

Current Biology

Genomic Evidence Establishes Anatolia as the Source of the European Neolithic Gene Pool

Highlights

- Kum6 shows a strong population continuity with present-day Sardinia
- Kum6 expresses connections to the central Eurasian gene pool
- Kum6 shares notable affinity with the Iceman, a 5,300-year-old southern European
- Genetic affinities to both East and West suggest continuous contact with Anatolia

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In Brief

Genetic data from Neolithic Anatolia link European Neolithization to Anatolia and indicate a vivid area of communication from Western Asia into Europe. Later admixture events in Anatolia have made people from southern Europe, and especially Sardinia, the best living representatives of Neolithic populations in Anatolia.



Genomic Evidence Establishes Anatolia as the Source of the European Neolithic Gene Pool

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<http://dx.doi.org/10.1016/j.cub.2015.12.019>

SUMMARY

Anatolia and the Near East have long been recognized as the epicenter of the Neolithic expansion through archaeological evidence. Recent archaeological studies on Neolithic European human remains have shown that the Neolithic expansion in Europe was driven westward and northward by migration from a supposed Near Eastern origin [1–5]. However, this expansion and the establishment of numerous culture complexes in the Aegean and Balkans did not occur until 8,500 before present (BP), over 2,000 years after the initial settlements in the Neolithic core area [6–9]. We present ancient genome-wide sequence data from 6,700-year-old human remains excavated from a Neolithic context in Kumtepe, located in northwestern Anatolia near the well-known (and younger) site Troy [10]. Kumtepe is one of the settlements that emerged around 7,000 BP, after the initial expansion wave brought Neolithic practices to Europe. We show that this individual displays genetic similarities to the early European Neolithic gene pool and modern-day Sardinians, as well as a genetic affinity to modern-day populations from the Near East and the Caucasus. Furthermore, modern-day Anatolians carry signatures of several admixture events from different populations that have diluted this early Neolithic farmer component, explaining why modern-day Sardinian populations, instead of modern-day Anatolian populations, are genetically more similar to the people that drove the Neolithic expansion into Europe. Anatolia's central geographic location appears to have served as a connecting point, allowing a complex contact network with other areas of the Near East and Europe throughout, and after, the Neolithic.

RESULTS AND DISCUSSION

As the earliest Neolithic sites in Anatolia predate the indications of Neolithization in Europe, it was already recognized a century ago that the Neolithic lifestyle must have spread from here to neighboring regions, although the mode of transmission was debated [11, 12]. In recent years, the topic has generated new momentum through archaeogenetic research, providing evidence for migrations being responsible for the spread of Neolithic life ways [1, 3–5]. According to the archaeological record, the Neolithic period in Anatolia spans over 6,000 years—from 11,000 before present (BP) to around 5,000 BP [6]. After the initial settlements in the Neolithic core area, including the central Anatolian plateau, the development continued within the area but without further expanding the borders [7] while maintaining complex interactions with the Levant [6]. Settlements and pottery finds along the Anatolian west coast from 8,500 BP until 7,500 BP indicate a westward expansion with a large impact on the local demography and the establishment of numerous culture complexes [8, 9]. The site of Kumtepe, located in northwestern Anatolia and established around 7,000 BP [13], is one of the settlements that emerged after this expansion.

We generated genome-wide sequence data from two Neolithic individuals excavated at the site of Kumtepe and obtained ~0.13× genome coverage for Kum6 (6,700 BP) and ~0.01× for Kum4 (5,500–4,800 BP) (Tables S1 and S2). The sequence data showed evidence of nucleotide misincorporations characteristic of post-mortem degradation [14] (Figure S1). Given the low genome coverage of Kum4, it was only possible to use this individual to corroborate the patterns observed in Kum6 (see the Supplemental Experimental Procedures, section S4). We obtained mitochondrial genomes with coverage of 21× for individual Kum6 and 1.5× for Kum4. Kum6 carries the H2a mitochondrial haplogroup (Supplemental Experimental Procedures, section 3.2; Table S3), a haplogroup commonly found in modern-day Eastern Europeans and Caucasians [15]. Haplogroup H is the most common haplogroup in Europe and the Near East, and it is thought to have originated in the Near East 25,000–30,000 years ago [15]. It is also frequently observed in

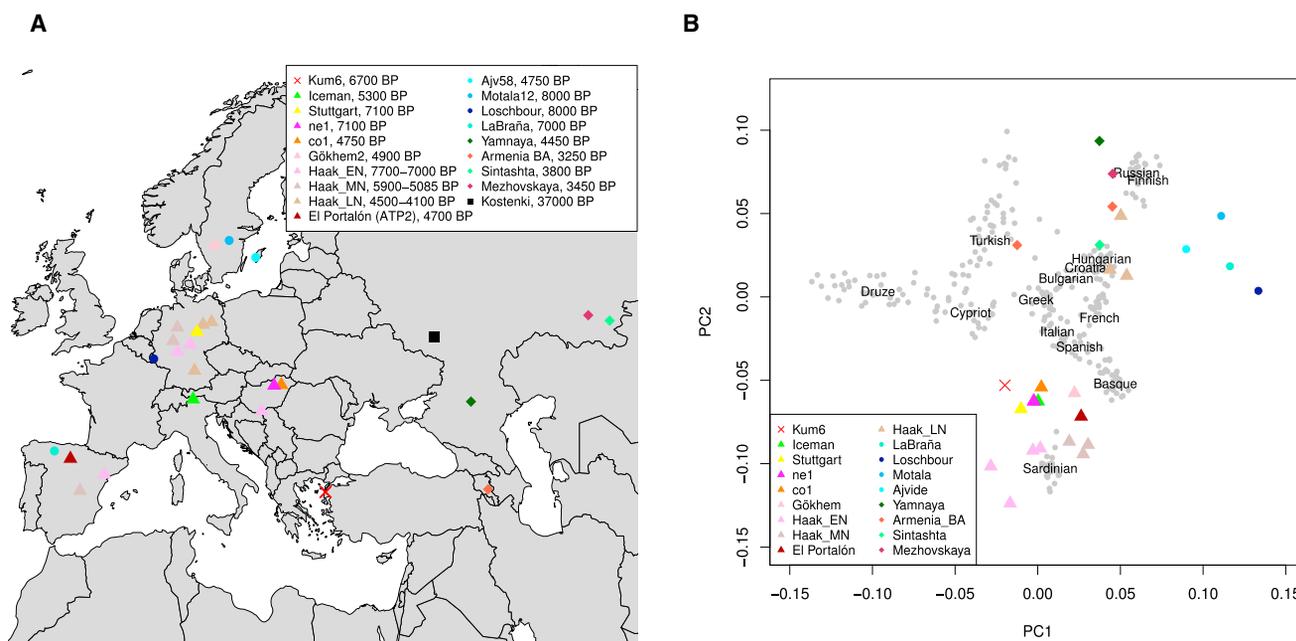


Figure 1. Overview of Samples Included in This Study

(A) Map showing geographic location of ancient western Eurasian samples and radiocarbon dates. Asian samples from Ust-Ishim and Karasuk were included in some analyses, as well. Individuals used in this study were clustered contextually using different symbols: squares indicate Paleolithic individuals, circles indicate individuals from hunter-gatherer contexts, triangles indicate individuals from farming contexts, and diamonds indicate Bronze Age individuals. See also [Table S4](#) for the number of SNPs yielded per individual for the analyses, [Table S1](#) for radiocarbon dates for Kum6, and [Tables S2 and S3](#) and [Figure S1](#) for contamination estimates and sequence statistics for Kum6.

(B) PCA of the ancient individuals together with modern-day individuals from western Eurasian populations. See also [Figure S2](#) for individual PCAs.

early farmers of Europe [16] and the Near East [17]. We estimated mtDNA contamination [18] of Kum6 and found low levels of contamination (2.0%, with a 95% confidence interval of 0.0%–5.9%) ([Table S3](#)).

Genome coverage as low as 0.13 \times has previously been shown to provide important and clear insights into population history [5, 19]. We intersected the genomic data from Kum6 with a reference panel of genome-wide autosomal SNP array data including 594,924 SNPs genotyped in 312 modern-day Eurasians [20]. Principal component analysis (PCA) was conducted for each ancient individual, including 25 publicly available ancient western Eurasians [3, 4, 19, 21–24]. ([Figures 1A and S2](#); [Table S4](#)). As observed in previous studies [3–5] there is a clear genetic differentiation between hunter-gatherers and early farmers ([Figure 1B](#)). The Anatolian Kum6 individual falls close to the early and middle Neolithic European farmers, showing a tendency toward modern-day Near Eastern populations. Interestingly, Kum6 does not group with any modern-day Anatolian populations. These results were confirmed by outgroup f_3 statistics where, among modern-day groups, Kum6 shows the greatest genetic similarity to Sardinians, Greeks, and Cypriots, whereas modern-day Anatolian populations display lower levels of genetic affinity to Kum6 ([Figure 2](#)). Kum6 also falls between modern-day West Asians and Europeans when additional modern-day populations are included in the analysis ([Figure S2E](#)).

In order to infer population structure and admixture signatures among Kum6 and other populations, as well as other ancient individuals, we conducted a maximum-likelihood clustering

analysis using ADMIXTURE [25] ([Figures 3 and S5](#)). We included all modern-day Eurasian and North African populations, a set of ancient European farmers and hunter-gatherers with more than 1 \times coverage [3, 4, 21–23], a Yamnayan individual and individuals with the largest number of SNPs from each Neolithic group from a recent SNP capture study [24], six Bronze Age Asian individuals [19], and three Paleolithic individuals [26–28]. For a model with nine clusters ($K = 9$; results for higher numbers of clusters are similar, [Figure S3](#)), three major ancestry components were observed in the ancient individuals. The first one (blue), observed as the main component in all hunter-gatherers, is also found as a minor contribution to all farmers, which is in line with the observed admixture from hunter-gatherers into farmers [3]. The second (orange) and the third (green) components were observed mostly in farmers to varying degrees ($\sim 5\%$ – 68% and $\sim 0.06\%$ – 45% for $K = 9$, respectively). The orange component is mainly found in present-day Western Europeans, whereas the third component (green) is mostly found in the modern-day Near East and Caucasus, and the highest proportion of this third component among Neolithic individuals was observed in Kum6 ($\sim 45\%$ for $K = 9$). The notion that this component is West Asian is also supported by its presence in a Bronze Age Armenian sample (51%), which contains less than 2% of the orange component. Interestingly, this “West Asian” component (green) is not related to the potential genetic material brought to Europe by migration during the Bronze Age and recently connected to the Yamnaya culture [19, 24], visualized in [Figure 3](#) as light blue, and it is observed in high frequency in modern-day people

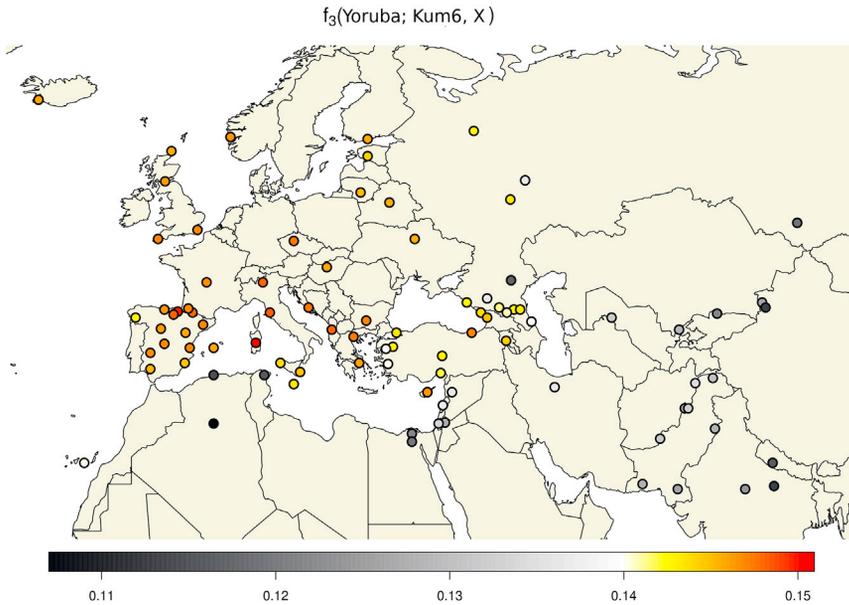


Figure 2. The Western Anatolian Neolithic Kum6 Individual's Affinities with Modern-Day Populations

Shared genetic drift between modern-day individuals and Kum6.

from southern Asia. The elevated “West Asian” affinity of Kum6 is likely to be the cause of the genetic differentiation observed between Kum6 and all other ancient farmers shown in the PCA plot (Figure 1B). A clear decline was observed in the values of the green component over time (average of ~29% in Early Neolithic, ~14% in Middle Neolithic, and 2% in Late Neolithic), which is consistent with increased admixture with hunter-gath-

erer groups [3]. Our results suggest that the two ancestry components of ancient farmers (orange and green in Figure 3) were established at an early stage, probably before the first farmers expanded into Europe, and were maintained in Europe up until the end of Middle Neolithic and that both components are present in various modern-day European populations. Therefore, these observations directly link the early European Neolithic gene pool to western Anatolia.

We computed *D* statistics [20] to further investigate additional genetic relationships between ancient Europeans with sufficient sequencing coverage (>1x) and Kum6. All proposed tree topologies where the Tyrolean Iceman [20] was included as one of the in-groups were rejected ($2 < |Z| < 4.6$), suggesting gene flow or a more recent shared ancestry between Kum6 and the Tyrolean Iceman (Figure 4A). A similar tendency was observed with a Middle Neolithic Hungarian farmer [23], (co1), contemporary with the Tyrolean Iceman, despite the limited

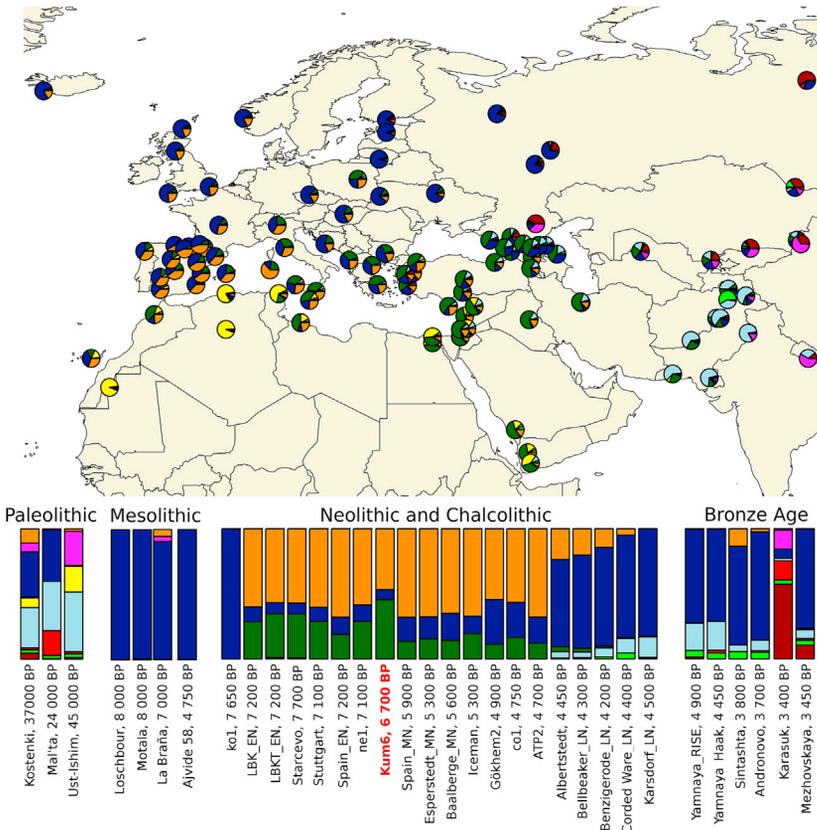


Figure 3. Ancestry Proportions Inferred from Model-Bases Clustering

The map shows population ancestry proportions for modern-day western Eurasian and North African populations determined using ADMIXTURE for $K = 9$ (note that eastern Eurasian populations were included in the analysis, as well; see Figure S3 for the full set of results). Ancestry proportions for the ancient individuals are shown as bar charts in the lower panel. Ajv58 was grouped with the Mesolithic samples (despite being dated to the Neolithic time period) since it is genetically and culturally more similar to European Mesolithic hunter-gatherers. Ko1 is, genetically, very similar to the hunter-gather gene pool [23], but it has been excavated from a farmer context. ADMIXTURE results for other values of K are shown in Figure S3.

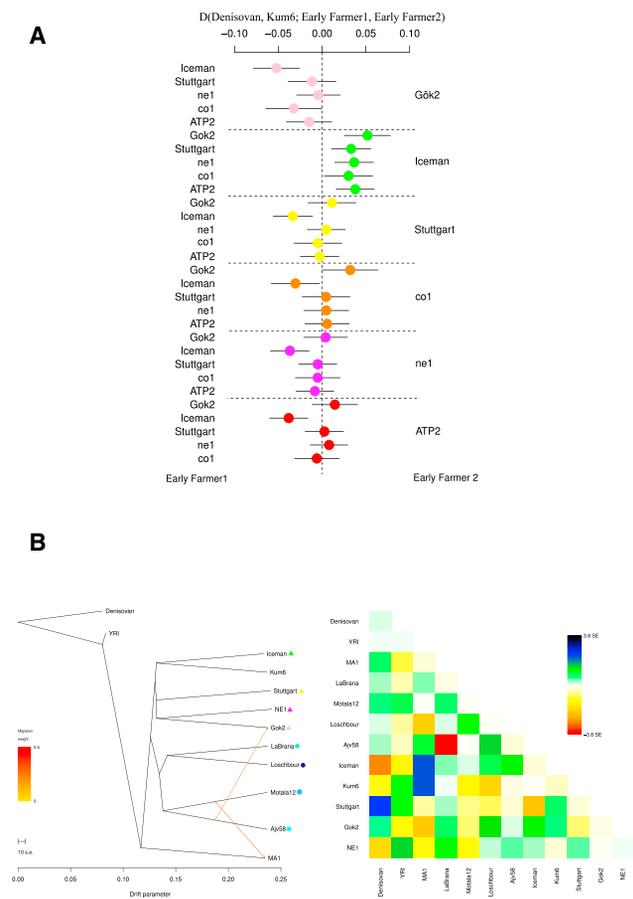


Figure 4. Affinities among Ancient Farmers and Admixture Graph Inference

(A) *D* statistics testing the consistency of proposed tree topologies of the shape (outgroup, Kum6; early farmer 1, early farmer 2) using genetic data. Negative values suggest strong affinities between Kum6 and early farmer 1 (labels on the left), whereas positive values indicate that Kum6 is closer to early farmer 2 (labels on the right). Significant differences from zero can be interpreted as rejection of the tree topology. Error bars represent ± 2 SEs. See also Table S5.

(B) TreeMix plot for Kum6 allowing two migration events. The symbols indicate the two different cultural groups: triangles indicate individuals from farming contexts, and circles indicate individuals from hunter-gatherer contexts.

resolution due to the low coverage of Kum6 and co1. The observed genetic affinity between the Tyrolean Iceman and Kum6 could be interpreted as additional contacts between western Anatolia and Neolithic Europe at a later stage. This scenario is congruent with mitochondrial [29] and archaeozoological [30] studies, as well as the archaeological indications of multiple waves of contact between the Balkans and Anatolia [7, 9, 31–33]. A continuous contact between northwestern Anatolia and southeastern Europe is not surprising, given the archaeological record, although it has not been detected in the genomic data previously.

Furthermore, the Bronze Age Yamnayan component suggested to be a part of the Corded ware expansion [19, 24] is not present in Kum6, and thus is not producing any increased affinity to the ancestors of the Yamnaya culture from north of the Caucasus (D-Denisovan, Yamnaya_RISE; Kum6, early farmer),

all $Z > -1.7$). Contacts to the east, independent of Yamnaya ancestry [19, 24] are, however, supported by (1) higher affinity of Kum6 to some Bronze Age Asian cultures when compared to Mesolithic Europeans and (2) higher affinities of Bronze Age Asians to Kum6 compared to early Neolithic Europeans (Table S5). A comparison of Kum6 to an Asian Upper Paleolithic individual (Ust-Ishim [27]) and a European Upper Paleolithic sample (Kostenki14 [28]) confirms that Kum6 shows more affinity to early Europeans ($Z = 5$). Stronger affinities of Kostenki14 to Kum6 than to early Neolithic Europeans (Table S5), however, suggest that Kum6 contains genomic components not found in early Neolithic Europeans. Kum6 is also grouped with farmers in a model-based population-tree analysis [34] (Figure 4B), and the inferred migration edges point to the same conclusion as the *D* statistics results, as well as manifest the expected signals from previously published observations [3, 4].

Our findings show a direct link between Anatolia and the early European Neolithic gene pool similar to recently published data [35]. The genetic composition of Kum6 indicates, however, that this individual is a representative of the local population in the area 6,700 BP, as there are European hunter-gatherer traces in this individual, although at lower levels than in any other studied Neolithic farmer. In addition, we also observe limited genetic material from the later Bronze Age expansions (sometimes linked to the Yamnaya culture [19, 24]), and instead a larger genetic component related to people that are linked to the east (these components are all in addition to the strong genetic affinities to early European farmers, as discussed above). Interestingly, the genetic similarity to the Tyrolean Iceman and the eastern component detected in Kumtepe indicate an intense entanglement of contacts from the East and into Europe, with western Anatolia at the center. Most modern-day European populations display ancestries from Mesolithic hunter-gatherers, early Neolithic farmers, and in some cases traces of additional admixture from different sources [3, 4, 23, 24] (Figure 3). Modern-day Anatolian groups display a variety of admixture traces originating from groups in the Middle East, Central Asia, and Siberia, which cause Kum6 to be genetically more similar to modern-day Europeans than to modern-day Anatolians. These results show a large diversity in Anatolian groups that is consistent with previous population genetic studies of modern-day Anatolians. Influences from South Caucasus and the Near East [36] and major population movements, including the arrival of Turkic tribes [37, 38], have most likely contributed to the genetic makeup of modern-day Anatolians, whereas the affinity to present-day Sardinians could be the result of the relative isolation of that particular population after the initial Neolithic expansion [39]. Thus, the observed genetic continuity between Kum6 and the modern-day inhabitants of southern Europe, and especially Sardinia, is likely to be the result of these population movements that occurred in Anatolia.

EXPERIMENTAL PROCEDURES

DNA was extracted from 12 bone pieces from eight individuals (Supplemental Experimental Procedures, section S2). DNA extracts were built into Illumina libraries and sequenced on the Illumina HiSeq2500 (Supplemental Experimental Procedures, sections S2 and S3). Reads were mapped to the human reference genome builds 36 and 37.1 (Supplemental Experimental Procedures, section S3). Principal component analysis was carried out to investigate

affinities to modern-day populations (Supplemental Experimental Procedures, section S6.1). Shared genetic drift with modern-day populations and ancient individuals were estimated using outgroup f_3 statistics [20] (Supplemental Experimental Procedures, section S6.2). Tree-like population models were tested using D statistics [20] (Supplemental Experimental Procedures, section S6.3). Admixture graphs were fitted to the observed allele frequencies using TreeMix [34] (Supplemental Experimental Procedures, section S7). Model-based clustering was performed using ADMIXTURE [25] and the Human Origins dataset [20] (Supplemental Experimental Procedures, section S8).

ACCESSION NUMBERS

The accession number for the sequence data reported in this paper is European Nucleotide Archive: PRJEB12155.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and five tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.12.019>.

AUTHOR CONTRIBUTIONS

A.G., M.J., and J.S. designed and supervised the study. A.O., C.V., E.M.S., H.M. performed the experiments. A.O. and T.G. analyzed population-genetic data with additional contributions from H.M. (mtDNA). H.K. excavated the samples and did osteological assessments. H.K. and J.S. provided the archaeological interpretations. W.A. provided the archaeological material. A.O., T.G., and A.G. wrote and edited the manuscript with input from all authors.

ACKNOWLEDGMENTS

The authors would like to thank the Directors of Project Troia, the late Prof. Dr. Manfred O. Korfmann, under whose direction the material from Kumtepe was discovered and excavated, and Prof. Dr. Ernst Pernicka, who authorized release of the material for study, as well as the General Directorate of Museums and Monuments in the Ministry of Culture and Tourism of the Republic of Turkey for permissions to conduct research in Turkey. The authors would also like to thank Pontus Skoglund for initial screening analyses and discussions. This work has been funded by Swedish Foundation for Humanities and Social Sciences grant no. M13-0904:1, Swedish Research Council grant no. 2013-1905, the Wenner Gren Foundation, La Trobe Bridging Fellowship, and European Research Council grant no. 311413. Sequencing was conducted at the Uppsala University SNP&SEQ Technology Platform. Computational analyses were carried out at the Swedish National Infrastructure for Computing (SNIC-UPPMAX, projects b2013236 and b2013240).

Received: November 20, 2015

Revised: December 7, 2015

Accepted: December 9, 2015

Published: December 31, 2015

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