The Genomic Formation of South and Central Asia

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Abstract

The genetic formation of Central and South Asian populations has been unclear because of an absence of ancient DNA. To address this gap, we generated genome-wide data from 362 ancient individuals, including the first from eastern Iran, Turan (Uzbekistan, Turkmenistan, and Tajikistan), Bronze Age Kazakhstan, and South Asia. Our data reveal a complex set of genetic sources that ultimately combined to form the ancestry of South Asians today. We document a southward spread of genetic ancestry from the Eurasian Steppe, correlating with the archaeologically known expansion of pastoralist sites from the Steppe to Turan in the Middle Bronze Age (2300-1500 BCE). These Steppe communities mixed genetically with peoples of the Bactria Margiana Archaeological Complex (BMAC) whom they encountered in Turan (primarily descendants of earlier agriculturalists of Iran), but there is no evidence that the main BMAC population contributed genetically to later South Asians. Instead, Steppe communities integrated farther south throughout the 2nd millennium BCE, and we show that they mixed with a more southern population that we document at multiple sites as outlier individuals exhibiting a distinctive mixture of ancestry related to Iranian agriculturalists and South Asian hunter-gathers. We call this group *Indus Periphery* because they were found at sites in cultural contact with the Indus Valley Civilization (IVC) and along its northern fringe, and also because they were genetically similar to post-IVC groups in the Swat Valley of Pakistan. By co-analyzing ancient DNA and genomic data from diverse present-day South Asians, we show that *Indus Periphery*-related people are the single most important source of ancestry in South Asia—consistent with the idea that the *Indus Periphery* individuals are providing us with the first direct look at the ancestry of peoples of the IVC—and we develop a model for the formation of present-day South Asians in terms of the temporally and geographically proximate sources of *Indus Periphery*-related, Steppe, and local South Asian hunter-gatherer-related ancestry. Our results show how ancestry from the Steppe genetically linked Europe and South Asia in the Bronze Age, and identifies the populations that almost certainly were responsible for spreading Indo-European languages across much of Eurasia.

One Sentence Summary: Genome wide ancient DNA from 357 individuals from Central and South Asia sheds new light on the spread of Indo-European languages and parallels between the genetic history of two sub-continents, Europe and South Asia.

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149   **Main text**

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151   **Ancient DNA Data and Analysis Strategy**

152

153   We generated whole-genome ancient DNA data from 362 previously unreported ancient

154   individuals and higher quality data from 17 previously reported individuals. Almost all derive

155   from three broad regions: 132 from Iran and the southern part of Central Asia (present-day

156   Turkmenistan, Uzbekistan, and Tajikistan, which we call Turan; "Iran/Turan"), 165 from the

157   western and central Steppe and northern forest zone encompassing present day Kazakhstan and

158   Russia ("Forest Zone/Steppe"), and 65 from northern Pakistan ("South Asia"). Our dataset

159   includes the first published ancient DNA data from 1) Chalcolithic and Bronze Age eastern Iran

160   and Turan (5600-1200 BCE from 12 sites); 2) early ceramic-using hunter-gatherers from the

161   western Siberian forest zone (6200-4000 BCE from 2 sites); 3) Chalcolithic and Bronze Age

162   pastoralists from the Steppe east of the Ural mountains, including the first ancient data from

163   Bronze Age Kazakhstan (4700-1000 BCE from 20 sites); and 4) the first ever ancient DNA from

164   South Asia from Iron Age and historical settlements in the Swat Valley of Pakistan (1200 BCE –

165   1 CE from 7 sites) (**Fig. 1, Supplementary Materials, Data S1**). To generate these data, we

166   prepared samples in dedicated clean rooms, extracted DNA (*1, 2*), constructed libraries for

167   Illumina sequencing (*3, 4*), and screened them using previously described procedures (*5-7*). We

168   enriched the libraries for DNA overlapping around 1.24 million single nucleotide polymorphisms

169   (SNPs), sequenced the products on Illumina instruments, and performed quality control (**Data

170   S1**) (*5, 6, 8*). We also report 186 new direct radiocarbon dates on human bone (**Data S2**). After

171   grouping individuals based on archaeological and chronological information and merging with

172   previously reported data, our dataset included 612 ancient individuals that we then co-analyzed

173   with genome-wide data from present-day individuals genotyped at around 600,000 SNPs, 1,789

174   of which were from 246 ethnographically-distinct groups in South Asia (**Data S3;

175   Supplementary Materials**) (*9-11*). We restricted analyses to ancient samples covered by at least

176   15,000 SNPs. We use *Italic* font to refer to genetic groupings and normal font to indicate

177   archaeological cultures or sites.

178

179   We carried out principal component analysis (PCA) by projecting the ancient individuals onto

180   the patterns of genetic variation in present-day Eurasians (**Fig. 1**) (*12, 13*). This revealed three

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181 major groupings, closely corresponding to the geographic regions of the Forest Zone/Steppe,

182 Iran/Turan and South Asia, a pattern we replicate in ADMIXTURE clustering (*14*). To test

183 formally whether populations differ significantly in their ancestry within regions, we used

184 symmetry-f_4-statistics measuring whether pairs of populations differ in their degree of allele

185 sharing to a third population, and admixture-f_3-statistics to test formally for mixture

186 (**Supplementary Materials**). We tested the fit of mixture models using *qpAdm*, which evaluates

187 whether all possible f_4-statistics relating a set of tested populations to outgroup populations is

188 consistent with mixtures of a pre-specified number of sources and if so estimates proportions of

189 ancestry (*5*). We can model almost every population as a mixture of seven deeply divergent

190 "distal" ancestry sources (usually closely related to populations for which we have data, but in

191 some cases deeply related):

192

193 • "Anatolian agriculturalist-related": represented by 7th millennium BCE western Anatolian

194 agriculturalists (*6*)

195 • "Western European Hunter-Gatherer (*WHG*)-related": represented by Mesolithic western

196 Europeans (*5, 10, 15, 16*)

197 • "Iranian agriculturalist-related": represented by 8th millennium BCE pastoralists from the

198 Zagros Mountains of Iran (*17, 18*)

199 • "Eastern European Hunter-Gatherer (*EHG*)-related": represented by hunter-gatherers from

200 diverse sites in Eastern Europe (*5, 6*)

201 • "West Siberian Hunter-Gatherer (*West_Siberian_HG*)-related": a newly documented deep

202 source of Eurasian ancestry represented here by three samples

203 • "East Asian-related": represented in this study by Han Chinese

204 • "Ancient Ancestral South Indian (*AASI*)-related": a hypothesized South Asian Hunter-Gatherer

205 lineage related deeply to present-day indigenous Andaman Islanders (*19*)

206

207 We also used *qpAdm* to identify "proximal" models for each group as mixtures of temporally

208 preceding groups. This often identified multiple alternative models that were equally good fits to

209 the data. These analyses were nevertheless useful because we could identify patterns that were

210 qualitatively consistent across models. The discussion that follows presents an overview of these

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211   analyses, while the **Supplementary Materials** presents the full details. **Table 1** summarizes the

212   key findings that emerge from our analysis.

213

214   **Iran/Turan**

215   We analyzed our newly generated data together with previously published data to examine the

216   genetic transformations that accompanied the spread of agriculture eastward from Iran beginning

217   in the 7th millennium BCE (*20, 21*). Our analysis confirms that early Iranian agriculturalists from

218   the Zagros Mountains harbor a distinctive type of West Eurasian ancestry (*17, 18*) (**Fig. 1**), while

219   later groups across a broad geographic region were admixed between this type of ancestry and

220   that related to early Anatolian agriculturalists. (In this paper we use the term "agriculturalists" to

221   refer both to crop cultivation and/or herding, and accordingly refer to the people of the Zagros

222   Mountains who kept domesticated goats as agriculturalists (*17, 22, 23*).) We show that there was

223   a west-to-east cline of decreasing Anatolian agriculturalist-related admixture ranging from ~70%

224   in Chalcolithic Anatolia to ~33% in eastern Iran, to ~3% in far eastern Turan (**Fig. 1**;

225   **Supplementary Materials**). The timing of the establishment of this cline is consistent with the

226   dates of spread of wheat and barley agriculture from west to east (in the 7th to 6th millennia

227   BCE), suggesting the possibility that individuals of Anatolian ancestry may have contributed to

228   spreading agriculturalist economies not only westward to Europe, but also eastward to Iran (*21,*

229   *24, 25*). An increase of Anatolian agriculturalist-related ancestry was also proposed for the Pre-

230   Pottery agriculturalists from the Levant in comparison to the earlier Natufian hunter-gatherers

231   (*17*), further supporting this hypothesis. However, without data on the distribution of

232   Anatolian/Iranian-agriculturalist ancestry in early agriculturalists in Mesopotamia, it is difficult

233   to determine when the cline was established. In the far eastern part of this cline (eastern Iran and

234   Turan) we also detect admixture related to *West_Siberian_HG*, proving that North Eurasian

235   admixture impacted Turan well before the spread of Yamnaya-related Steppe pastoralists

236   (*Steppe_EMBA*).

237

238   From Bronze Age Turan, we report 69 ancient individuals (2300-1400 BCE) from four urban

239   sites of the Bactria Margiana Archaeological Complex (BMAC) and its immediate successors.

240   The great majority of individuals fall in a genetic cluster that is similar, albeit not identical, to the

241   preceding groups in Turan in harboring a large proportion of early Iranian agriculturalist-related

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242   ancestry (~60% in the *BMAC*) with smaller components of Anatolian agriculturalist-related

243   ancestry (~21%) and *West_Siberian_HG*-related ancestry (~13%) suggesting that the main

244   *BMAC* cluster coalesced from preceding pre-urban populations in Turan (which in turn likely

245   derived from earlier eastward spreads from Iran). The absence in the *BMAC* cluster of the

246   *Steppe_EMBA* ancestry that is ubiquitous in South Asia today—along with *qpAdm* analyses that

247   rule out *BMAC* as a substantial source of ancestry in South Asia (**Fig. 3A**)—suggests that while

248   the BMAC was affected by the same demographic forces that later impacted South Asia (the

249   southward movement of Middle to Late Bronze Age Steppe pastoralists described in the next

250   section), it was also bypassed by members of these groups who hardly mixed with *BMAC* people

251   and instead mixed with peoples further south. In fact, the data suggest that instead of the main

252   *BMAC* population having a demographic impact on South Asia, there was a larger effect of gene

253   flow in the reverse direction, as the main *BMAC* genetic cluster is slightly different from the

254   preceding Turan populations in harboring ~5% of their ancestry from the *AASI*.

255

256   We also observe outlier individuals at multiple sites, revealing interactions among populations

257   that would be difficult to appreciate without the large sample sizes reported here.

258

259   First, around ~2300 BCE in Turan, we observe two outliers at the BMAC site of Gonur with

260   *West_Siberian_HG*-related ancestry of a type that we observe at multiple sites in Kazakhstan

261   over the preceding and succeeding millennia. The most plausible explanation is that this ancestry

262   is that of indigenous populations associated with the Kelteminar culture, the native hunter-

263   gatherers of the region who covered a vast area of Central Asia before the BMAC (*26*). Future

264   ancient DNA data from Kelteminar contexts will make it possible to determine whether it is

265   indeed the case that the genetic ancestry of Kelteminar people was similar to that of

266   *West_Siberia_HG*. Importantly, in the 3rd millenium BCE we do not find any individuals with

267   ancestry derived from Yamnaya-related Steppe pastoralists in Turan. Thus, *Steppe_EMBA*

268   ancestry was not yet widespread across the region.

269

270   Second, between 2100-1700 BCE, we observe BMAC outliers from three sites with

271   *Steppe_EMBA* ancestry in the admixed form typically carried by the later Middle to Late Bronze

272   Age Steppe groups (*Steppe_MLBA*). This documents a southward movement of Steppe ancestry

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273 through this region that only began to have a major impact around the turn of the 2nd millennium

274 BCE.

275

276 Third, between 3100-2200 BCE we observe an outlier at the BMAC site of Gonur, as well as two

277 outliers from the eastern Iranian site of Shahr-i-Sokhta, all with an ancestry profile similar to 41

278 ancient individuals from northern Pakistan who lived approximately a millennium later in the

279 isolated Swat region of the northern Indus Valley (1200-800 BCE). These individuals had

280 between 14-42% of their ancestry related to the AASI and the rest related to early Iranian

281 agriculturalists and *West_Siberian_HG*. Like contemporary and earlier samples from Iran/Turan

282 we find no evidence of Steppe-pastoralist-related ancestry in these samples. In contrast to all

283 other Iran/Turan samples, we find that these individuals also had negligible Anatolian

284 agriculturalist-related admixture, suggesting that they might be migrants from a population

285 further east along the cline of decreasing Anatolian agriculturalist ancestry. While we do not

286 have access to any DNA directly sampled from the Indus Valley Civilization (IVC), based on (a)

287 archaeological evidence of material culture exchange between the IVC and both BMAC to its

288 north and Shahr-i-Sokhta to its east (*27*), (b) the similarity of these outlier individuals to post-

289 IVC Swat Valley individuals described in the next section (*27*), (c) the presence of substantial

290 *AASI* admixture in these samples suggesting that they are migrants from South Asia, and (d) the

291 fact that these individuals fit as ancestral populations for present-day Indian groups in *qpAdm*

292 modeling, we hypothesize that these outliers were recent migrants from the IVC. Without ancient

293 DNA from individuals buried in IVC cultural contexts, we cannot rule out the possibility that the

294 group represented by these outlier individuals, which we call *Indus_Periphery*, was limited to the

295 northern fringe and not representative of the ancestry of the entire Indus Valley Civilization

296 population. In fact, it was certainly the case that the peoples of the Indus Valley were genetically

297 heterogeneous as we observe one of the *Indus_Periphery* individuals having ~42% *AASI*

298 ancestry and the other two individuals having ~14-18% *AASI* ancestry (but always mixes of the

299 same two proximal sources of *AASI* and Iranian agriculturalist-related ancestry). Nevertheless,

300 these results show that *Indus_Periphery* were part of an important ancestry cline in the wider

301 Indus region in the 3rd millennium and early 2nd millennium BCE. As we show in what follows,

302 peoples related to this group had a pivotal role in the formation of subsequent populations in

303 South Asia.

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304

305   Using a newly developed approach for estimating dates of admixture in ancient genomes (an

306   adaptation of a previous method to measure ancestry covariance among pairs of neighboring

307   positions in the genome; **Supplementary Materials**), we estimate that the time of admixture

308   between Iranian agriculturalist-related ancestry and AASI ancestry in the three *Indus_Periphery*

309   samples was 53 ± 15 generations ago on average, corresponding to a 95% confidence interval of

310   about 4700-3000 BCE assuming 28 years per generation (*28*). This places a minimum date on the

311   first contact between these two types of ancestries.

312

313   **The Steppe**

314   Three individuals from the West Siberian forest zone with direct dates ranging from 6200 BCE

315   to 4000 BCE play an important role in this study as they are representatives of a never-before-

316   reported mixture of ancestry that we call *West_Siberian_HG*: ~30% derived from *EHG*, ~50%

317   from Ancestral North Eurasians (defined as being related deeply to 22000-15000 BCE Siberians

318   (*29, 30*)), and ~20% related to present-day East Asians. This ancestry type also existed in the

319   southern Steppe and in Turan, as it formed about 80% of the ancestry of an early 3rd millennium

320   BCE agro-pastoralist from Dali, Kazakhstan, and also contributed to multiple outlier individuals

321   from 2nd millennium sites in Kazakhstan and Turan (**Fig. 2**).

322

323   Using the *West_Siberian_HG* individuals as a reference population along with other pre-

324   Chalcolithic groups that have been previously reported in the ancient DNA literature, we

325   document the presence of a genetically relatively homogeneous population spread across a vast

326   region of the eastern European and trans-Ural Steppe between 2000-1400 BCE (*Steppe_MLBA*)

327   (*17*). Many of the samples from this group are individuals buried in association with artifacts of

328   the Corded Ware, Srubnaya, Petrovka, Sintashta and Andronovo complexes, all of which

329   harbored a mixture of *Steppe_EMBA* ancestry and ancestry from European Middle Neolithic

330   agriculturalists (*Europe_MN*). This is consistent with previous findings showing that following

331   westward movement of eastern European populations and mixture with local European

332   agriculturalists, there was an eastward reflux back beyond the Urals (*6, 16, 31*). Our new dataset

333   enhances our understanding of the *Steppe_MLBA* cluster by including many sites in present-day

334   Kazakhstan and as far east as the Minusinsk Basin of Russia—and in doing so allows us to

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335 appreciate previously undetected substructure. All previously reported samples fall into a

336 subcluster we call *Steppe_MLBA_West* that harbors ~26% *Europe_MN* ancestry and ~74%

337 *Steppe_EMBA* ancestry. With our newly reported data we now also detect a previously

338 unappreciated subcluster, *Steppe_MLBA_East,* which is significantly differentiated (p=7×10^{-6}

339 from *qpAdm*), with ~8% *West_Siberian_HG*-related ancestry and proportionally less of the other

340 ancestry components, suggesting that people carrying *Steppe_MLBA_West* ancestry admixed

341 with *West_Siberian_HG*-related peoples as they spread further east.

342

343 As in Iran/Turan, the outlier individuals provide key additional information.

344

345 First, our analysis of 50 newly reported individuals from the Kamennyi Ambar V cemetery from

346 the Sintashta culture reveals three groups of outliers, in addition to the main cluster of 40

347 individuals. These outliers have elevated proportions of *Steppe_EMBA*, *West_Siberian_HG* or

348 *East Asian*-related ancestry (and direct dates that are contemporaneous with the other

349 individuals), thereby showing that this fortified site harbored people of diverse ancestries living

350 side-by-side.

351

352 Second, samples from three sites from the southern and eastern end of the Steppe dated to 1600-

353 1500 BCE (Dashti-kozy, Taldysay and Kyzlbulak) show evidence of significant admixture from

354 Iranian agriculturalist-related populations, demonstrating northward gene flow from Turan into

355 the Steppe at the same time as there was southward movement of *Steppe_MLBA* ancestry

356 through Turan and into South Asia. These findings are consistent with evidence of a high degree

357 of human mobility both to the north and south along the Inner Asian Mountain Corridor (*32, 33*).

358

359 Third, we observe samples from multiple sites dated to 1700-1500 BCE (Maitan, Kairan,

360 Oy_Dzhaylau and Zevakinsikiy) that derive up to ~25% of their ancestry from a source related to

361 present-day East Asians and the remainder from *Steppe_MLBA*. A similar ancestry profile

362 became widespread in the region by the Late Bronze Age, as documented by our time transect

363 from Zevakinsikiy and samples from many sites dating to 1500-1000 BCE, and was ubiquitous

364 by the Scytho-Sarmatian period in the Iron Age (*34*). This observation decreases the probability

365 that populations in the 1st millennium BCE and 1st millennium CE—including Scythians,

11

366   Kushans, and Huns, sometimes suggested as sources for the Steppe ancestry influences in South

367   Asia today (*17*)—contributed to the majority of South Asians, which have negligible East Asian

368   ancestry in our analysis. It is possible that there were unsampled groups in Central Asia with

369   negligible East Asian admixture that could have migrated later to South Asia. However, at least

370   some (possibly all) of the Steppe pastoralist ancestry in South Asia owes its origins to southward

371   pulses in the 2nd millennium BCE, as indeed we prove directly through our observation of this

372   ancestry in the Swat Iron Age individuals dating to ~1000 BCE (discussed further below).

373

374   **South Asia**

375   Previous work has shown that the Indian Cline—a gradient of different proportions of West

376   Eurasian related ancestry in South Asia—can be well modeled as having arisen from a mixture

377   of two statistically reconstructed ancestral populations (the *ANI* and the *ASI*), which mixed

378   mostly after 2000 BCE (*35, 36*). Ancient DNA analysis has furthermore revealed that the

379   populations along the Indian Cline actually descend more deeply in time from at least three

380   ancestral populations (*17*), with ancestry from groups related to early Iranian agriculturalists,

381   *Steppe_EMBA*, and *Onge*.

382

383   To shed light on the mixture events that transformed this minimum of three ancestral populations

384   into two (the *ANI* and *ASI*), we used *qpAdm* to search for triples of source populations—the

385   *AASI*, all sampled ancient Iran/Turan-related groups, and all sampled ancient Steppe groups—

386   that could fit as sources for South Asians. As South Asian test populations we used an Indian

387   Cline group with high ANI ancestry (*Punjabi.DG*), one with high ASI ancestry (*Mala.DG*), early

388   Iron Age Swat Valley samples *(Swat Protohistoric Grave Type - SPGT),* and Early Historic Swat

389   Valley samples *(Butkara_IA)*. **Fig. 3A** shows that the only models that fit all four test South

390   Asians groups are combinations that involve the *AASI*, *Indus_Periphery* and *Steppe_MLBA* (in

391   the analyses that follow, we therefore pooled the *Steppe_MLBA*). The evidence that the

392   *Steppe_MLBA* cluster is a plausible source for the Steppe ancestry in South Asia is also

393   supported by Y chromosome evidence, as haplogroup R1a which is of the Z93 subtype common

394   in South Asia today (*37, 38*) was of high frequency in *Steppe_MLBA* (68%) (*16*), but rare in

395   *Steppe_EMBA* (absent in our data).

396

12

397   To obtain a richer understanding of the ancestry of the entire Indian Cline, we took advantage of

398   previously published genome-wide data from 246 ethnographically diverse groups from South

399   Asia (*11*), from which we sub-selected 140 groups that fall on a clear gradient in PCA to

400   represent the Indian Cline (the other groups either fall off the cline due to additional African or

401   East Asian-related ancestry or had small sample size or heterogeneous ancestry). The per-group

402   *qpAdm* estimates for the proportions of ancestry from these three sources are statistically noisy.

403   We therefore developed new methodology that allows us to jointly fit the data from all Indian

404   Cline groups within a hierarchical model. The analysis confirms that the great majority of all

405   groups on the Indian Cline can be jointly modeled as a mixture of two populations, and the

406   analysis also produces an estimate of the functional relationship between the ancestry

407   components. Setting *Steppe_MLBA* to its smallest possible proportion of zero to estimate the

408   minimum fraction of *Indus_Periphery* ancestry that could have existed in the *ASI,* we obtain

409   ~39%. Setting *AASI* to its smallest possible proportion of zero to estimate the maximal fraction

410   of *Indus_Periphery* ancestry that could have existed in the *ANI,* we obtain ~72%. In fact, we find

411   four tribal groups from southern India (*Palliyar, Ulladan, Malayan,* and *Adiyan*) with close to

412   the maximum mathematically allowed proportion of *Indus_Periphery*-related ancestry, and we

413   find a population in northern Pakistan (*Kalash*) with close to the minimum. Thus, nearly

414   unmixed descendants of the *ASI* and *ANI* exist as isolated groups in South Asia today.

415

416   We built an admixture graph using *qpGraph* co-modeling *Palliyar* (as a representative of the

417   *ASI*) and *Juang* (an Austroasiatic speaking group in India with low West Eurasian-relatedness),

418   and show that it fits when the ASI have ~27% Iranian agriculturalist-related ancestry and the

419   *Juang* also harbor ancestry from an AASI population without Iranian admixture (**Fig. 3**). This

420   model is also notable in showing that early Iranian agriculturalists fit without *AASI* admixture,

421   and thus the patterns we observe are driven by gene flow into South Asia and not the reverse

422   (**Fig. 3**; **Supplementary Materials**). The fitted admixture graph also reveals that the deep

423   ancestry of the indigenous hunter-gather population of India represents an anciently divergent

424   branch of Asian human variation that split off around the same time that East Asian, *Onge* and

425   *Australian* aboriginal ancestors separated from each other. This finding is consistent with a

426   model in which essentially all the ancestry of present-day eastern and southern Asians (prior to

13

427 West Eurasian-related admixture) derives from a single eastward spread, which gave rise in a

428 short span of time to the lineages leading to *AASI*, East Asians, *Onge*, and *Australians* (*19*).

429

430 Using admixture linkage disequilibrium, we estimate a date of 107 ± 11 generations ago for the

431 Iranian agriculturalist and AASI-related admixture in the *Palliyar*, corresponding to a 95%

432 confidence interval of 1700-400 BCE assuming 28 years per generation (*28*). This date is

433 consistent with a previous estimate of 110 ± 12 generations ago for the *Kalash* (*39*). These

434 results suggest that the *ASI* and *ANI* were both largely unformed at the beginning of the 2nd

435 millennium BCE, and imply that the *ASI* may have formed in the course of the spread of West

436 Asian domesticates into peninsular India beginning around 3000 BCE (where they were

437 combined with local domesticates to form the basis of the early agriculturalist economy of South

438 India (*40*)), or alternatively in association with eastward spread of material culture from the

439 Indus Valley after the IVC declined (*41*). Further evidence for a Bronze Age formation of the

440 *ASI* comes from our analysis of Austroasiatic-speaking groups in India such as *Juang,* who have

441 a higher ratio of *AASI*-to-Iranian agriculturalist-related ancestry than the *ASI* (**Fig. 3**,

442 **Supplementary Materials**). Austroasiatic speakers likely descend from populations that arrived

443 in South Asia in the 3rd millennium BCE (based on hill cultivation systems associated with the

444 spread of Austroasiatic languages (*20*)), and our genetic results show that when Austroasiatic

445 speakers arrived they mixed with groups with elevated ratios of *AASI*- to Iranian-agriculturalist-

446 related ancestry than are found in the *ASI,* showing that the *ASI* had not yet overspread

447 peninsular India.

448

449 Finally, we examined our Swat Valley time transect from 1200 BCE to 1 CE. While the earliest

450 group of samples (*SPGT*) is genetically very similar to the *Indus_Periphery* samples from the

451 sites of *Gonur* and *Shahr-i-Sokhta,* they also differ significantly in harboring *Steppe_MLBA*

452 ancestry (~22%). This provides direct evidence for *Steppe_MLBA* ancestry being integrated into

453 South Asian groups in the 2nd millennium BCE, and is also consistent with the evidence of

454 southward expansions of *Steppe_MLBA* groups through Turan at this time via outliers from the

455 main *BMAC* cluster from 2000-1500 BCE. Later samples from the Swat time transect from the

456 1st millennium BCE had higher proportions of Steppe and AASI derived ancestry more similar to

14

457   that found on the Indian Cline, showing that there was an increasing percolation of Steppe

458   derived ancestry into the region and additional admixture with the ASI through time.

459

460   **Implications for Archaeology and Linguistics**

461   Our evidence that a population with both Iranian agriculturalist and South Asian hunter gatherer

462   ancestry (*Indus_Periphery*) was established in the 3rd millennium BCE—and that its Iranian

463   agriculturalist-related and *AASI* ancestry sources mixed at an average time of around 4700-3000

464   BCE—shows that this type of Iranian agriculturalist-related ancestry must have reached the

465   Indus Valley by the 4th millennium BCE. However, it is very possible that Iranian agriculturalist-

466   related ancestry was widespread in South Asia even earlier, as wheat and barley agriculture as

467   well as goat and sheep herding spread into South Asia after the 7th millennium BCE, as attested

468   at sites such as Mehrgarh in the hills surrounding the Indus Valley (*20, 21*), and these

469   domesticates could have been carried by movements of people. Regardless of when these

470   agricultural species arrived, the genetic data show that *Indus_Periphery*-related ancestry

471   contributed in large proportions to both the *ANI* and *ASI*, and that these two groups both formed

472   in the 2nd millennium BCE, overlapping the decline of the IVC and major changes in settlement

473   patterns in the northern part of the Indian subcontinent (*41*). A parsimonious hypothesis is that as

474   *Steppe_MLBA* groups moved south and mixed with *Indus_Periphery*-related groups at the end of

475   the IVC to form the *ANI,* other *Indus_Periphery*-related groups moved further south and east to

476   mix with *AASI* groups in peninsular India to form the *ASI*. This is consistent with suggestions

477   that the spread of the IVC was responsible for dispersing Dravidian languages (*42-44*), although

478   scenarios in which Dravidian languages derive from pre-Indus languages of peninsular India are

479   also entirely plausible as *ASI* ancestry is mostly derived from the *AASI*.

480

481   Our results also shed light on the question of the origins of the subset of Indo-European

482   languages spoken in India and Europe (*45*). It is striking that the great majority of Indo-European

483   speakers today living in both Europe and South Asia harbor large fractions of ancestry related to

484   Yamnaya Steppe pastoralists (corresponding genetically to the *Steppe_EMBA* cluster)*,*

485   suggesting that "Late Proto-Indo-European"—the language ancestral to all modern Indo-

486   European languages—was the language of the Yamnaya (*46*). While ancient DNA studies have

487   documented westward movements of peoples from the Steppe that plausibly spread this ancestry

15

488   to Europe (*5, 31*), there has not been ancient DNA evidence of the chain of transmission to South

489   Asia. Our documentation of a large-scale genetic pressure from *Steppe_MLBA* groups in the 2nd

490   millennium BCE provides a prime candidate, a finding that is consistent with archaeological

491   evidence of connections between material culture in the Kazakh middle-to-late Bronze Age

492   Steppe and early Vedic culture in India (*46*).

493

494   Our analysis also provides an entirely new line of evidence for a linkage between Steppe

495   ancestry and Indo-European culture. When we used *qpAdm* to test if a mixture of ANI and ASI is

496   a fit to the data for all 140 Indian Cline groups, we found 10 groups with poor fits and a

497   significantly elevated ratio of *Steppe_MLBA*- to *Indus_Periphery*-related ancestry compared to

498   the expectation for the model (Z≥3). We found the strongest two signals in *Brahmin_Tiwari*

499   (p=2×10^{-5}) and *Brahmin_UP* (p=4×10^{-5}), and more generally there was a striking enrichment of

500   a Z≥3 signals in groups of traditionally priestly status in northern India (57% of groups with Z≥3

501   were *Brahmins* or *Bhumihars* even though these groups comprised only 11% of the 74 groups we

502   analyzed in northern India). Although the enrichment for Steppe ancestry is not found in the

503   southern Indian groups, the Steppe enrichment in the northern groups is striking as *Brahmins* and

504   *Bhumihars* are among the traditional custodians of texts written in early Sanskrit. A possible

505   explanation is that the influx of *Steppe_MLBA* ancestry into South Asia in the mid-2nd

506   millennium BCE created a meta-population of groups with different proportions of Steppe

507   ancestry, with ones having relatively more Steppe ancestry having a central role in spreading

508   early Vedic culture. Due to strong endogamy in South Asia—which has kept some groups

509   isolated from their neighbors for thousands of years (*35*)—some of this substructure within

510   Indian population still persists.

511

512   We finally highlight a remarkable parallel between the prehistory of two sub-continents of

513   Eurasia: South Asia and Europe. In both regions, West Asian agricultural technology spread

514   from an origin in the Near East in the 7th and 6th millennia BCE (**Fig. 4**). In South Asia this

515   occurred via the Iranian plateau, and in Europe via western Anatolia, with the technological

516   spreads mediated in both cases by movements of people. An admixed population was then

517   formed by the mixing of incoming agriculturalists and resident hunter-gatherers—in South Asia

518   eventually giving rise to the *Indus_Periphery* and *ASI* and in Europe the Middle Neolithic

16

519   genetic cluster *Europe_MN*. In both Europe and South Asia, populations related to the *Yamnaya*

520   Steppe pastoralists arrived after this agriculturalist and hunter-gatherer admixture took place,

521   interacting with local populations to produce mixed groups, which then mixed further with

522   already resident agriculturalist populations to produce genetic groupings such as those found

523   associated with Corded Ware and central European Bell Beaker artifacts in much of Europe, and

524   the *ANI* genetic cluster in South Asia. These mixed groups then mixed further to produce the

525   major gradients of ancestry in both regions. Future studies of populations from South Asia and

526   the linguistically related Iranian world will extend and add nuance to the model presented here.

527   **Figure Legends**

528

529   **Fig. 1 Overview of ancient DNA data.** (**A**) Number of newly reported samples passing our

530   analysis thresholds and their date range is shown by site. (**B**) Locations, color-coded by analysis

531   grouping. (**C**) Projections of ancient samples onto PCA axes computed using present-day

532   Eurasians. (**D**) ADMIXTURE analysis, with components maximized in *West_Siberian_HG*,

533   Anatolian agriculturalists, Iranian agriculturalists, indigenous South Asians and *WHG* in blue,

534   orange, teal, red and green, respectively. (**E**) Y-chromosome haplogroups. N, Neolithic; C,

535   Chalcolithic; BA, Bronze Age; IA, Iron Age; H, Historic; E/M/L, Early/Middle/Late; o, outlier.

536

537   **Fig. 2 Modeling results.** (**A**) Admixture events originating from 7 "Distal" populations leading

538   to the formation of the modern Indian cloud shown geographically. Clines or 2-way mixtures of

539   ancestry are shown in rectangles, and clouds (3-way mixtures) are shown in ellipses. (**B**) A

540   schematic model of events originating from 7 "Distal" populations leading to the formation of

541   the modern Indian cline, shown chronologically. (**C**) Admixture proportions as estimated

542   using *qpAdm* for populations reflected in A and B.

543

544   **Fig. 3 The Genomic Origins of Indians.** (**A**) We used *qpAdm* to model four groups that are

545   representative of major sources of South Asian ancestry over the last few thousand years

546   (*Punjabi.DG*, *Mala.DG*, *SPGT*, and *Butkara_IA*) as mixtures of *Onge,* an Iran/Turan-related

547   population, and a Steppe-related group, and report the minimum p-value (highlighting cases at

548   p>0.01). The only working models involve a combination of *Indus_Periphery* and a

549   *Steppe_MLBA* group (note that the *Steppe_MLBA_West* group includes a subset

550   *Sintashta_MLBA* and *Srubnaya*). (**B**) For all 140 Indian Cline groups, we give *Maximum A*

551   *Posteriori* fits for this model. Significant outliers ($|Z|>2$) are shown, and include a cluster of

552   *Brahmins* (filled circles) and *Bhumihars* (filled squares) with excess Steppe pastoralist-related

553   ancestry compared to others with similar West Eurasian ancestry proportion. (**C**) Admixture

554   graph fit supports Iranian agriculturalist-related admixture into South Asia but no gives evidence

555   of AASI-related admixture into ancient Iran; dotted lines show admixture events.

556

18

557   **Fig. 4 A Tale of Two Subcontinents.** The prehistory of South Asia and Europe are parallel in

558   both being impacted by two successive spreads, the first from the Near East after 7000 BCE

559   bringing agriculturalists who mixed with local hunter-gatherers, and the second from the Steppe

560   after 3000 BCE bringing people who spoke Indo-European languages and who mixed with those

561   they encountered during their migratory movement. Mixtures of these mixed populations then

562   produced the rough clines of ancestry present in both South Asia and in Europe today (albeit

563   with more variable proportions of local hunter-gatherer-related ancestry in Europe than in India),

564   which are (imperfectly) correlated to geography. The plot shows in contour lines the time of the

565   expansion of Near Eastern agriculture. Human movements and mixtures, which also plausibly

566   contributed to the spread of languages, are shown with arrows.

19

567 **Table 1 Summary of Key Findings**

Iran/Turan

1. There was a west-to-east gradient of ancestry across Eurasia in the Chalcolithic and Bronze Ages, with more Anatolian agriculturalist-related ancestry in the west and more *West_Siberian_HG* or *AASI*-related ancestry in the east, all superimposed on primary ancestry related to early Zagros agriculturalists. The establishment of the Anatolian ancestry gradient corresponds to the spread of crop-based agriculture across this region, raising the possibility that people of Anatolian ancestry spread this technology east just as they helped spread it west into Europe. However, Anatolian agriculturalist-related ancestry is absent in the *Indus_Periphery* samples, showing that if such people were instrumental in bringing crop farming eastward to Iran, diffusion of ideas brought it further east to South Asia.

2. The primary population of the BMAC was largely derived from preceding local Chalcolithic peoples and had little if any Steppe pastoralist ancestry of the type that is ubiquitous in South Asia today. Instead of being a source for South Asia, the *BMAC* received admixture from South Asia.

3. Outlier analysis shows no evidence of Steppe pastoralist ancestry in groups surrounding BMAC sites prior to 2100 BCE, but suggests that between 2100-1700 BCE, the BMAC communities were surrounded by peoples carrying such ancestry.

4. We document a distinctive ancestry type—58%-86% Iranian agriculturalist-related ancestry with little Anatolian agriculturalist-related admixture, and 14%-42% *AASI* ancestry—that was present at two sites known to be in close cultural contact with the Indus Valley Culture (IVC). Combined with similar ancestry about a millennium later in the post-IVC Swat Valley, this documents an *Indus_Periphery* population during the flourishing of the IVC, which we show formed by admixture 4700-3000 BCE.

The Steppe

1. In the Kazakh Steppe and Minusinsk Basin during the Middle to Late Bronze Age, ancestry typical of pastoralists in the western Steppe (*Steppe_MLBA_West*) admixed with ancestry related to earlier *West_Siberian_HG*-related groups to form a distinctive *Steppe_MLBA_East* cluster.

2. Outlier analysis shows that by 1600 BCE in the Middle to Late Bronze Age of the Kazakh Steppe, there were numerous individuals with admixture from Turan, providing genetic evidence of northward movement into the Steppe in this period.

3. By 1500 BCE, there were numerous individuals in the Kazakh Steppe with East Asian-related admixture, the same type of ancestry that was widespread by the Scythian period (*34*). This ancestry is hardly present in the two primary ancestral populations of South Asia—*ANI* and *ASI*—suggesting that Steppe ancestry widespread in South Asia derived from earlier southward movements.

South Asia

1. After exploring a wide range of models of present-day and ancient South Asia, we identify a unique class of models that fits geographically and temporally South Asians: a mixture of *AASI*, *Indus_Periphery,* and *Steppe_MLBA*. We reject *BMAC* as a primary source of ancestry in South Asians.

2. A population of which the *Indus_Periphery* samples were a part played a pivotal role in the formation of the two proximal sources of ancestry in South Asia, the *ANI* and *ASI*. Both ends of the Indian Cline had major components of *Indus_Periphery* admixture: ~39% for the *ASI* and ~72% for the *ANI*. Today there are groups in South Asia with very similar ancestry to the *ASI* and *ANI*.

3. Much of the formation of both the *ASI* and *ANI* occurred in the 2nd millennium BCE. Thus, the events that formed both the *ASI* and *ANI* overlapped the decline of the IVC.

4. The *ASI* were not a clade with the earlier hunter-gatherer populations of South Asia (*AASI*), but harbored significant amounts of ancestry related to early Iranian agriculturalists, likely transmitted though the IVC.
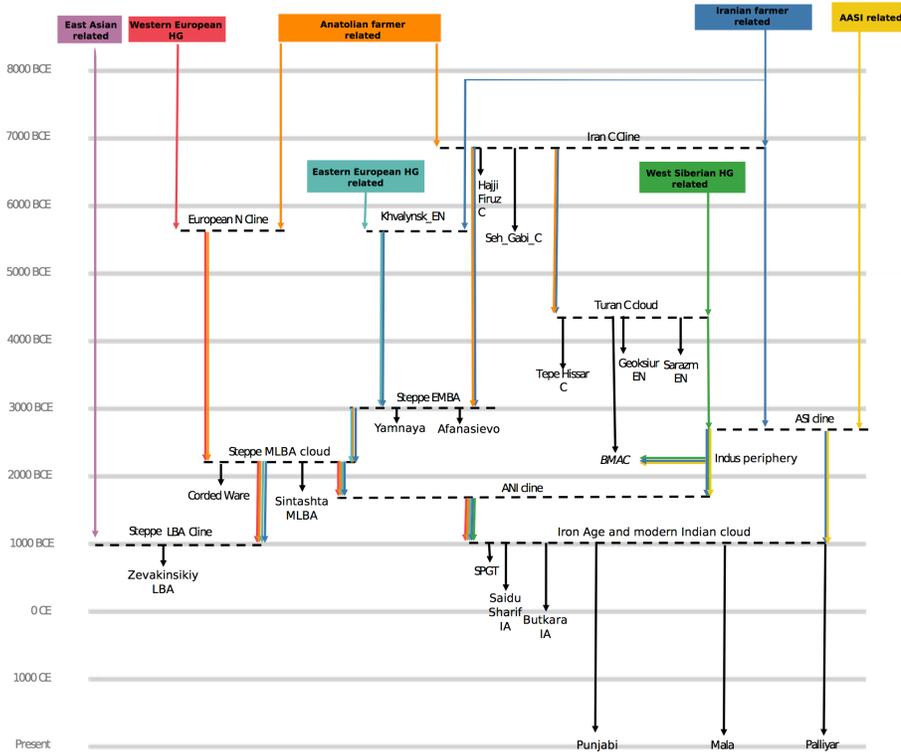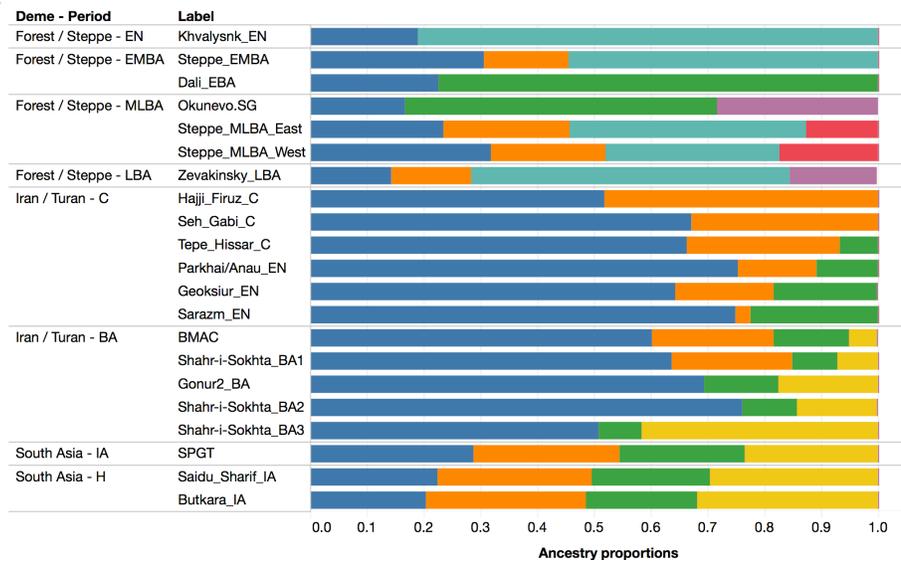
568

20

Fig. 1 Overview of ancient DNA data
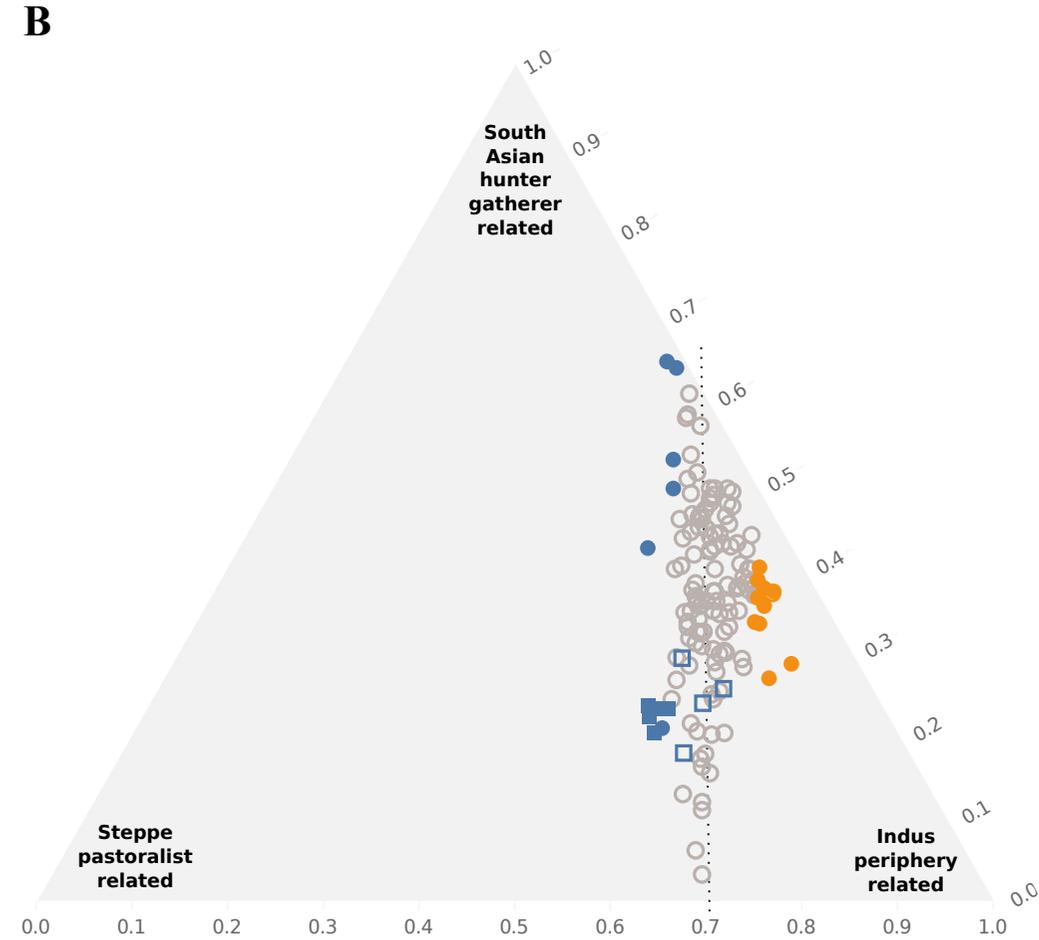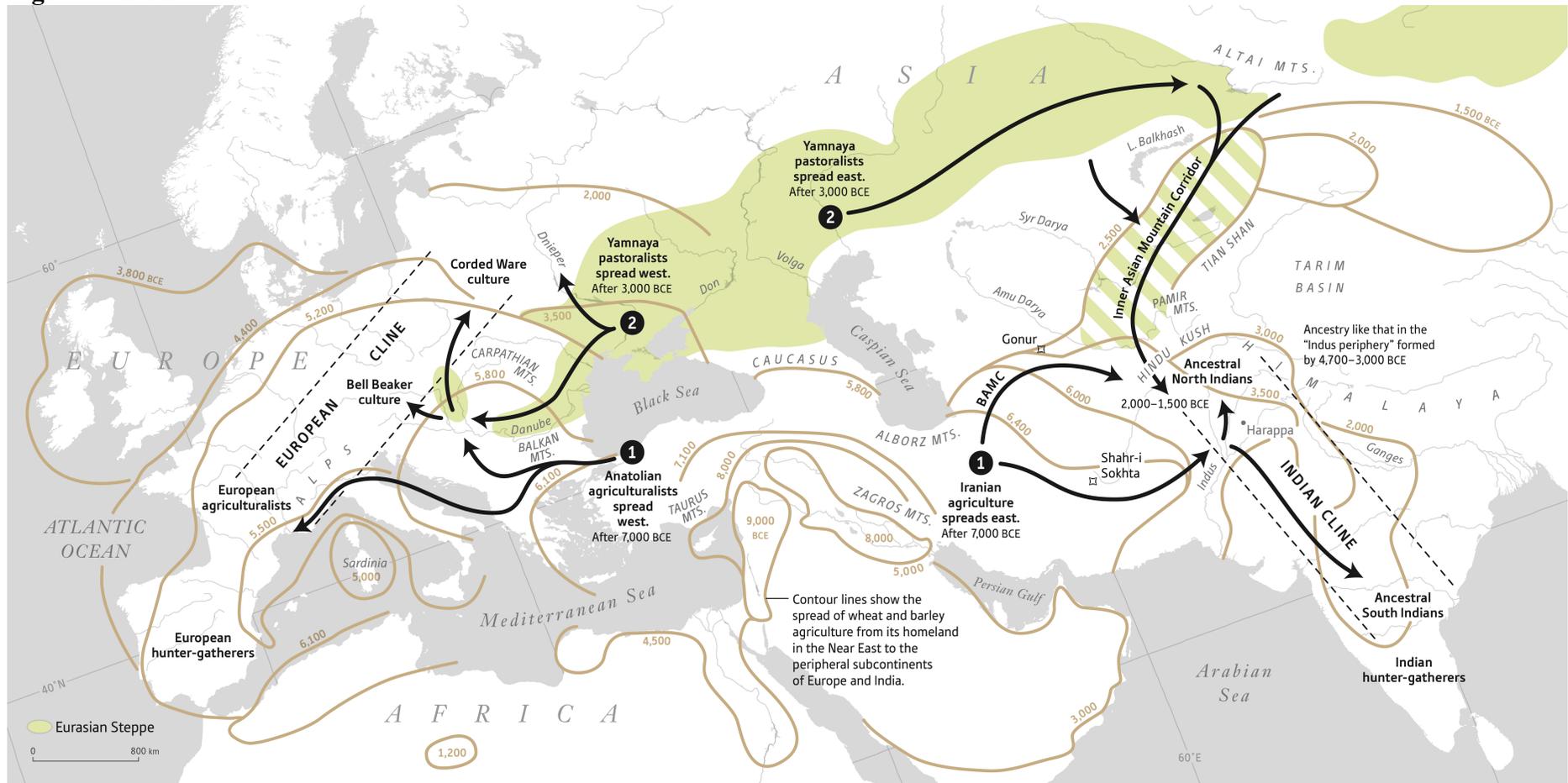
Fig. 2 Modeling results

Fig. 3 The genomic origin of Indians

A

Tested combinations	CHG	Armenia_EBA	Hajji_Firuz_C	Seh_Gabi_C	Tepe_Hissar_C	Geoksiur_EN	BMAC	Indus_Periphery
Khvalynsk_EN	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Steppe_EMBA	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Okunevo.SG	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Sintashta_MLBA	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1877
Srubnaya	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2024
Steppe_MLBA_West	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1599
Steppe_MLBA_East	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0834
Steppe_LBA	0.0000	0.0000	0.0000	0.0000	0.0001	0.0045	0.0000	0.0000

B

C

Fig. 4 A Tale of Two Subcontinents

Labels on map:

ALTAI MTS.

A S I A

Yamnaya pastoralists spread east. After 3,000 BCE ②

L. Balkhash

Syr Darya

1,500 BCE

2,000

TARIM BASIN

Inner Asian Mountain Corridor

2,500

PAMIR MTS.

TIAN SHAN

Volga

Don

3,500

2,000

Dnieper

Yamnaya pastoralists spread west. After 3,000 BCE ②

Corded Ware culture

CLINE

CARPATHIAN MTS. 5,800

EUROPEAN

Bell Beaker culture

Amu Darya

Gonur

HINDU KUSH

3,000

Ancestry like that in the "Indus periphery" formed by 4,700–3,000 BCE

CAUCASUS 5,800

Caspian Sea

Ancestral North Indians 2,000–1,500 BCE

3,500

HARAPPA

H I M A L A Y A

2,000

3,800 BCE

4,400

5,200

60°

E U R O P E

Danube

BALKAN MTS.

Black Sea

BAMC

6,000

6,400

Indus

Harappa

Ganges

5,500

European agriculturalists

A L P S

①

Anatolian agriculturalists spread west. After 7,000 BCE

7,100

8,000

ALBORZ MTS.

①

Iranian agriculture spreads east. After 7,000 BCE

Shahr-i Sokhta

INDIAN CLINE

6,100

ATLANTIC OCEAN

Sardinia 5,000

TAURUS MTS.

ZAGROS MTS.

9,000 BCE

8,000

40°N

European hunter-gatherers

6,100

Mediterranean Sea

4,500

5,000

Persian Gulf

Contour lines show the spread of wheat and barley agriculture from its homeland in the Near East to the peripheral subcontinents of Europe and India.

Ancestral South Indians

A F R I C A

1,200

Arabian Sea

Indian hunter-gatherers

3,000

60°E

🟩 Eurasian Steppe

0   800 km

1   **Materials and Methods**

2

3   **Ancient DNA Laboratory Work** We prepared powder from human skeletal remains either at field

4   sites using a method for extracting petrous bone powder by drilling directly from the cranial base

5   (*47*), or in dedicated clean rooms at Harvard Medical School, University College Dublin, or the

6   Max Planck Institute for Evolutionary Anthropology in Leipzig Germany.

7

8   All the molecular work except for that of a single sample (Darra-i-Kur) was carried out at Harvard

9   Medical School (HMS). At HMS, we extracted DNA using a method that is optimized to retain

10   small DNA fragments (*1, 2*). We converted the DNA into a form that could be sequenced using a

11   double-stranded library preparation protocol, usually pre-treating with the enzyme Uracil-DNA

12   Glycosylase (UDG) to reduce the characteristic cytosine-to-thymine errors in ancient DNA (*4*). For

13   some libraries, we substituted the MinElute columns used for cleaning up reactions with magnetic

14   beads, and the MinElute column-based PCR cleanup at the end of library preparation with SPRI

15   beads (*48, 49*). We enriched the libraries both for sequences overlapping mitochondrial DNA (*50*),

16   and for sequences overlapping about 1.24 million nuclear targets after two rounds of enrichment (*5,*

17   *6, 8*). We sequenced the enriched products on an Illumina NextSeq500 using v.2 150 cycle kits for

18   2×76 cycles and 2×7 cycles, and sequenced up to the point that the expected number of new SNPs

19   covered per 100 additional read pairs sequenced was approximately less than 1.

20

21   To analyze the data computationally, we separated read pairs into individuals based on searching

22   for the expected two indices and two barcodes, allowing up to one mismatch from the expected

23   sequence in each case. We removed adapters and merged together sequences requiring a 15 base

24   pair overlap (allowing up to one mismatch), using a modified version of Seqprep

25   (https://github.com/jstjohn/SeqPrep), which takes the highest quality base in the merged regions.

26   We mapped the resulting single-ended sequences were mapped to the GRCh37 human reference

27   (from the 1000 Genomes project) using the *samse* command of the Burrows-Wheeler Aligner tool

28   (*BWA*) (version 0.6.1) (*51*). We trimmed two nucleotides from the end of each sequence, and then

29   randomly selected a single sequence at each site covered by at least one sequence in each

30   individual to represent their genotype at that position ("pseudo-haploid" genotyping). For each

31   sample we generated "pseudo-haploid" calls at the 1.24 million target sites, selecting sequences

32   that have a minimum mapping quality of MAPQ≥10, restricting to nucleotides with a minimum

33   base quality of 20, and trimming 2 base pairs from each end of the reads.

34

35 For Darra-i-Kur, we prepared a single-stranded DNA library (L5082) at the Max-Planck-Institute

36 for Evolutionary Anthropology (MPI-EVA) in Leipzig, Germany, as part of a previous project (*52*).

37 The previous study only analyzed mitochondrial DNA, and for the current study, the library was

38 enriched for molecules overlapping target the same panel of 1.24 million nuclear targets using two

39 rounds of hybridization capture (*5, 6, 8*). We sequenced the enriched libraries on 2 lanes of an

40 Illumina HiSeq2500 platform in a double index configuration (2x76 cycles) (*53*), and we called

41 sites using *FreeIbis* (*54*). We merged overlapping paired-end and trimmed using *leeHom* (*55*). We

42 used *BWA* to align the captured data to the human reference genome (GRCh37 from the 1000

43 Genomes project) (*51*). Only sequences showing a perfect match to the expected index combination

44 were retained for downstream analyses.

45

46 We assessed evidence for ancient DNA authenticity by measuring the rate of damage in the first

47 nucleotide (flagging individuals as potentially contaminated if they had a less than 3% cytosine to

48 thymine substitution rate in the first nucleotide for a UDG-treated library and less than 10%

49 substitution rate for a non-UDG-treated library). We used *contamix* to determine evidence of

50 contamination based on polymorphism in mitochondrial DNA (*56*), and ANGSD to determine

51 evidence of contamination based on polymorphism on the X chromosome in males (*57*).

52

53 **Principal component analysis (PCA)** We carried out PCA using the *smartpca* package of

54 *EIGENSOFT* 7.2.1 (*13*). We used default parameters and added two options (lsqproject:YES and

55 numoutlieriter:0 options) in order to project our ancient samples onto the PCA space. We used two

56 basis sets for the projection: the first based on 1,340 present-day Eurasians genotyped on the

57 Affymetrix Human Origins array, and the second based on a subset of 991 present-day West

58 Eurasians (*5, 10, 58*). These projections are shown repeatedly in the **Supplementary Materials**,

59 and the whole-Eurasian projection is shown in **Fig. 1**. As part of this analysis, we also computed

60 the F_{ST} between groups using the parameters inbreed:YES and fstonly:YES.

61

62 **ADMIXTURE clustering analysis** Using PLINK2 (*59*), we first pruned our dataset using the --

63 geno 0.7 option to ensure that we only performed our analysis on sites that had at least 70% of

64 samples with a called genotype. We then ran ADMIXTURE (*14*) with 10 replicates, reporting the

65 replicate with the highest likelihood. We show results for K=6 in **Fig. 1**, as we found in practice

66 that this provides the most resolution for disambiguating the sources of pre-Chalcolithic ancestry in

67 our newly reported samples.

68

69 **f-statistics** We used the *qp3pop* and *qpDstat* packages in ADMIXTOOLS to compute f_3-statistics

70 and f_4-statistics. We used the inbreed:YES parameter to compute f_3-statistics as a test for admixture

71 with an ancient population as a target, with all published and newly reported ancient genomes as

72 sources. Using the f4Mode:YES parameter in *qpDstat,* we also computed two sets of f_4-symmetry

73 statistics to evaluate if pairs of populations are consistent with forming a clade relative to a

74 comparison population. The first is a statistic where we compare all possible pairs of newly

75 reported ancient groups (*Reported1* and *Reported2*) to a panel of *Test* populations that encompass

76 diverse pre-Chalcolithic and more widespread genetic variation (*Test* is one of

77 *Iran_Ganj_Dareh_Neolithic, Karelia_HG, Han, Onge, LBK_EN, AfontovaGora3,*

78 *Ukraine_Mesolithic*). Thus, we compute a statistic of the form f_4(*Reported1, Reported2; Test,*

79 *Mbuti* African outgroup). The second is a comparison of each newly reported group in turn against

80 all possible pairs of *Test* populations, using statistics of the form f_4(*Test1*, *Test2*; *Reported*, *Mbuti*).

81

82 **Formally modeling admixture history** We used the *qpAdm* methodology (*5*) in the

83 ADMIXTOOLS package to estimate the proportions of ancestry in a *Test* population deriving from

84 a mixture of *N* 'reference' populations by exploiting (but not explicitly modeling) shared genetic

85 drift with a set of 'Outgroup' populations. We set the details:YES parameter, which reports a

86 normally distributed Z-score for the fit (estimated with a block jackknife).

87

88 **Hierarchical model of the Indian Cline** We used *qpAdm* as described above to obtain estimates

89 for the proportion of Steppe-related, Iranian agriculturalist-related and *AASI*-related ancestries and

90 their relevant covariance matrices for each population on the Indian cline. We then jointly modeled

91 these estimates using a bivariate normal model (since the three proportions sum to 100%) and

92 inferred the mean and covariance of the two parameters across all samples on the Indian cline using

93 maximum likelihood estimation. Then, using this inferred matrix, we tested whether the cline could

94 be modeled by a mixture of two populations, the *ANI* and the *ASI*, in two ways. First, we examined

95 whether the covariance matrix is singular, implying that knowledge of one estimated proportion of

96 ancestry of one of the ancestry components revealed knowledge of the other two, as expected in a

97 two-way mixture. Second, if we were able to establish that this was the case, we examined the

98 difference between the expected and observed ratios of the ancestry proportions of individual

99 populations in this generative model obtained from fitting all the populations simultaneously. This

100 process resulted in a handful of populations deviating from expectation, as discussed in the main

101 text and **Supplementary Materials**.

102   **Supplementary Materials:**

103   Materials and Methods

104   Online Tableau Server for visualizing data.

105   Data S1-S3

106

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