern [Korean, Japanese, Tungusic (except northernmost Oroqen), and Mongolic (except westernmost Kalmyk) speakers] and the western part [West Xiōngnú (~2,150 BP)[36], Tiānshān Hun (~1,500 BP)[36], Turkic-speaking Karakhanid (~1,000 BP)[36] and Tuva, and Kalmyk]. Whereas the East Asian ancestry of populations in the western part has entirely belonged to the Devil's Cave lineage till now, populations in the eastern part have received the genomic influence from an Amis-related lineage (17.4–52.1%) posterior to the presence of the Devil's Cave population roughly in the same region (~7,600 BP)[12]. Analogically, archaeological record has documented the transmission of wet-rice cultivation from coastal China (Shāndōng and/or Liáoníng Peninsula) to Northeast Asia, notably the Korean Peninsula (Mumun pottery period, since ~3,500 BP) and the Japanese archipelago (Yayoi period, since ~2,900 BP)[2]. Especially for Japanese, the Austronesian-related linguistic influence in Japanese[37] may indicate a potential contact between the Proto-Japonic speakers and population(s) affiliating to the coastal lineage. Thus, our results imply that a southern-East-Asian-related lineage could be arguably associated with the dispersal of wet-rice agriculture in Northeast Asia at least to some extent.

DISCUSSION

In this study, we discover an explicit genomic pattern regarding Hmong-Mien speakers and other southern East Asians, which has been established in Neolithic and is still detectable in present-day populations. After the separation from the northern East Asians, southern East Asians further divided into two lineages genetically, concordant with archaeological and linguistic evidence. While the inland lineage is associated with the farming dispersal from the middle Yangtze and the dissemination of the Austroasiatic and Hmong-Mien language family, the coastal lineage is more correlated with the agriculture expansion out of Yangtze Delta and the diffusion of the Austronesian and (at least partially) Kra-Dai language family. Especially for Hmong-Mien speakers, the dispersal from inland southern China and subsequent genetic contact with neighboring populations (particularly Tibeto-Burman- and Kra-Dai-speaking ones) majorly form the demographic history of Hmong-Mien-speaking populations.

It has been long debated how the Neolithic cultures relate to major language families in East Asian, and several assumptions have been proposed, notably the connection between the Dàxī culture and Proto-Hmong-Mien[30], the Dàwènkǒu culture and Pre-Austronesian[30], as well as the Yǎngsháo[34]/Císhān[35] culture and Proto-Sino-Tibetan. Even though the ancient genomic data from the Neolithic farming heartlands in China have not been sufficiently reported yet, we still recover a general demographic pattern using both the genetic diversity preserved in present-day populations

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and ancient samples from peripheral regions of East Asia, which can be served as a foothold for further archaeogenetic studies on the demographic dynamic of the southern East Asia. Although we observe a good matching among the evidence from genetics, linguistics, and archaeology, we still cautiously refer the proposed ancestral populations to an approximate geographical range instead of determined archaeological sites. Thus, we predict further studies on directly sampled ancient genomes from these sites will shed new light on resolving a clearer genetic landscape of this region, especially the demographic history since the Neolithic revolution.

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REFERENCE

1 Stevens, C. J. & Fuller, D. Q. The spread of agriculture in Eastern Asia: Archaeological bases for hypothetical farmer/language dispersals. *Language Dynamics and Change* **7**, 152-186 (2017).

2 Barnes, G. L. *Archaeology of East Asia: the rise of civilization in China, Korea and Japan*. (Oxbow Books, 2015).

3 McColl, H. *et al.* The prehistoric peopling of Southeast Asia. *Science* **361**, 88-92 (2018).

4 Lipson, M. *et al.* Ancient genomes document multiple waves of migration in Southeast Asian prehistory. *Science* **361**, 92-95 (2018).

5 Diamond, J. & Bellwood, P. Farmers and their languages: the first expansions. *Science* **300**, 597-603 (2003).

6 Bellwood, P. in *The peopling of East Asia* 41-54 (Routledge, 2005).

7 Ratliff, M. *Hmong-Mien language history*. (Pacific linguistics, Research School of Pacific and Asian Studies, The …, 2010).

8 Wen, B. *et al.* Genetic structure of Hmong-Mien speaking populations in East Asia as revealed by mtDNA lineages. *Molecular biology and evolution* **22**, 725-734 (2004).

9 Cai, X. *et al.* Human Migration through Bottlenecks from Southeast Asia into East Asia during Last Glacial Maximum Revealed by Y Chromosomes. *PLoS One* **6**, e24282, doi:10.1371/journal.pone.0024282 (2011).

10 Baxter, W. H. & Sagart, L. *Old Chinese: A new reconstruction*. (Oxford University Press, 2014).

11 Patterson, N., Price, A. L. & Reich, D. Population structure and eigenanalysis. *PLoS genetics* **2**, e190 (2006).

12 Sikora, M. *et al.* The population history of northeastern Siberia since the Pleistocene. *Nature*, doi:10.1038/s41586-019-1279-z (2019).

13 Reich, D. *Who We Are and How We Got Here: Ancient DNA and the new science of the human past*. (Oxford University Press, 2018).

14 Alexander, D. H., Novembre, J. & Lange, K. Fast model-based estimation of ancestry in unrelated individuals. *Genome research* **19**, 1655-1664 (2009).

15 Pickrell, J. K. & Pritchard, J. K. Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS genetics* **8**, e1002967 (2012).

16 Patterson, N. J. *et al.* Ancient admixture in human history. *Genetics*, genetics. 112.145037 (2012).

17 Mallick, S. *et al.* The Simons genome diversity project: 300 genomes from 142 diverse populations. *Nature* **538**, 201 (2016).

18 Yan, S. *et al.* Y Chromosomes of 40% Chinese Descend from Three Neolithic Super-Grandfathers. *PLoS One* **9**, e105691, doi:10.1371/journal.pone.0105691 (2014).

19 Blench, R., Sagart, L. & Sanchez-Mazas, A. *The peopling of East Asia: putting together archaeology, linguistics and genetics*. (Routledge, 2005).

20 Silva, F. *et al.* Modelling the Geographical Origin of Rice Cultivation in Asia Using the Rice Archaeological Database. *PLOS ONE* **10**, e0137024, doi:10.1371/journal.pone.0137024 (2015).

21 Chi, Z. & Hung, H.-c. The emergence of agriculture in southern China. *Antiquity* **84**, 11-25 (2010).

22 Bellwood, P. S. First farmers: the origins of agricultural societies. (2005).

23 Lipson, M. *et al.* Reconstructing Austronesian population history in island Southeast Asia. *Nature communications* **5**, 4689 (2014).

24 Ostapirat, W. in *23rd Annual Meeting of the Southeast Asian Linguistic Society, Chulalongkorn University.*

25 Blench, R. Was there an Austroasiatic presence in Island Southeast Asia prior to the Austronesian expansion? *Bulletin of the Indo-Pacific Prehistory Association* **30**, 133-144 (2010).

26 Simanjuntak, T. The western route migration: A second probable Neolithic diffusion to Indonesia. *Terra Australis* **45**, 201-212 (2017).

27 Luo, Y. in *The Tai-Kadai Languages* 25-44 (Routledge, 2004).

28 Ostapirat, W. 7. Macrophyletic Trees of East Asian Languages Re examined. *Senri Ethnological Studies* **98**, 107-121 (2018).

29 Zhang, X. *et al.* An updated phylogeny of the human Y-chromosome lineage O2a-M95 with novel SNPs. *PLoS One* **9**, e101020 (2014).

30 Fuller, D. Q. Pathways to Asian civilizations: Tracing the origins and spread of rice and rice cultures. *Rice* **4**, 78 (2011).

31 Haak, W. *et al.* Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature* **522**, 207 (2015).

32 de Barros Damgaard, P. *et al.* The first horse herders and the impact of early Bronze Age steppe expansions into Asia. *Science* **360**, eaar7711 (2018).

33 Reich, D., Thangaraj, K., Patterson, N., Price, A. L. & Singh, L. Reconstructing Indian population history. *Nature* **461**, 489-U450, doi:10.1038/nature08365 (2009).

34 Zhang, M., Yan, S., Pan, W. & Jin, L. Phylogenetic evidence for Sino-Tibetan origin in northern China in the Late Neolithic. *Nature*, doi:10.1038/s41586-019-1153-z (2019).

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35 Sagart, L. *et al.* Dated language phylogenies shed light on the ancestry of Sino-Tibetan. *Proceedings of the National Academy of Sciences*, 201817972 (2019).

36 de Barros Damgaard, P. *et al.* 137 ancient human genomes from across the Eurasian steppes. *Nature* **557**, 369 (2018).

37 Robbeets, M. Austronesian influence and Transeurasian ancestry in Japanese: A case of farming/language dispersal. *Language Dynamics and Change* **7**, 210-251 (2017).

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METHOD

Samples. Totally, we collected 903 Y-chromosomal samples [including 458 published[38] samples of 17 loci Y-chromosomal Short Tandem Repeat (Y-STR)] from healthy males, 624 mitochondrial samples, and 415 autosomal samples from unrelated volunteers in Húnán, China. All the samples are either blood or saliva. All the participants had signed the consent forms before joining the study. The study was approved by the ethics committee for biological research at the School of Life Science at Fudan University.

Molecular Methods. We used the DP-318 Kit (Tiangen Biotechnology, Beijing) to extract the genomic DNA with the manufacturer's protocol. We used AmpFlSTR® YFiler™ PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA) to amplify and genotype 17 Y-STR loci (DYS19, DYS389a/b, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, Y-GATA H4, and DYS385a/b) of all Y-chromosomal samples. We used the ABI 3730 and ABI 3130 Genetic Analyzers (Applied Biosystems) to analyze the amplification products. We used Genscan v. 3.7 and Genotyper v. 3.7 (Applied Biosystems) to analyze electrophoresis results. We selected 936 Y-chromosomal single nucleotide polymorphisms (Y-SNPs) for genotyping, according to Y-haplogroup polymorphism in the East Asian populations. Then we designed single-stranded nucleic acid bait libraries (Roche Nimblegen, Pleasanton, CA, USA) to capture these Y-SNPs. We sent ~500 of our Y-chromosomal samples for next-generation sequencing on the Illumina HiSeq2000 platform (Illumina, San Diego, CA, USA) and assigned haplogroups according to their Y-SNP results. For other Y-chromosomal samples, the haplogroups were inferred by Y-STR comparison. Other details of our next-generation sequencing process are identical to those previously described[18]. All 624 mitochondrial samples and 415 autosomal samples were extracted and performed similar processes of next-generation sequencing applied for Y-chromosomal samples to attain complete mitochondrial DNA sequences and selected autosomal single nucleotide polymorphisms (SNPs), respectively. Mitochondrial sequences were aligned to the Cambridge Reference Sequence (rCRS) by Kalign[39] and manually edited by BioEdit 7.0.4.1[40], with haplogroups assigned in accordance with *phylotree* Build 17[41].

Genomic dataset assembly. We used three distinct datasets in population genetic analysis: (1) a dataset for all the 415 newly reported samples from Central China, covering 9,752 SNPs and referred as "10k dataset"; (2) a dataset based on Human Origin (HO) dataset from Reich Lab (https://reich.hms.harvard.edu/datasets)[42,43] and combining other ancient and modern samples[3,4,12,17,32,36,44-56], covering 590,388 SNPs, and referred as "600k dataset"; (3) a dataset based on Simons Genome Diversity Project[17], combining other ancient and modern samples[3,4,12,32-46], covering 1,233,013 SNPs, and referred as "1240k dataset".

Principle Component Analysis (PCA). We performed PCA in *smartpca* program of EIGENSOFT[11] with parameters as default parameters and killr2: YES, r2thresh: 0.1, r2genlim: 0.02, lsqproject: YES, shrinkmode: YES. We used 10k dataset for PCA of population in Húnán, and a combination of 10k and 1240k dataset for East-Asia-wide PCA (leaving 4,911 SNPs). For East-Asia-wide PCA, principle components are constructed by modern samples with high-coverage ancient samples [Shamanka_EN (DA249)[32], a Devil's Cave individual[12] (NEO240), Chokhopani[45], and Laos_LN_BA[3] (LA364)] being projected.

Model-based clustering. We first applied PLINK[57] with the parameter indep-pairwise 50 5 0.2 to prune out the Linkage Disequilibrium (LD), yielding 3995 SNPs (10k dataset). Then, we performed the ADMIXTURE[14] from K = 2 to K = 10. We find the likelihood increasing and when $K \geqslant 4$, there is a heterogeneous composition within Pahng, which is inconsistent with the result from PCA and f_3 statistics. Therefore, we assume the best modeling reaches when K = 3.

***f*-statistics.** We utilized the *qp3Pop* and *qpDstat* program of ADMIXTOOLS[16] to calculate f_3- and *D*-statistics with default parameters. Based on the definition in Raghavan *et al.*, 2014[52], we calculated the outgroup f_3(Mbuti; Hmong, X) to measure the shared drift between Hmong and Eurasian populations, using the 1240k dataset. Based on the result of outgroup f_3-statistics, we computed the main *D* (X_1, Mbuti; Hmong, X_2), where X_1 = Eurasia-wide populations and X_2 = populations shared the most genetic drift with Hmong with additional *D*-statistics.

TreeMix analysis. We used the TreeMix[15] to generate the admixture graph, with k = 10 and *noss* for the 10k dataset, and k = 1000 and *noss* for the 600k dataset. We set migration events from m = 1 to m = 10 and reported the result of m = 4.

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Coalescence time estimation. In total, 93 complete mitochondrial sequences were selected for phylogenetic analysis of haplogroup B5, including 52 sequences from this study and 41 sequences [33 of B5, 1 of B4'5*[58] (Tiányuán), 1 of B4, and 6 of outgroup of B4'5] from phylotree Build 17[41]. The mutations of C indels 309.1C(C)-315.1C, AC indels at 515-522, C indels at 956-960, 3107, 8281-8289, A16182c, A16183c, 16189 and 16193.1C(C) were excluded from phylogeny reconstruction, manually performed using BioEdit 7.0.4.1[40]. We applied ARB[59] to generate the phylogeny using the Maximum Parsimony (MP) method. Bayesian time estimation for the most recent common ancestor (TMRCA) of B5 and its subclades were performed by BEAST v1.10.0[60]. The monophyletic option was set according to the previously generated MP phylogeny. The age of 40.0 kya for Tiányuán belonging to mitochondrial haplogroup B4'5* was chosen for time calibration[58]. For substitution model, HKY model was chosen with estimated base frequencies and Site Heterogeneity Model was set to eight Gamma categories. Strict clock model was set, and Bayesian skyline model was applied as prior[61]. The run was conducted 10 million iterations with sampling every 1000 steps, with the first 10% burnt in. All the effective sample sizes (ESS) were higher than 200. We applied similar procedure to the full-sequencing Y-chromosomal data for Bayesian TMRCA estimation.

Admixture graph fitting by *qpGraph*. We used the *qpGraph* program of ADMIXTOOLS[16] to create the admixture graph based on the result of *D*-statistics, with the default parameters and useallsnps: YES, outpop: NULL. We used 600k dataset to fit the admixture graph, leaving 496,352 SNPs. We used the backbone graph described in McColl *et al.*, 2018[3] as the initial graph. To avoid the case of a "basal" admixture, only Mbuti and Eurasian samples without significant Denisovan ancestry retained in the graph. Since all the Eurasian samples in the graph (except Ust'-Ishim and Tiányuán) do not harbor significantly excess Neanderthal ancestry compared with each other[3], we suppose that the interference of Neanderthal ancestry can only affect fitness between Mbuti and Eurasian samples, and Tiányuán or Ust'-Ishim with the others. Thus, such interference cannot significantly bias the main result of our *qpGraph* fitting.

Inference of mixture proportions. We used the *qpWave*[62] and *qpAdm*[31] program of ADMIXTOOLS to estimate the fitness and proportion of admixture components. We used the Devil's Cave samples[12], Amis, the Laotian Hòabìnhian individual (LA368)[3], Afanasievo[56], Namazga[32], and Kolyma[12] as reference populations. We used Mbuti, Peştera cu Oase1[53], Ust'-Ishim[48], Tiányuán[47], Kostenki[49], Yana_UP[12], Naṭūfian_N[43], Anatolia_N[54], Motala_HG[54,55], Önge[17], Papuan, Eskimo, and Mixe as outgroups.

Inbreeding coefficient F_{ST}. Using 10k dataset, we performed *smartpca* program of EIGENSOFT[11] to estimate pairwise F_{ST} with default parameters and fstonly: YES.

Abbreviations. We use the following abbreviated labels addressing populations throughout the paper: AAA, Ancestral Austroasiatic; AAN, Ancestral Austronesian; AHM, Ancestral Hmong-Mien; AKD, Ancestral Kra-Dai; South_EA, southern East Asian; North_EA, northern East Asians; First_MSEA, First Mainland Southeast Asian; Second_MSEA, Second Mainland Southeast Asian; Insular_AN, Insular Austronesian; SEAH, southern East Asian ancestry of Hàn; Aus, Australasian; UP, Upper Pleistocene; N, Neolithic; EN, Early Neolithic; LN, Late Neolithic; BA, Bronze Age; IA, Iron Age; HG, hunter-gatherer.

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38 Shu, L. *et al.* Genetic analysis of 17 Y-STR loci in Han, Dong, Miao and Tujia populations from Hunan province, central-southern China. *Forensic science international. Genetics* **19**, 250 (2015).
39 Lassmann, T. & Sonnhammer, E. L. Kalign–an accurate and fast multiple sequence alignment algorithm. *BMC bioinformatics* **6**, 298 (2005).
40 Hall, T. A. in *Nucleic acids symposium series.* 95-98 ([London]: Information Retrieval Ltd., c1979-c2000.).
41 van Oven, M. & Kayser, M. Updated Comprehensive Phylogenetic Tree of Global Human Mitochondrial DNA Variation. *Human Mutation* **30**, E386-E394, doi:10.1002/humu.20921 (2009).
42 Lazaridis, I. *et al.* Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* **513**, 409 (2014).
43 Lazaridis, I. *et al.* Genomic insights into the origin of farming in the ancient Near East. *Nature* **536**, 419 (2016).
44 Consortium, G. P. A global reference for human genetic variation. *Nature* **526**, 68 (2015).
45 Jeong, C. *et al.* Long-term genetic stability and a high-altitude East Asian origin for the peoples of the high valleys of the Himalayan arc. *Proceedings of the National Academy of Sciences* **113**, 7485-7490 (2016).
46 Rasmussen, M. *et al.* The genome of a Late Pleistocene human from a Clovis burial site in western Montana. *Nature* **506**, 225 (2014).
47 Yang, M. A. *et al.* 40,000-year-old individual from Asia provides insight into early population structure in Eurasia. *Current Biology* **27**, 3202-3208. e3209 (2017).
48 Fu, Q. *et al.* Genome sequence of a 45,000-year-old modern human from western Siberia. *Nature* **514**, 445 (2014).
49 Fu, Q. *et al.* The genetic history of ice age Europe. *Nature* **534**, 200 (2016).
50 Meyer, M. *et al.* A high-coverage genome sequence from an archaic Denisovan individual. *Science* **338**, 222-226 (2012).
51 Prüfer, K. *et al.* The complete genome sequence of a Neanderthal from the Altai Mountains. *Nature* **505**, 43 (2014).
52 Raghavan, M. *et al.* Upper Palaeolithic Siberian genome reveals dual ancestry of Native Americans. *Nature* **505**, 87 (2014).
53 Fu, Q. *et al.* An early modern human from Romania with a recent Neanderthal ancestor. *Nature* **524**, 216 (2015).
54 Mathieson, I. *et al.* Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* **528**, 499 (2015).
55 Mittnik, A. *et al.* The genetic prehistory of the Baltic Sea region. *Nature communications* **9**, 442 (2018).
56 Allentoft, M. E. *et al.* Population genomics of bronze age Eurasia. *Nature* **522**, 167 (2015).
57 Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American journal of human genetics* **81**, 559-575 (2007).
58 Fu, Q. *et al.* A Revised Timescale for Human Evolution Based on Ancient Mitochondrial Genomes. *Current Biology* **23**, 1-7 (2013).
59 Ludwig, W. *et al.* ARB: a software environment for sequence data. *Nucleic Acids Research* **32**, 1363-1371 (2004).
60 Suchard, M. A. *et al.* Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution* **4**, vey016 (2018).
61 Drummond, A. J., Rambaut, A., Shapiro, B. & Pybus, O. G. Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular biology and evolution* **22**, 1185-1192 (2005).
62 Reich, D. *et al.* Reconstructing Native American population history. *Nature* **488**, 370-+, doi:10.1038/nature11258 (2012).

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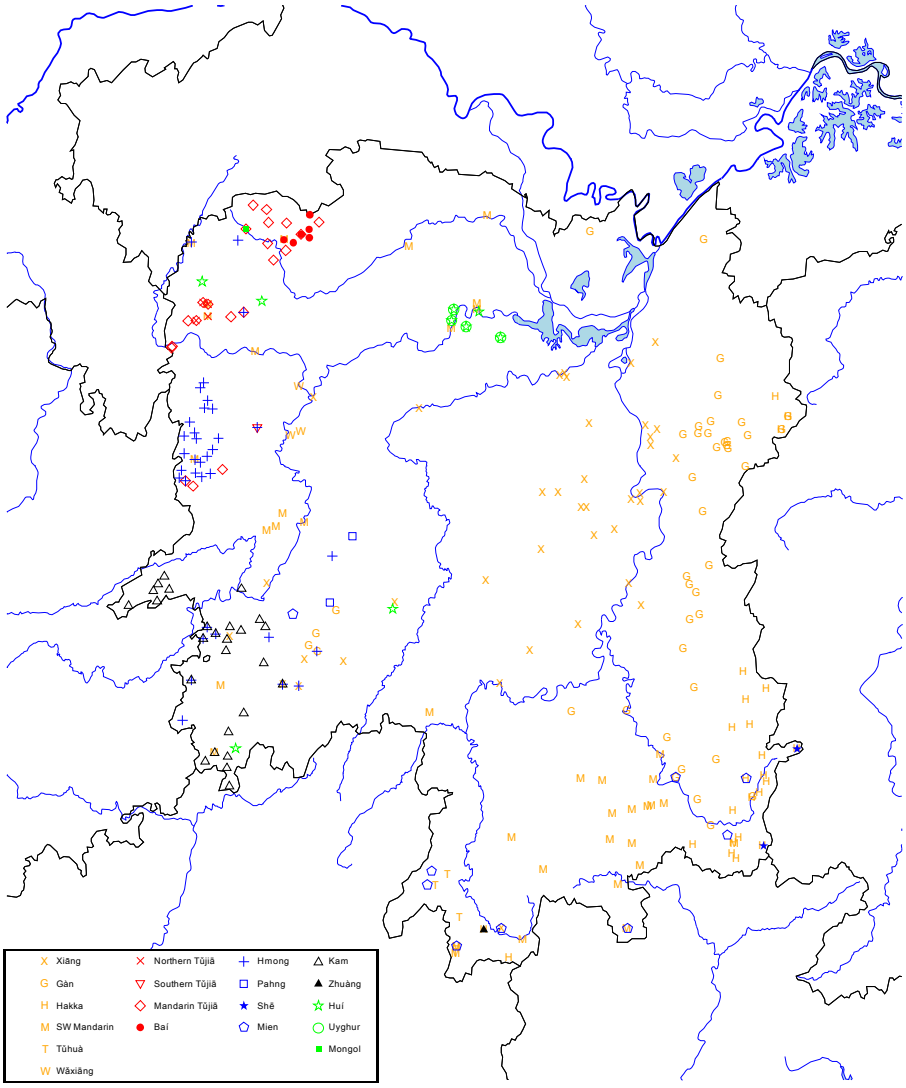
(A)



(B)

Population	Ethnicity	Language	Language branch	Language family
Xiāng	Hàn	Chinese, Xiāng Dialect	Sinitic	Sino-Tibetan
Gàn		Chinese, Gàn Dialect		
Hakka		Chinese, Hakka Dialect		
MandarinSW		Chinese, Southwest Mandarin Dialect		
Tǔhuà	Hàn or Yáo	Chinese, Tǔhuà Dialect		
Wǎxiāng	Hàn, Miáo or Yáo	Wǎxiāng		
BáiH	Bái in Húnán	Chinese, Southwest Mandarin Dialect		
TǔjiāN	Tǔjiā	Tǔjiā, Northern Dialect	Tibeto-Burman	
TǔjiāS		Tǔjiā, Southern Dialect		
TǔjiāM		Tǔjiā (*historical*), Southwest Mandarin (*present*)		
Hmong	Miáo (Hmong)	Xiong	Hmongic	Hmong-Mien
Pahng	Yáo	Hm Nai		
Mien		Iu Mien	Mienic	
Kam	Dòng (Kam)	Kam, Southern Dialect	Kam-Sui	Kra-Dai
Zhuàng	Zhuàng	Zhuàng	Tai	
Huí	Huí	Southwest Mandarin or Xiāng (*present*)	Sinitic	Sino-Tibetan
Uyghur	Uyghur	Old Uyghur (*historical*), Southwest Mandarin (*present*)		

Figure 1| Geographic and linguistic information of sampled populations. (A) Geographic distribution of populations in Central China. Locations are accurate to township level. **(B)** Recognized ethnicity and linguistic affiliation of every population.

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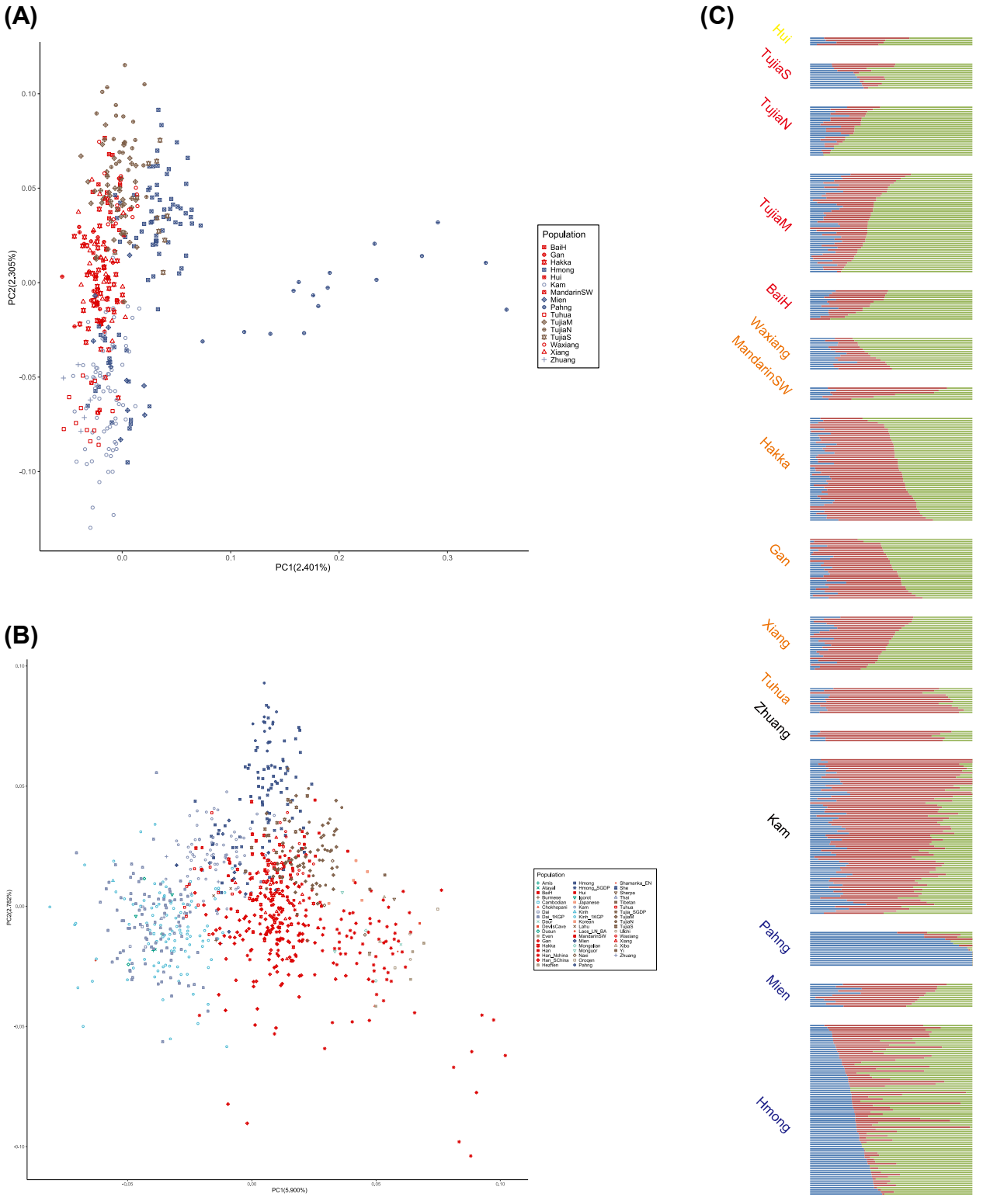

Figure 2 | Genomic structure of Húnán in relation to East-Asia-wide diversity. (A to **B)** Principal component analysis (PCA) (A) restricted to populations in Húnán and (B) together with a wide-ranging panel of 473 modern East Asians and four projected ancient samples. **(C)** Model-based clustering equipped with ADMIXTURE. When K=3, there is a high intra-population consistency of genetic composition, and the distribution of ancestral components is highly correlated with linguistic affiliation: blue, Hmong-Mien; green, Sino-Tibetan; red, Kra-Dai.

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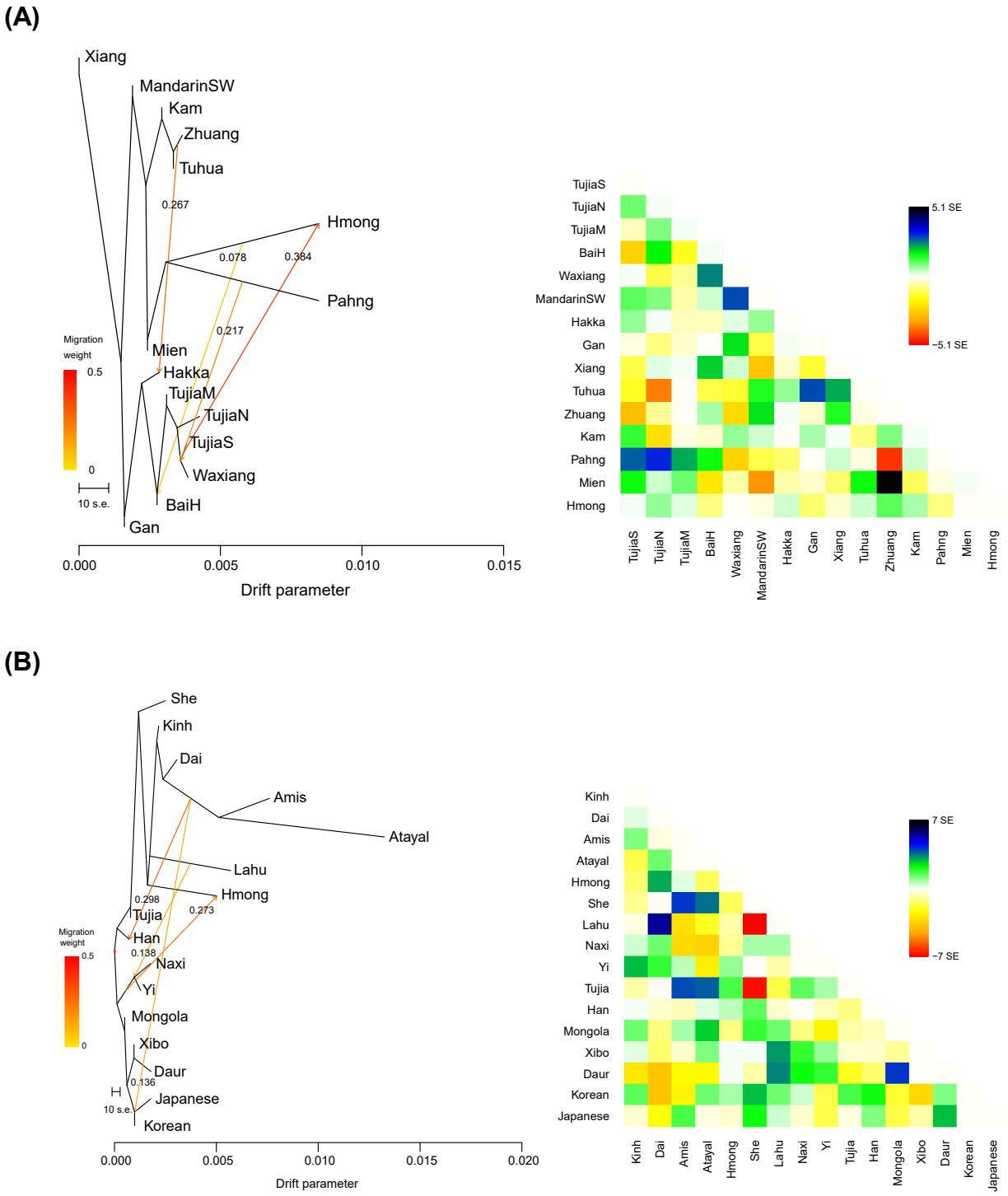

Figure 3 | Unrooted TreeMix analysis modelling a tentative phylogenetic relationship. TreeMix graph of **(A)** populations in Húnán and **(B)** East-Asia-wide populations.

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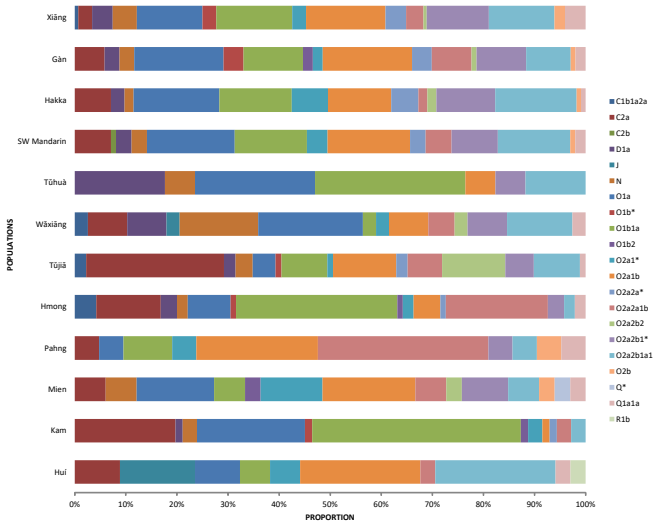
(A)



(B)

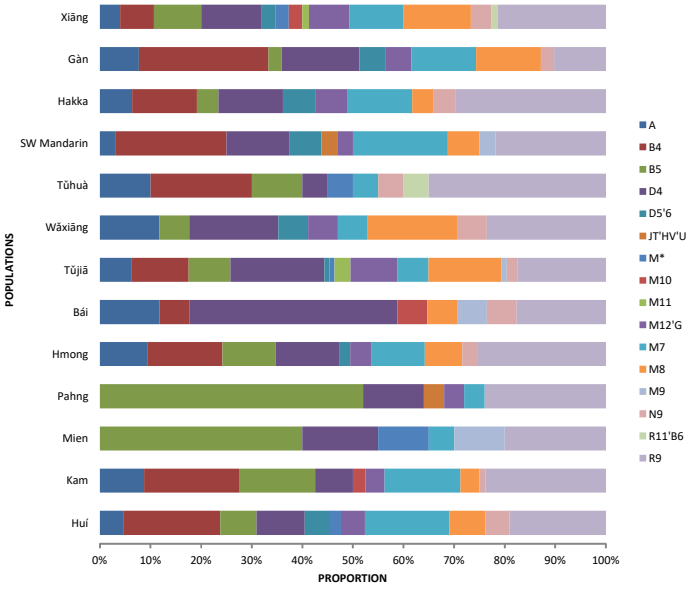


Figure 4 | Distribution of uniparentally inherited lineages in Húnán. Proportion of **(A)** Y-chromosomal and **(B)** mitochondrial haplogroups in Central China populations.

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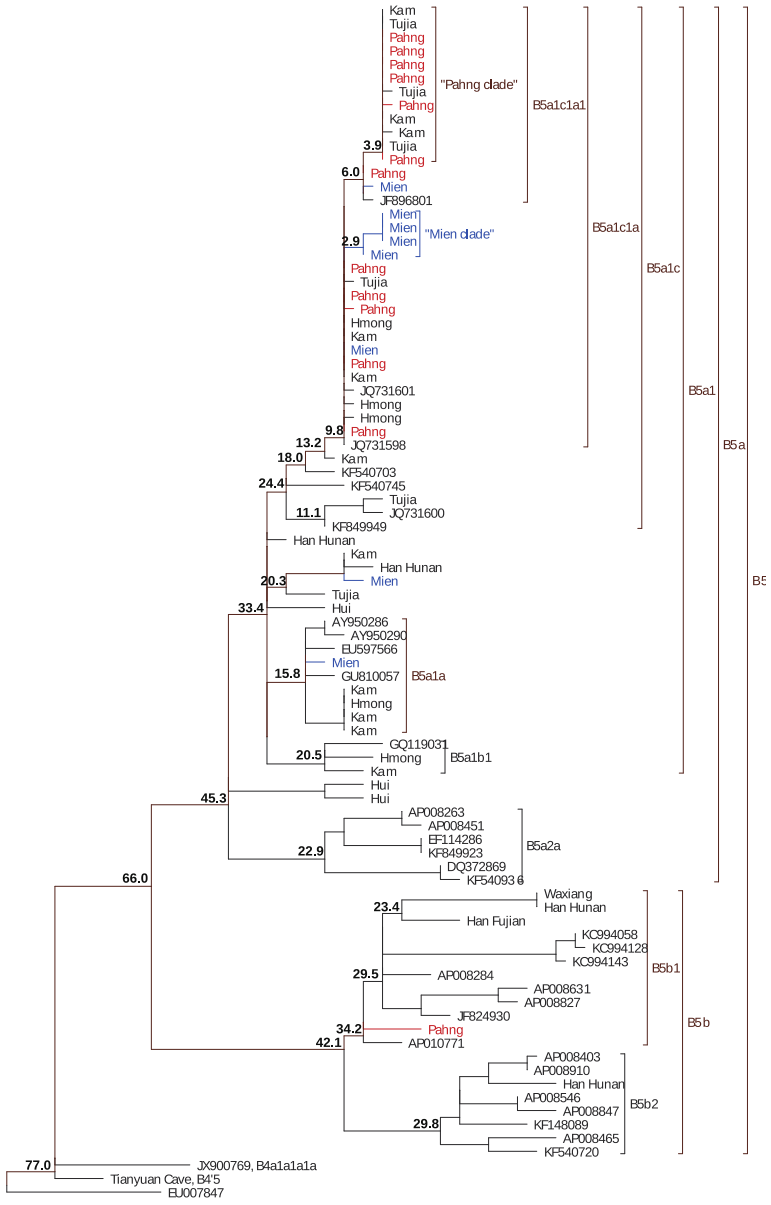
(A)



(B)

Haplogroup	Sampling Range	Divergence time
Cα2a1a-F10273	Tùjiā	3.89
	Húnán	5.45
O1b1a-PK4	—	14.29
O1b1a1-M95	—	11.66
O1b1a1a-F1252	—	11.17
O1b1a1a1-F2924	—	10.08
O1b1a1a1a-M88	—	6.34
O1b1a1a1a1-F2758	—	5.40
O1b1a1a2-F5504	—	2.11
O2a2a1b-M7	—	14.71
O2a2a1b2-F5511	Amis	0.99
O2a2a1b1a-F1276	—	13.56
O2a2a1b1a1-F2309	—	11.46
O2a2a1b1a1b-N5	Hmong, Shē (Fújiàn)	2.33

Figure 5 | Coalescent time estimation of uniparental lineages. (A) Phylogeny and divergence time estimates for mitochondrial haplogroup B5. We use maximum parsimony to establish the phylogeny and Bayesian inference to estimate the divergence time (kya). **(B)** Divergence time estimates of motif Y-haplogroups.

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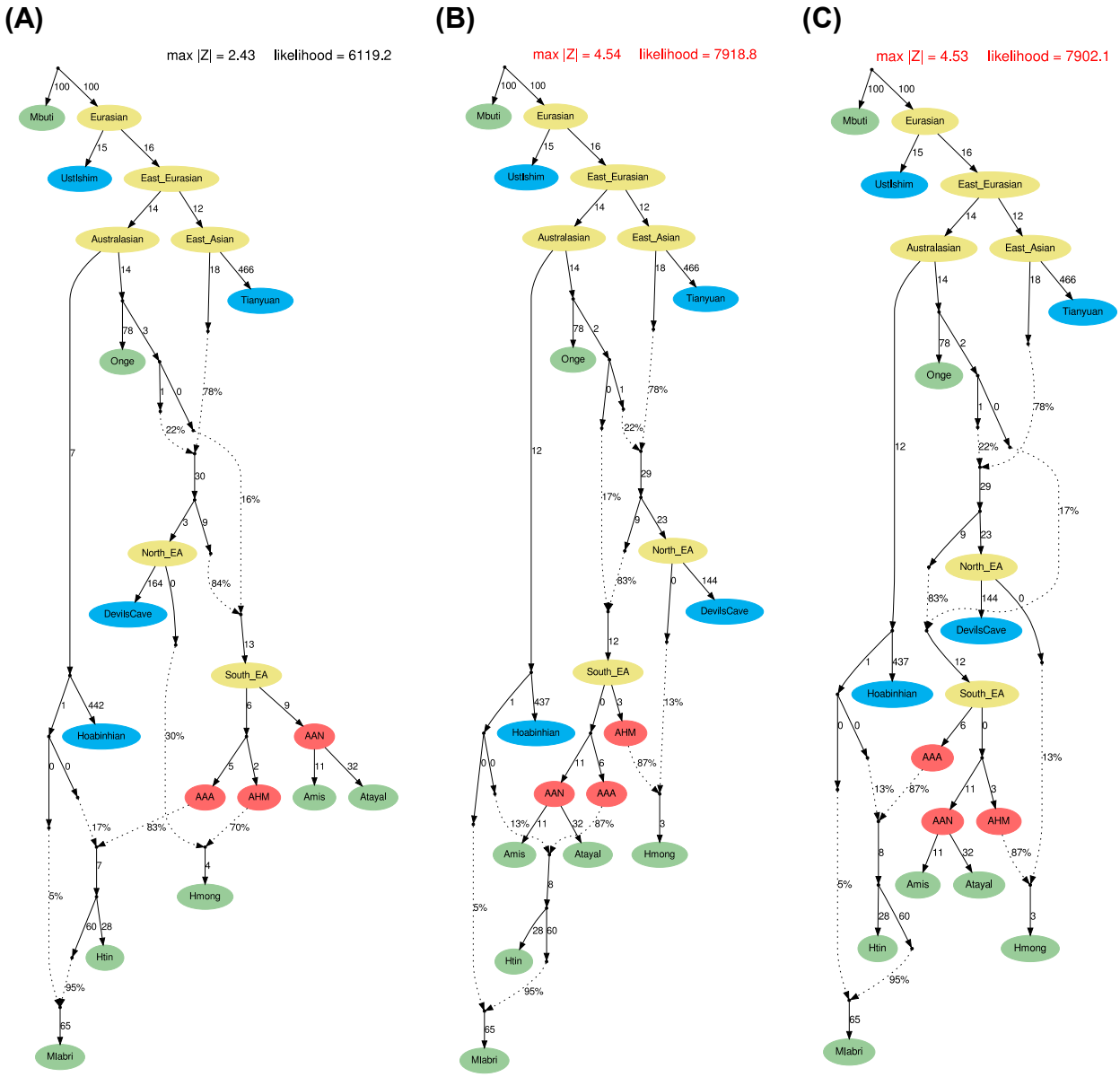

Figure 6 | Admixture Graph modelling the phylogenetic relationship among AHM, AAA, and AAN. (A) AAN as the outgroup; **(B)** AHM as the outgroup; **(C)** AAA as the outgroup. Graph (A) is the best model given following criteria: (1) a feasible worst-fitting Z-score (|Z| < 3), (2) the smallest score of likelihood, and (3) without zero-length internal branch. Therefore, modeled by *qpGraph*, AHM is phylogenetically closer to AAA than AAN. We note that the worst-fitting Z-score increases [Z = 3.02 for f_4(Tianyuan, Amis; Mlabri, Hmong)] if we do not model an increasing contribution from the Hòabìnhian lineage to Mlabri than Htin for graph (A).

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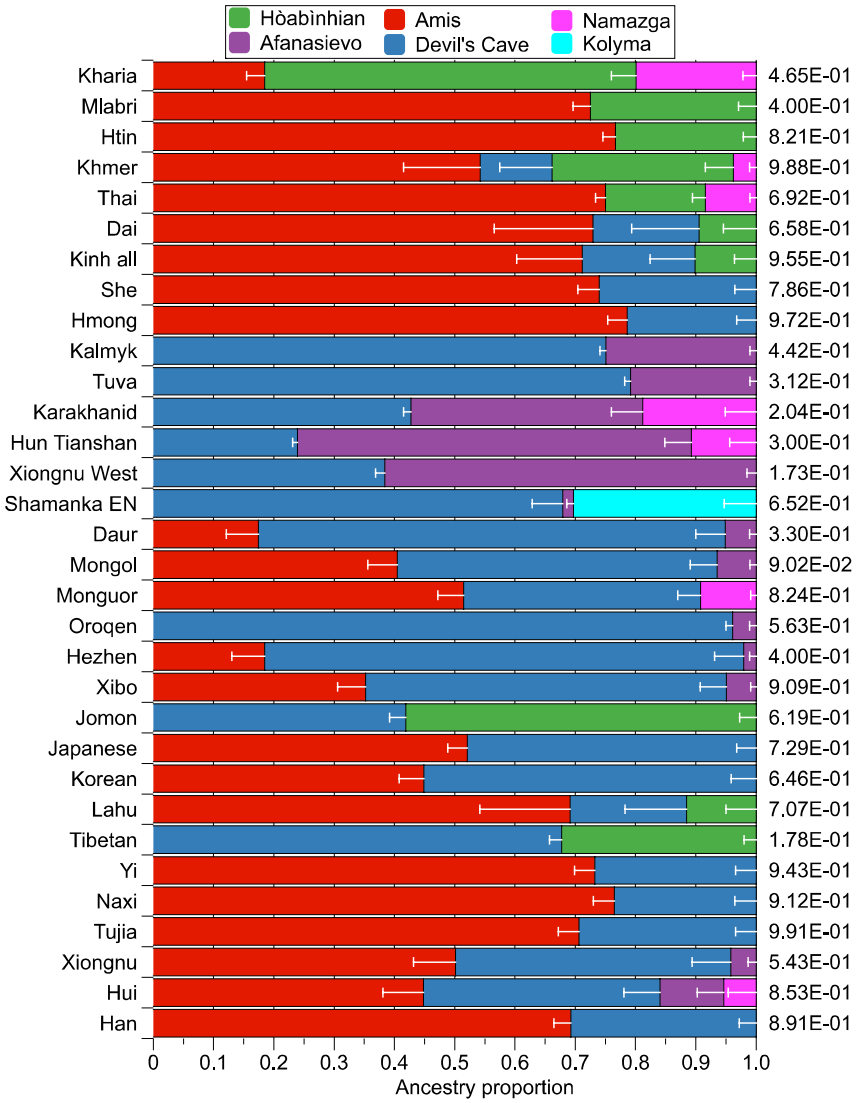

Figure 7| Spatial and temporal distribution of ancestries in East Asians. Reference populations and corresponding hypothesized ancestral populations: (1) Devil's Cave (~7,600 BP), the northern East Asian lineage; (2) Amis, the southern East Asian lineage (= AHM + AAA + AAN); (3) Hòabìnhian (~7,900 BP), a lineage related to Andamanese and indigenous hunter-gatherer of MSEA; (4) Kolyma (~9,800 BP), "Ancient Palaeo-Siberians"; (5) Afanasievo (~4,800 BP), steppe ancestry; (6) Namazga (~5,200 BP), the lineage of Chalcolithic Central Asian. Here, we report the best-fitting results of *qpAdm* based on following criteria: (1) a feasible *p*-value (> 0.05), (2) feasible proportions of all the ancestral components (mean > 0 and standard error < mean), and (3) with the highest *p*-value if meeting previous conditions.

Populations	Pahng does not have significant non-Hmong-Mien ancestry		Hmong has extra Sino-Tibetan-related ancestry		Mien has both Kra-Dai- and Hmong-Mien-related ancestries				TujiaS has extra Hmong-Mien-related ancestry		Han in Hunan has both Sino-Tibetan- and Kra-Dai-related ancestries					
	f_3(Pahng; Hmong, X)		f_3(Hmong; Pahng, X)		f_3(Mien; Pahng, X)		f_3(Mien; Hmong, X)		f_3(TujiaS; TujiaN, X)		f_3(Xiang; TujiaN, X)		f_3(Gan; TujiaN, X)		f_3(Hakka; TujiaN, X)	
	mean	Z-score	mean	Z-score	mean	Z-score	mean	Z-score	mean	Z-score	mean	Z-score	mean	Z-score	mean	Z-score
Xiang	0.01854	16.911	0.00125	2.109	0.00035	0.401	0.00075	1.015	0.00186	1.876	n/a	n/a	-0.00060	-0.875	-0.00041	-0.689
Gan	0.01825	17.307	0.00128	2.094	0.00044	0.480	0.00071	0.957	0.00117	1.180	-0.00124	-2.264	n/a	n/a	-0.00154	-2.636
Hakka	0.01906	18.564	-0.00002	-0.043	-0.00017	-0.171	0.00062	0.746	0.00144	1.539	0.00046	0.728	-0.00022	-0.324	n/a	n/a
MandarinSW	0.01949	16.510	0.00104	1.544	0.00093	0.876	0.00179	2.202	0.00096	0.911	0.00053	0.827	-0.00016	-0.254	-0.00036	-0.546
Waxiang	0.02084	16.973	-0.00044	-0.759	0.00106	1.060	0.00343	3.930	0.00092	1.051	0.00342	4.520	0.00279	3.246	0.00224	3.571
Tuhua	0.01814	16.581	0.00176	3.265	-0.00026	-0.249	-0.00052	-0.633	0.00019	0.163	-0.00296	-4.489	-0.00270	-3.499	-0.00285	-3.909
BaiH	0.01957	16.032	-0.00002	-0.037	0.00159	1.554	0.00293	3.392	0.00343	3.530	0.00207	2.743	0.00297	4.047	0.00187	3.336
TujiaM	0.01987	17.592	-0.00045	-0.805	0.00023	0.258	0.00159	1.957	0.00264	3.045	0.00315	3.468	0.00326	3.732	0.00230	4.393
TujiaN	0.01991	17.382	-0.00043	-0.606	0.00066	0.663	0.00222	2.675	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
TujiaS	0.02031	17.996	0.00027	0.454	0.00263	2.510	0.00375	4.121	n/a	n/a	0.00387	4.047	0.00418	4.559	0.00315	5.160
Hmong	n/a	n/a	n/a	n/a	0.00132	1.505	n/a	n/a	-0.00002	-0.019	0.00082	1.334	0.00161	2.280	0.00066	1.374
Pahng	n/a	n/a	n/a	n/a	n/a	n/a	0.00132	1.505	-0.00094	-0.863	-0.00030	-0.426	0.00007	0.083	0.00012	0.164
Mien	0.01813	15.537	0.00172	2.735	n/a	n/a	n/a	n/a	0.00077	0.685	-0.00047	-0.711	-0.00032	-0.436	-0.00051	-0.715
Kam	0.01726	16.128	0.00187	3.255	0.00041	0.444	-0.00024	-0.291	-0.00038	-0.368	-0.00242	-4.433	-0.00220	-2.996	-0.00272	-4.304
Zhuang	0.02046	14.722	-0.00013	-0.157	-0.00243	-1.932	-0.00084	-0.846	0.00177	1.362	-0.00189	-2.360	-0.00040	-0.456	-0.00256	-3.405

Table 1 | Admixture f_3 statistics of Central China populations. The key discoveries revealed by corresponding f_3 statistics are mentioned in the header. f_3 statistics with a relatively negative value ($< -4.0\times10^{-5}$) and Z-score ($Z < -0.5$, one-tailed $p < 0.309$) are shaded.

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Categories		Populations	*D* (X, Mbuti; Hmong, Tǔjia)	*D* (X, Mbuti; Hmong, Hàn)	*D* (X, Mbuti; Hmong, Shē)	*D* (X, Mbuti; Hmong, Dǎi)	*D* (X, Mbuti; Hmong, Amis)
Northern East Asia	Ancient	*DevilsCave_N*	-1.222	-2.650	-0.891	6.072	2.989
		Shamanka_EN	-1.139	-1.170	0.023	7.279	5.465
		Lokomotiv_EN	-0.243	-0.680	0.401	6.830	4.916
		Ust'-Ida_LN	-2.159	-2.262	-1.060	5.550	2.721
		Shamanka_EBA	-1.610	-1.767	-0.558	6.948	3.581
		Okunevo_BA	-1.035	-1.586	-0.650	3.005	2.269
		Xiōngnú	-0.167	-1.582	-1.652	4.974	1.621
		Xiōngnú_West	-0.049	-0.616	0.019	3.319	1.340
		Chokhopani	-0.119	0.540	-0.229	6.869	4.886
	Turkic	Yakut	-1.925	-2.163	-1.062	7.832	4.562
		Tubalar	-1.217	-1.677	-0.403	5.636	4.532
		Kyrgyz	-2.010	-2.170	-1.224	6.142	4.140
		Tuva	-2.210	-2.467	-1.259	7.276	3.952
		Altai	-1.048	-2.341	-0.799	6.118	3.696
		Dolgan	-1.886	-2.107	-0.599	6.205	3.054
		Uyghur	-1.654	-1.881	-1.871	4.073	2.554
		Uzbek	-0.961	-1.533	-0.745	3.580	2.787
	Mongolic	Monguor	-1.661	-1.152	-1.194	7.569	4.871
		Daur	-1.348	-1.805	-1.336	7.870	4.578
		Kalmyk	-2.231	-2.222	-1.247	6.982	3.947
		Mongol	-2.169	-2.555	-2.672	6.460	3.304
		Hazāra	-0.427	-1.204	-0.506	4.803	3.426
	Tungusic	Hezhen	-1.561	-1.968	-1.501	8.084	4.397
		Xībó	-1.888	-2.120	-1.677	7.016	4.126
		Oroqen	-1.807	-2.307	-1.691	7.378	4.116
		Ulchi	-1.838	-2.565	-1.502	7.429	3.598
		Even	-1.343	-2.450	-0.867	5.763	3.409
	Uralic	Mansi	-1.342	-1.473	-1.062	4.654	2.289
		Nganasan	-1.409	-2.900	-1.157	7.147	3.431
	Tibeto-Burman	Sherpa	-0.318	0.006	0.921	7.438	5.681
		Tibetan	-0.024	0.507	0.329	7.954	7.028
		Nàxī	-1.222	-0.412	-1.059	6.224	5.054
		Yí	-0.229	0.699	-0.130	6.873	4.628
	Japan & Korea	Japanese	-1.668	-2.676	-2.654	5.857	1.359
		Korean	-1.886	-2.454	-2.289	6.472	2.650
Eastern and Central China		Tǔjiā	n/a	1.243	-0.842	5.149	1.663
		Hàn	-0.915	n/a	-2.637	3.693	0.981
		Shē	0.257	0.537	n/a	3.069	-0.113
Austronesian	Taiwan	Amis	-0.019	1.016	-3.040	-3.614	n/a
		Atayal	-0.529	-0.049	-3.206	-4.252	-21.719
	Luzon & Visayas	*Phl534*	-0.627	0.627	-1.322	-0.214	-5.286
		Kankanaey	0.381	1.427	-2.066	-3.016	-22.350
		Tagalog	-0.238	-0.327	-2.762	-2.669	-15.202
		Ilocano	1.220	1.767	-1.631	-1.738	-18.687
		Visayan	0.182	1.336	-1.202	-3.034	-17.044
	Sumatra & Borneo	Malay	2.008	3.305	-0.288	-4.763	-8.919
		Dusun	1.401	2.726	-1.283	-4.454	-16.001
		Murut	1.617	2.565	-1.108	-4.711	-17.170
Southwest China & Mainland Southeast Asia	Ancient	*Laos_LN_BA (LA364)*	2.992	5.101	0.672	-4.926	-0.642
		Laos_BA	3.116	4.087	1.049	-3.434	-1.627
		Vietnam_N (Mán Bạc)	2.719	3.928	-0.578	-4.646	-1.283
		Thailand_LN_IA (Ban Chiang)	2.137	3.822	1.342	-1.310	0.136
		Vietnam_LN	1.678	2.825	-0.220	-3.525	-2.090
		Vietnam_BA (Đông Sơn)	1.713	2.482	-0.545	-3.726	-2.932
		Vietnam_BA (Núi Nạp)	1.834	1.879	0.636	-1.392	-2.918
	Austroasiatic	Htin	3.951	5.650	1.239	-4.880	-0.870
		Mlabri	2.020	4.469	1.195	-5.482	-1.497
		Khmer	2.087	3.068	-0.292	-4.304	-3.289
		Kinh_all	1.273	2.418	-1.334	-2.412	-3.132
	Kra-Dai	Dǎi	2.466	3.009	-0.795	n/a	-3.758
		Thai	1.478	2.590	-1.533	-3.869	-3.236
	Tibeto-Burman	Burmese	0.346	1.691	-0.292	1.983	2.118
		Lāhù	1.525	3.307	0.274	-0.596	0.485
Eastern Siberia	Chukotko-Kamchatkan	Chukchi	-1.598	-2.341	-1.125	6.332	3.555
		Itelmen	-2.329	-2.755	-1.384	5.799	2.955

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	Yukaghir	Koryak	-2.540	-2.531	-1.867	6.060	3.581
		Yukaghir	-1.713	-2.513	-1.249	6.758	3.910
North America	Eskimo-Aleut	Aleut	-0.338	-1.485	-0.237	2.992	1.798
		Eskimo	-2.067	-2.793	-1.776	5.238	3.634
	Na-Dene	Tlingit	-0.261	-0.994	0.312	3.881	2.791
First Americas		*Anzick*	1.153	0.381	0.114	3.686	2.078
		Pima	-1.026	-2.173	-1.722	3.924	1.560
		Mayan	-1.070	-1.880	-1.739	4.807	1.648
		Mixe	-0.452	-1.488	-1.204	5.097	2.502
		Mixtec	-0.552	-1.874	-1.448	4.913	1.803
		Zapotec	-0.892	-1.907	-1.399	4.712	2.180
		Quechua	-1.124	-2.304	-1.561	3.990	1.619
		Karitiâna	-0.786	-1.156	-2.063	3.938	1.107
		Piapoco	-0.049	-1.528	-1.581	3.560	1.260
		Surui	-0.299	-1.108	-0.789	4.059	1.621
Australasians		*Hòabìnhian (LA368)*	0.552	0.230	-1.673	-2.048	-2.084
		Önge	-0.446	-0.139	-0.399	-1.984	-1.556
		Australian	0.506	1.068	-1.062	0.507	-1.311
		Papuan	0.391	0.804	-0.747	-0.409	-1.088
Ancient East Asians		*Tiányuán*	-0.637	-1.206	-0.998	-2.754	-0.683
		Jōmon	-0.676	-0.104	-0.717	1.263	-3.153

Table 2 | *D*-statistics detecting the major gene flow events in relation to Hmong. Significant ($|Z| > 3$, *i.e.*two-tailed $p < 0.0027$) and quasi-significant ($2.58 < |Z| \leq 3$, *i.e.*two-tailed $0.0027 \leq p < 0.01$) *D*-statistics are shaded in different shade.

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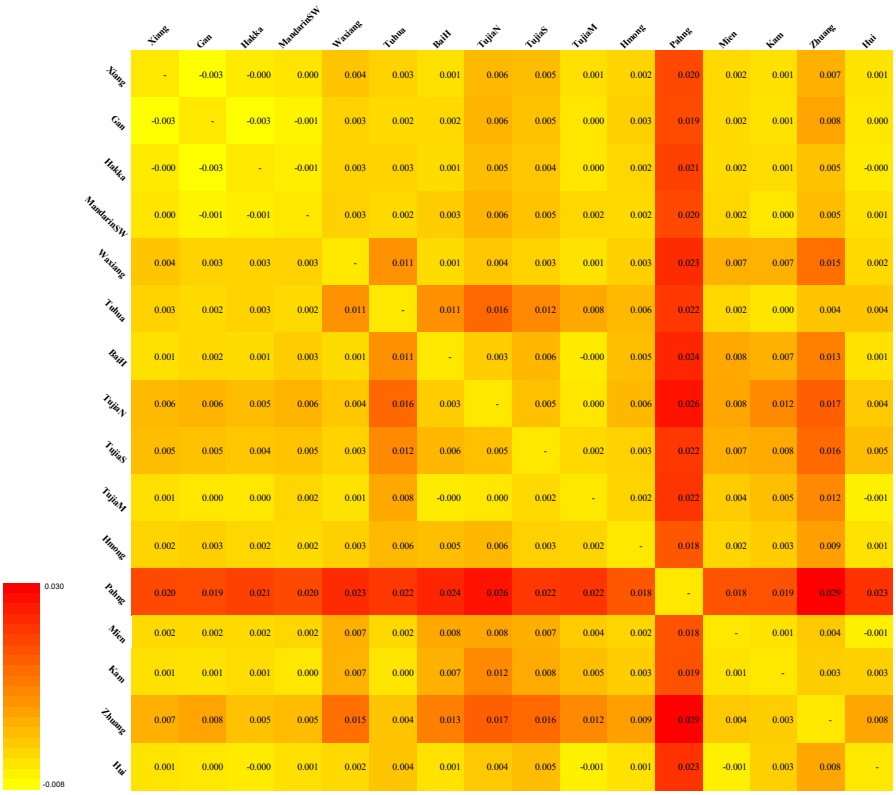

Extended Data Figure 1 | Pairwise F_{ST} of populations in Húnán.

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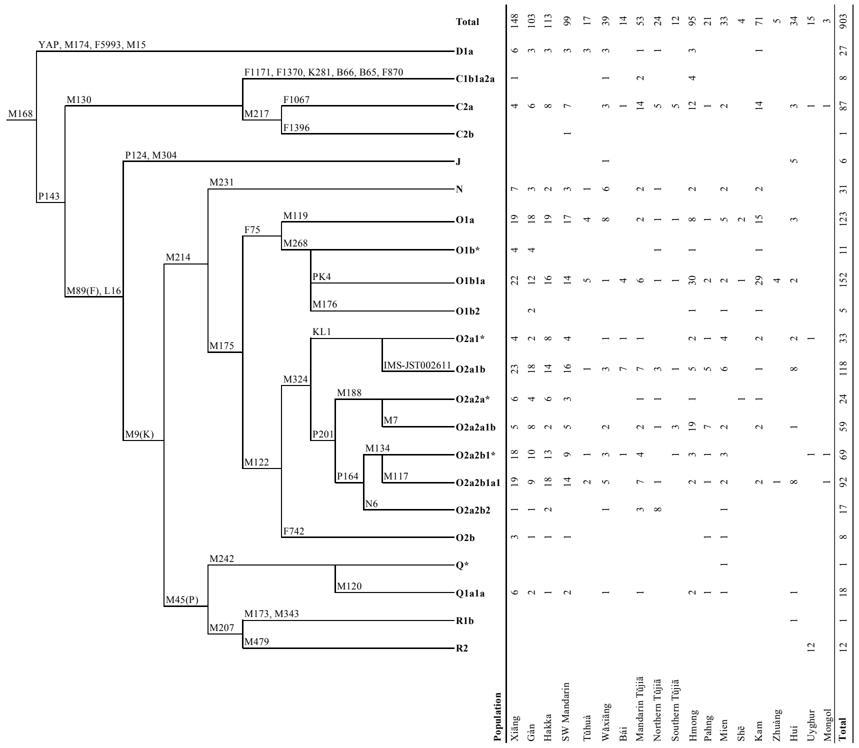
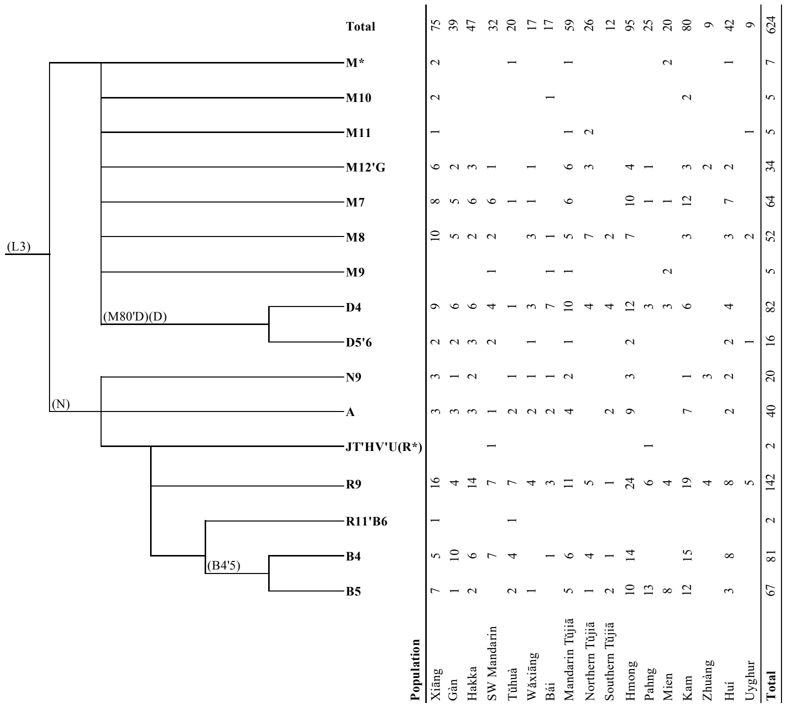
(A)



(B)



Extended Data Figure 2 | Phylogenies and distribution of (A) Y-chromosomal and (B) mitochondrial haplogroups in populations in Húnán.

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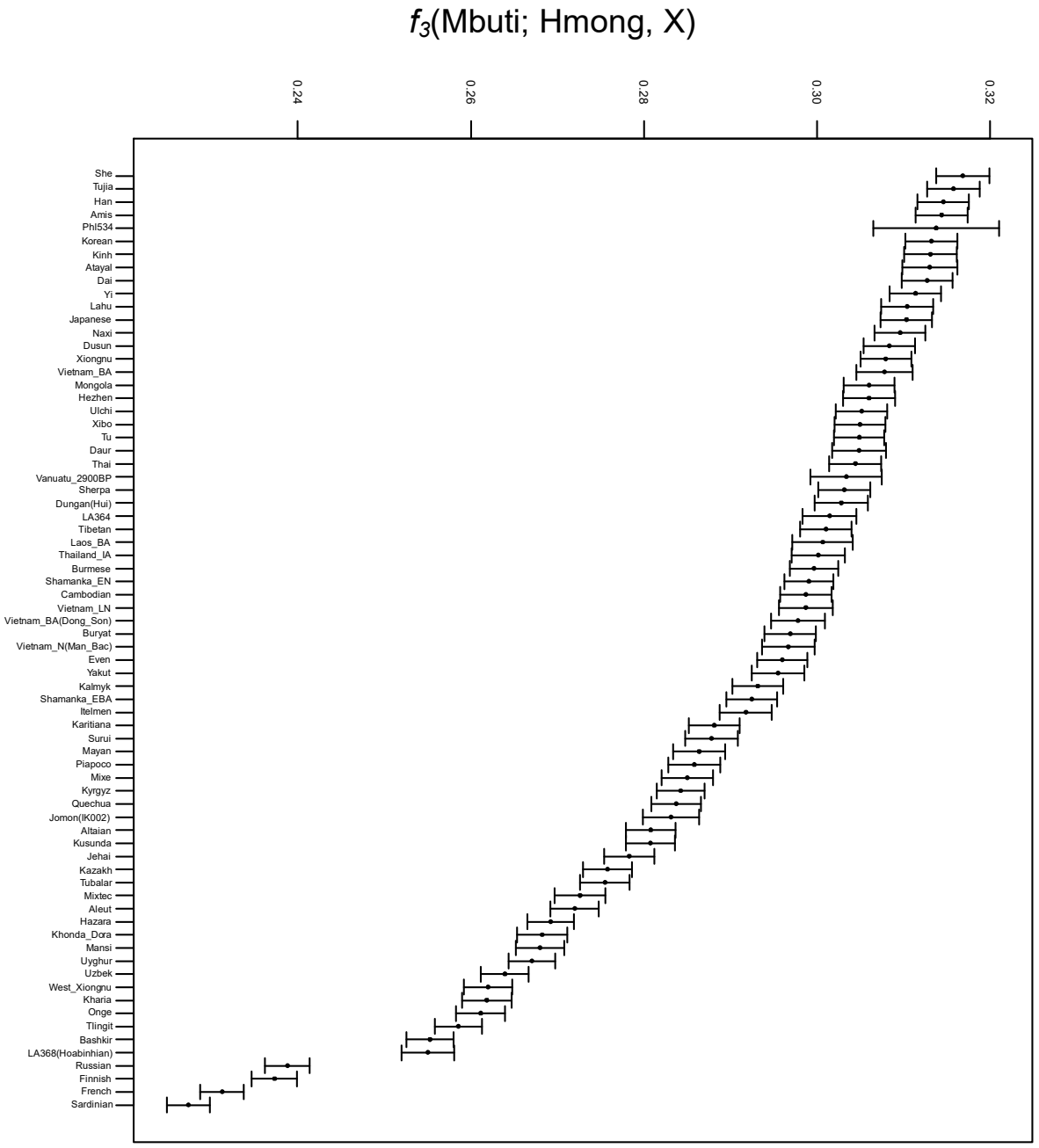

Extended Data Figure 3 | Outgroup f_3-statistics measuring shared drift between Hmong and other Eurasian populations. Mbuti is used as the outgroup.

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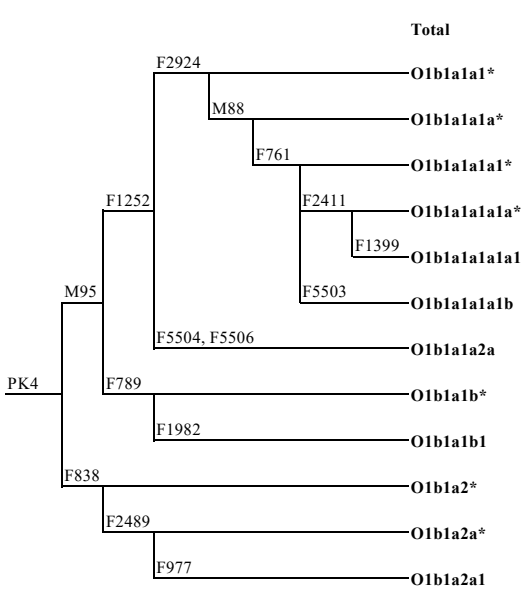

Population	O1b1a1a1*	O1b1a1a1a*	O1b1a1a1a1*	O1b1a1a1a1a*	O1b1a1a1a1a1	O1b1a1a1a1b	O1b1a1a2a	O1b1a1b*	O1b1a1b1	O1b1a2*	O1b1a2a*	O1b1a2a1	Total
Xiang	3	1	1							2		1	8
Gan	2												2
Hakka	3	2				1			1	1	1		9
SW Mandarin	6												6
Tuhua	3												3
Mandarin Tujia	3					1				1		1	6
Northern Tujia				1									1
Southern Tujia	1												1
Bai	2	1				1							4
Hmong	4		2				23			1			30
Pahng								1					1
Mien		1											1
Kam	13	1		1		8			3	2			29
Zhuang	2			2									4
Total	42	6	3	4	1	11	23	1	4	7	1	2	105

Extended Data Figure 4 | Distribution of subgroups of Y-haplogroup O1b1a-PK4 in Húnán.

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Category	Population	Sample size
Sinitic	Xiāng	25
	Gàn	28
	Hakka	47
	MandarinSW	6
	Huí	4
	Tǔhuà	12
	Wǎxiāng	15
	BáiH	14
Tibeto-Burman	TǔjiāN	23
	TǔjiāS	12
	TǔjiāM	46
Hmong-Mien	Hmong	79
	Pahng	16
	Mien	11
Kra-Dai	Kam	72
	Zhuàng	5
	Total	415

Extended Data Table 1 | Autosomal sample size of populations in Hunan.

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Populations	*D* (X, Mbuti; Hmong, Tujia)		*D* (X, Mbuti; Hmong, Han)		*D* (X, Mbuti; Hmong, She)		*D* (X, Mbuti; Hmong, Dai)		*D* (X, Mbuti; Hmong, Amis)	
	mean	Z-score	mean	Z-score	mean	Z-score	mean	Z-score	mean	Z-score
DevilsCave_N	-0.0027	-1.347	-0.0050	-3.028	-0.0026	-1.157	0.0105	4.998	0.0050	1.910
Shamanka_EN	-0.0022	-1.332	-0.0027	-2.006	-0.0015	-0.833	0.0106	6.201	0.0071	3.355
Lokomotiv_EN	-0.0031	-1.467	-0.0023	-1.302	-0.0028	-1.175	0.0109	4.712	0.0061	2.217
Ust'-Ida_LN	-0.0043	-2.008	-0.0030	-1.720	-0.0025	-1.087	0.0087	4.059	0.0011	0.407
Shamanka_EBA	-0.0037	-1.819	-0.0041	-2.479	-0.0029	-1.334	0.0094	4.338	0.0031	1.212
Okunevo_BA	-0.0037	-2.170	-0.0033	-2.403	-0.0046	-2.446	0.0033	1.863	0.0002	0.115
Xiōngnú	-0.0028	-1.356	-0.0044	-2.501	-0.0047	-2.083	0.0071	3.202	0.0005	0.170
Xiōngnú_West	-0.0005	-0.210	-0.0004	-0.212	-0.0019	-0.784	0.0057	2.453	-0.0005	-0.196
Chokhopani	-0.0015	-0.618	-0.0012	-0.598	-0.0034	-1.335	0.0129	5.149	0.0094	3.128
Laos_LN_BA (LA364)	0.0016	0.559	0.0030	1.334	-0.0009	-0.312	-0.0103	-3.753	-0.0047	-1.456
Laos_BA	0.0100	2.430	0.0062	1.925	-0.0027	-0.614	-0.0062	-1.524	-0.0087	-1.750
Vietnam_N (Mán Bạc)	0.0056	1.580	0.0067	2.213	0.0031	0.774	-0.0062	-1.672	0.0004	0.087
Thailand_LN_IA (Ban Chiang)	0.0081	1.146	0.0095	1.671	0.0002	0.033	0.0003	0.041	0.0021	0.245
Vietnam_LN	-0.0035	-1.008	0.0016	0.573	-0.0063	-1.712	-0.0055	-1.523	-0.0096	-2.245
Vietnam_BA (Đông Sơn)	0.0041	1.227	0.0028	0.977	-0.0051	-1.347	-0.0049	-1.351	-0.0064	-1.470
Vietnam_BA (Núi Nạp)	0.0005	0.144	-0.0009	-0.288	-0.0062	-1.517	-0.0061	-1.596	-0.0102	-2.297
Anzick	0.0005	0.224	-0.0025	-1.221	-0.0030	-1.125	0.0078	3.040	0.0024	0.785
Hòabìnhian_Laos (LA368)	-0.0012	-0.351	-0.0024	-0.886	-0.0091	-2.485	-0.0028	-0.774	-0.0113	-2.698
Tiányuán	-0.0042	-1.606	-0.0037	-1.714	-0.0047	-1.730	-0.0071	-2.556	-0.0060	-1.849
Jōmon	-0.0034	-1.326	-0.0003	-0.144	-0.0036	-1.300	0.0057	2.073	-0.0118	-3.525

Extended Data Table 2 | *D*-statistics with only transversions for tetrads including ancient samples in Table 2.

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D (X_1, Mbuti; X_3, X_4)							Notes
X_1	X_3	X_4	All SNPs		Transversions only		
			mean	Z-score	mean	Z-score	
Önge	Tiányuán	DevilsCave_N	-0.0161	**-3.148**	-0.0197	-2.580	Compared with Tiányuán, modern and Neolithic East Asians have additional Australasian ancestry.
		Hàn	-0.0214	**-4.689**	-0.0205	**-3.084**	
		Hmong	-0.0212	**-4.534**	-0.0193	-2.840	
		Amis	-0.0236	**-5.058**	-0.0232	**-3.397**	
		Atayal	-0.0242	**-5.050**	-0.0237	**-3.421**	
		Htin	-0.0255	**-5.395**	-0.0262	**-3.778**	
		Mlabri	-0.0241	**-4.560**	-0.0251	**-3.340**	
Önge	Hàn	Hmong	0.0002	0.139	0.0011	0.654	Hàn, Hmong, Amis, and Atayal do not significantly have more Australasian ancestry than each other.
		Amis	-0.0028	-1.668	-0.0032	-1.485	
		Atayal	-0.0028	-1.390	-0.0029	-1.138	
	Hmong	Amis	-0.0030	-1.556	-0.0043	-1.719	
		Atayal	-0.0030	-1.312	-0.0039	-1.419	
	Amis	Atayal	0.0000	-0.009	0.0004	0.132	
Hàn	DevilsCave_N	Amis	-0.0171	**-7.425**	-0.0107	**-3.267**	The major genomic component of Hàn, AHM, and AAA are phylogenetically closer to AAN than Devil's Cave ancestry.
		Atayal	-0.0152	**-6.219**	-0.0086	-2.505	
Hmong		Amis	-0.0214	**-8.579**	-0.0154	**-4.417**	
		Atayal	-0.0184	**-6.949**	-0.0118	**-3.234**	
Htin		Amis	-0.0317	**-12.337**	-0.0284	**-7.810**	
		Atayal	-0.0307	**-10.925**	-0.0271	**-6.929**	
Mlabri		Amis	-0.0330	**-10.807**	-0.0262	**-6.003**	
		Atayal	-0.0314	**-9.256**	-0.0241	**-5.144**	
DevilsCave_N	Hàn	Amis	0.0090	**5.185**	0.0100	**4.477**	Compared with AAN, Hàn and Hmong have additional North_EA ancestry.
		Atayal	0.0109	**5.679**	0.0122	**4.576**	
	Hmong	Amis	0.0057	2.989	0.0050	1.910	
		Atayal	0.0077	**3.614**	0.0072	2.482	
	Htin	Amis	-0.0215	**-9.367**	-0.0222	**-7.311**	Compared with AAN, Htin and Mlabri have additional non-East-Asian (likely Australasian) ancestry.
		Atayal	-0.0198	**-7.982**	-0.0207	**-5.978**	
	Mlabri	Amis	-0.0271	**-9.073**	-0.0250	**-6.126**	
		Atayal	-0.0254	**-7.867**	-0.0235	**-5.454**	
Kostenki	DevilsCave_N	Hàn	0.0017	0.508	0.0089	1.769	Compared with other East Asians, DevilsCave_N does not have additional West Eurasian ancestry.
		Hmong	0.0011	0.301	0.0109	2.083	
		Amis	0.0011	0.278	0.0057	1.012	
		Atayal	0.0021	0.507	0.0066	1.117	
		Htin	0.0010	0.257	0.0027	0.481	
		Mlabri	0.0031	0.671	0.0031	0.472	

Extended Data Table 3 | Additional *D*-statistics.

Test Population	Feasible	Best Model	*p*-value	Amis		Devil's_Cave		Hòabìnhian		Afanasievo		Namazga		Kolyma	
				mean	str_err	mean	str_err	mean	str_err	mean	str_err	mean	str_err	mean	str_err
Hàn	Yes	Yes	8.912E-01	0.6927	0.028	0.3073	0.028	NA	NA	NA	NA	NA	NA	NA	NA
Huí	Yes	Yes	8.526E-01	0.4480	0.067	0.3925	0.060	NA	NA	0.1062	0.044	0.0533	0.046	NA	NA
Xiōngnú	Yes	Yes	5.427E-01	0.5014	0.070	0.4567	0.064	NA	NA	0.0418	0.013	NA	NA	NA	NA
Tǔjiā	Yes	Yes	9.913E-01	0.7063	0.034	0.2937	0.034	NA	NA	NA	NA	NA	NA	NA	NA
Nàxī	Yes	Yes	9.118E-01	0.7649	0.035	0.2351	0.035	NA	NA	NA	NA	NA	NA	NA	NA
Yí	Yes	Yes	9.425E-01	0.7326	0.034	0.2674	0.034	NA	NA	NA	NA	NA	NA	NA	NA
Tibetan	Yes	Yes	1.782E-01	NA	NA	0.6775	0.020	0.3225	0.020	NA	NA	NA	NA	NA	NA
	No	No	1.235E-04	1.0005	0.033	NA	NA	-0.0005	0.033	NA	NA	NA	NA	NA	NA
	No	No	1.635E-01	-0.1374	0.420	0.7712	0.286	0.3662	0.136	NA	NA	NA	NA	NA	NA
Lāhù	Yes	Yes	7.074E-01	0.6916	0.150	0.1930	0.102	0.1154	0.050	NA	NA	NA	NA	NA	NA
Korean	Yes	Yes	6.460E-01	0.4488	0.041	0.5512	0.041	NA	NA	NA	NA	NA	NA	NA	NA
Japanese	Yes	Yes	7.293E-01	0.5208	0.032	0.4792	0.032	NA	NA	NA	NA	NA	NA	NA	NA
Jōmon	Yes	Yes	6.185E-01	NA	NA	0.4191	0.027	0.5809	0.027	NA	NA	NA	NA	NA	NA
	Yes	No	5.582E-01	0.6076	0.039	NA	NA	0.3924	0.039	NA	NA	NA	NA	NA	NA
	No	No	6.108E-01	0.3535	0.334	0.1798	0.230	0.4667	0.109	NA	NA	NA	NA	NA	NA
Xībó	Yes	Yes	9.090E-01	0.3519	0.046	0.5988	0.043	NA	NA	0.0493	0.009	NA	NA	NA	NA
Hezhen	Yes	Yes	4.004E-01	0.1845	0.054	0.7954	0.049	NA	NA	0.0201	0.011	NA	NA	NA	NA
Oroqen	No	No	4.657E-01	-0.0044	0.063	0.9651	0.057	NA	NA	0.0393	0.013	NA	NA	NA	NA
	Yes	No	5.833E-02	0.0671	0.054	0.9329	0.054	NA	NA	NA	NA	NA	NA	NA	NA
	Yes	Yes	5.631E-01	NA	NA	0.9615	0.011	NA	NA	0.0385	0.011	NA	NA	NA	NA
Monguor	Yes	Yes	8.242E-01	0.5151	0.043	0.3927	0.038	NA	NA	NA	NA	0.0922	0.009	NA	NA
Mongol	Yes	Yes	9.025E-02	0.4047	0.049	0.5309	0.045	NA	NA	0.0645	0.010	NA	NA	NA	NA
Daur	Yes	Yes	3.295E-01	0.1741	0.053	0.7751	0.049	NA	NA	0.0508	0.011	NA	NA	NA	NA
Shamanka_EN	Yes	Yes	6.520E-01	NA	NA	0.6796	0.051	NA	NA	0.0176	0.011	NA	NA	0.3028	0.053
	No	No	5.227E-01	0.0190	0.094	0.6538	0.152	NA		0.0154	0.017	NA	NA	0.3117	0.081
Xiongnu_West	No	No	1.252E-01	0.0124	0.083	0.3733	0.076	NA	NA	0.6143	0.017	NA	NA	NA	NA
	Yes	Yes	1.734E-01	NA	NA	0.3839	0.015	NA	NA	0.6161	0.015	NA	NA	NA	NA
Hun_Tianshan	Yes	Yes	3.000E-01	NA	NA	0.2392	0.008	NA	NA	0.6536	0.044	0.1072	0.044	NA	NA
	No	No	3.320E-01	-0.0805	0.055	0.3107	0.050	NA	NA	0.6272	0.046	0.1426	0.048	NA	NA

Karakhanid	Yes	Yes	2.045E-01	NA	NA	0.4271	0.012	NA	NA	0.3848	0.052	0.1881	0.051	NA	NA
	No	No	1.336E-01	0.0071	0.087	0.4211	0.080	NA	NA	0.3888	0.060	0.1829	0.064	NA	NA
Tuva	Yes	Yes	3.123E-01	NA	NA	0.7920	0.010	NA	NA	0.2080	0.010	NA	NA	NA	NA
	No	No	3.623E-01	-0.0694	0.058	0.8538	0.053	NA	NA	0.2156	0.012	NA	NA	NA	NA
Kalmyk	Yes	Yes	4.417E-01	NA	NA	0.7510	0.010	NA	NA	0.2490	0.010	NA	NA	NA	NA
	No	No	3.545E-01	0.0051	0.051	0.7464	0.047	NA	NA	0.2485	0.011	NA	NA	NA	NA
Hmong	Yes	Yes	9.715E-01	0.7861	0.032	0.2139	0.032	NA	NA	NA	NA	NA	NA	NA	NA
She	Yes	Yes	7.857E-01	0.7397	0.035	0.2603	0.035	NA	NA	NA	NA	NA	NA	NA	NA
Kinh_all	Yes	Yes	9.547E-01	0.7117	0.109	0.1874	0.075	0.1010	0.036	NA	NA	NA	NA	NA	NA
Dai	Yes	Yes	6.580E-01	0.7296	0.164	0.1759	0.112	0.0945	0.054	NA	NA	NA	NA	NA	NA
Thai	No	No	5.396E-01	0.6492	0.169	0.0696	0.115	0.1999	0.063	NA	NA	0.0813	0.012	NA	NA
	Yes	Yes	6.916E-01	0.7504	0.017	NA	NA	0.1654	0.021	NA	NA	0.0843	0.010	NA	NA
Khmer	Yes	Yes	9.877E-01	0.5425	0.127	0.1192	0.087	0.3009	0.047	NA	NA	0.0374	0.011	NA	NA
Htin	No	No	8.426E-01	0.6729	0.150	0.0657	0.105	0.2614	0.048	NA	NA	NA	NA	NA	NA
	Yes	Yes	8.213E-01	0.7669	0.021	NA	NA	0.2331	0.021	NA	NA	NA	NA	NA	NA
Mlabri	Yes	Yes	4.001E-01	0.7252	0.029	NA	NA	0.2748	0.029	NA	NA	NA	NA	NA	NA
	No	No	3.397E-01	0.5970	0.293	0.0901	0.204	0.3130	0.093	NA	NA	NA	NA	NA	NA
Kharia	Yes	Yes	4.649E-01	0.1849	0.030	NA	0.041	0.6160	0.022	NA	NA	0.1991	NA	NA	NA
	No	No	3.924E-01	0.4584	0.338	-0.2007	0.244	0.5305	0.116	NA	NA	0.2118	0.027	NA	NA

Extended Data Table 4 | Original *qpAdm* results.