

Ancient DNA Reveals Lack of Continuity between Neolithic Hunter-Gatherers and Contemporary Scandinavians

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Summary

The driving force behind the transition from a foraging to a farming lifestyle in prehistoric Europe (Neolithization) has been debated for more than a century [1–3]. Of particular interest is whether population replacement or cultural exchange was responsible [3–5]. Scandinavia holds a unique place in this debate, for it maintained one of the last major hunter-gatherer complexes in Neolithic Europe, the Pitted Ware culture [6]. Intriguingly, these late hunter-gatherers existed in parallel to early farmers for more than a millennium before they vanished some 4,000 years ago [7, 8]. The prolonged coexistence of the two cultures in Scandinavia has been cited as an argument against population replacement between the Mesolithic and the present [7, 8]. Through analysis of DNA extracted from ancient Scandinavian human remains, we show that people of the Pitted Ware culture were not the direct ancestors of modern Scandinavians (including the Saami people of northern Scandinavia) but are more closely related to contemporary populations of the eastern Baltic region. Our findings support hypotheses arising from archaeological analyses that propose a Neolithic or post-Neolithic population replacement in Scandinavia [7]. Furthermore, our data are consistent with the view that the eastern Baltic represents a genetic refugia for some of the European hunter-gatherer populations.

Results and Discussion

By 6,700 years before present (BP) the Neolithization process had influenced most of northern Europe [9]. However, Scandinavia (including Denmark) was still occupied by highly mobile hunter-gatherer groups. Although the hunter-gatherers of Denmark and southern Sweden adopted pottery early on, the Neolithization first took real shape with the appearance of the Funnel Beaker Cultural complex (FBC, also known as the Trichterbecher Kultur [TRB]) some 6,000 years BP (the oldest evidence possible dating back some 6,200 years BP [9]). At this time domestic cattle and sheep, cereal cultivation, and the characteristic TRB pottery were introduced into most of Denmark and southern parts of Sweden [6]. Nevertheless, the Neolithization process was slow in Scandinavia, and large areas remained populated by hunter-gatherer groups until the end of the 5th millennium BP.

One of these last hunter-gatherer complexes was the Pitted Ware culture (PWC), which can be identified by its single-inhumation graves distributed over the coastal areas of Sweden and the Baltic Sea islands that lie closest to the Swedish coast. Intriguingly, the PWC first appears in the archaeological record of Scandinavia after the arrival of the TRB (some 5,300 years BP) and existed in parallel with farmers for more than a millennium before vanishing about 4,000 years BP (Figure 1). This prolonged coexistence of hunter-gatherers and farmers in Scandinavia has been a focal point of debate within archaeology since 1909, when PWC human remains were used to argue for an early eastern influence on Neolithic Scandinavia, thus favoring relations to modern Saami people [10]. However, it has mainly been used as a key argument against both a rapid Neolithic transition and a large-scale population replacement between the Mesolithic and the present [7, 8].

Three main hypotheses have been proposed to explain the origin of the PWC: (1) it has an origin in the late Mesolithic hunter-gatherer complexes of northern Europe [11] that, given that Neolithic or post-Neolithic population replacement took place, would make them genetically distinct from modern Scandinavians; (2) the PWC arose from a reversion to the hunter-gathering subsistence strategy among TRB peoples [12], and, given that no population replacement took place in Scandinavia during the Neolithization process, PWC peoples are the direct ancestors of modern Scandinavians; and (3) the PWC originated in populations ancestral to modern Saami people of present-day northern Scandinavia [10, 13].

To investigate PWC ancestry components in modern Scandinavians and peoples of the Baltic region, we recovered ancient mitochondrial (mtDNA) sequences (316 bp of the D-loop) from the skeletons of 22 individuals deriving from the two different cultures (see Table S1 available online). Three of these were TRB (all from one passage tomb, Gökhem, dated to 5,500–4,500 years BP, Figure 1), and 19 belong to the PWC (recovered from three different sites on the Baltic island of Gotland dated to 4,800–4,000 years BP, Figure 1).

Quantitative real-time PCR was used to assess the total human mtDNA content in all samples (Tables S2 and S3, Figure S1) and to screen for appropriate molecular behavior (degradation ratio [14], Table S4). Amplicons were sequenced with the

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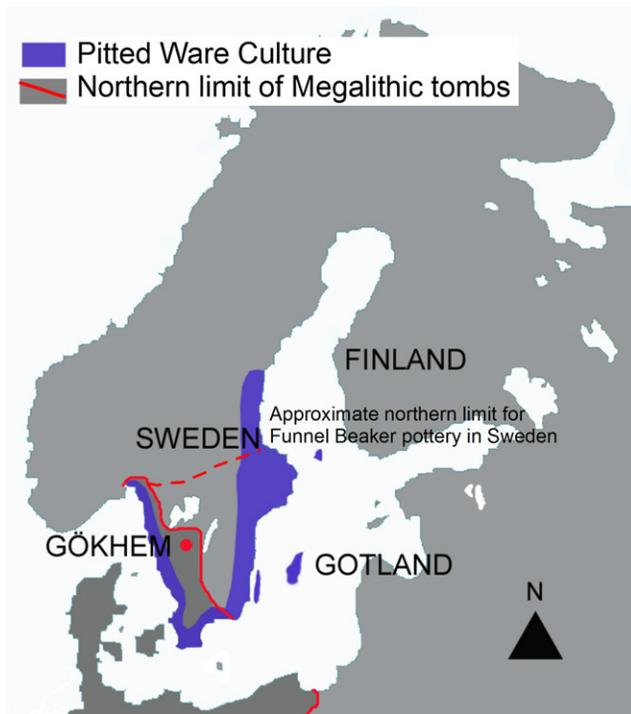


Figure 1. Scandinavia with the PWC and the Architectural Structures of the TRB Displayed

The three TRB sequences originate from Gökhem, Sweden, and the 19 PWC sequences originate from sites on Gotland, Sweden.

Roche Genome Sequencer FLX platform to retrieve synthetic clones [15] (Table S5). Sequences were regarded as authentic if they (1) originated from DNA extracts containing more than 1000 molecules of the quantified 80 bp fragment, (2) were supported by two independent extractions, (3) were based on a minimum of 20 synthetic FLX clone sequences, and (4) expressed a degradation ratio higher than 1 (Supplemental Data).

Reduced median networks [16] were used to graphically illustrate substitution differences among sequences and to enable sequence assignment to previously defined haplogroups [4, 17]. Haplogroups U4/H1b, U5, and U5a were found to have high incidence among the PWC but are all rare among contemporary Scandinavians and Saami (Figures 2A–2C). It is noteworthy that a high frequency of U lineages, especially U5, has been inferred for pre-Neolithic Europeans with the use of modern mtDNA data [18]. Interestingly, compared to the rest of Europe, the U haplogroups have relatively high frequencies among populations in the eastern Baltic region such as the Latvians and the Lithuanians (Figure 2C).

Analysis of molecular variance [19] (AMOVA) revealed that the PWC sequences are significantly differentiated from samples of contemporary Swedes [20] ($n = 289$, $F_{ST} = 0.05174$, $p < 0.001$), Saami [20] ($n = 38$, $F_{ST} = 0.25037$, $p < 10^{-6}$), Norwegians [21] ($n = 323$, $F_{ST} = 0.06148$, $p < 0.001$), Finns [22] ($n = 79$, $F_{ST} = 0.05327$, $p < 0.005$), Estonians [22] ($n = 117$, $F_{ST} = 0.04745$, $p < 0.003$), Lithuanians [22] ($n = 163$, $F_{ST} = 0.04022$, $p < 0.004$), and Latvians [22] ($n = 114$, $F_{ST} = 0.03622$, $p < 0.011$). To examine whether population differences could be accounted for by drift alone under the null hypothesis of population continuity, we performed coalescent simulations assuming a wide range of combinations of ancestral population size at the Upper Paleolithic colonization of Europe, 45,000 years ago,

and the time of arrival of farming in Scandinavia, 6,200 years ago. As a conservative measure, we assumed a relatively high (compared to other published estimates) mutation rate of 7.5×10^{-6} per site per generation [23] to ease the burden of explaining high F_{ST} values. We sampled sequences from each simulation according to the numbers and dates of the data considered here and calculated the proportion of simulated F_{ST} values that were greater than those observed (Supplemental Data).

The null hypothesis of population continuity between the PWC and modern Swedes can be rejected under a range of assumed ancestral population size combinations (including almost all that assume a Neolithic effective population size $> 15,000$), as can population continuity between the PWC and Norwegians under most assumed ancestral population size combinations (including almost all that assume a Neolithic effective population size $> 6,000$) (Figure 3). Population continuity between the PWC and modern Saami can be rejected under all assumed ancestral population size combinations. However, population continuity between the PWC and contemporary Baltic populations cannot be rejected under most assumed ancestral population size combinations (Supplemental Data).

These results indicate that the PWC hunter-gatherers are unlikely to be the main ancestors of either modern Scandinavians or Saami, despite their presence in Scandinavia at the early stages of Neolithization. On the contrary, the observed F_{ST} values indicate greater similarity between the PWC and modern eastern Baltic populations, and coalescent simulations confirm that those non-Scandinavian populations could plausibly be the direct descendants of PWC hunter-gatherers. Having only obtained three TRB sequences, we cannot exclude continuity with any of the modern populations.

Although complex demographic scenarios such as local population structuring, or sampling problems including close relatedness among individuals from the same site, might also explain the patterns of differentiation that we observe, we found no significant differentiation among the three PWC sites that we sampled (AMOVA pairwise $F_{ST} = -0.0189$; $p = 0.54733$; exact test of population differentiation global p value = 0.43421) and also note that the ubiquity of U4 and U5 types at those sites suggests that we are looking at patterns of genetic variation that extend beyond the local scale. It is noteworthy, however, that our interpretation is highly dependent on the assumed effective population size (N_e) at the onset of the Neolithic in Scandinavia 6,200 years BP. If N_e at this time were low ($< 6,000$ if modern Norwegians and Swedes share a common ancestry, $< 15,000$ for the unlikely event that the two populations have different ancestry in the region), then drift would be sufficient to explain the F_{ST} s for both modern Swedes and northeastern Baltic peoples. Furthermore, it may be possible that a relatively low level of admixture in Scandinavia between the PWC and the TRB could be sufficient to explain the differences observed between the PWC and modern Scandinavians. Currently, however, this remains untestable, because we lack an appropriate proxy for early farmers.

Given our results, it remains possible that the PWC represent remnants of a larger northern European Mesolithic hunter-gather complex. However, it appears unlikely that population continuity exists between the PWC and contemporary Scandinavians or Saami. Thus, our findings are in agreement with archaeological theories suggesting Neolithic or post-Neolithic population introgression or replacement in Scandinavia. To what extent this holds true for other parts of Europe requires further direct testing, although morphological [24, 25], ancient

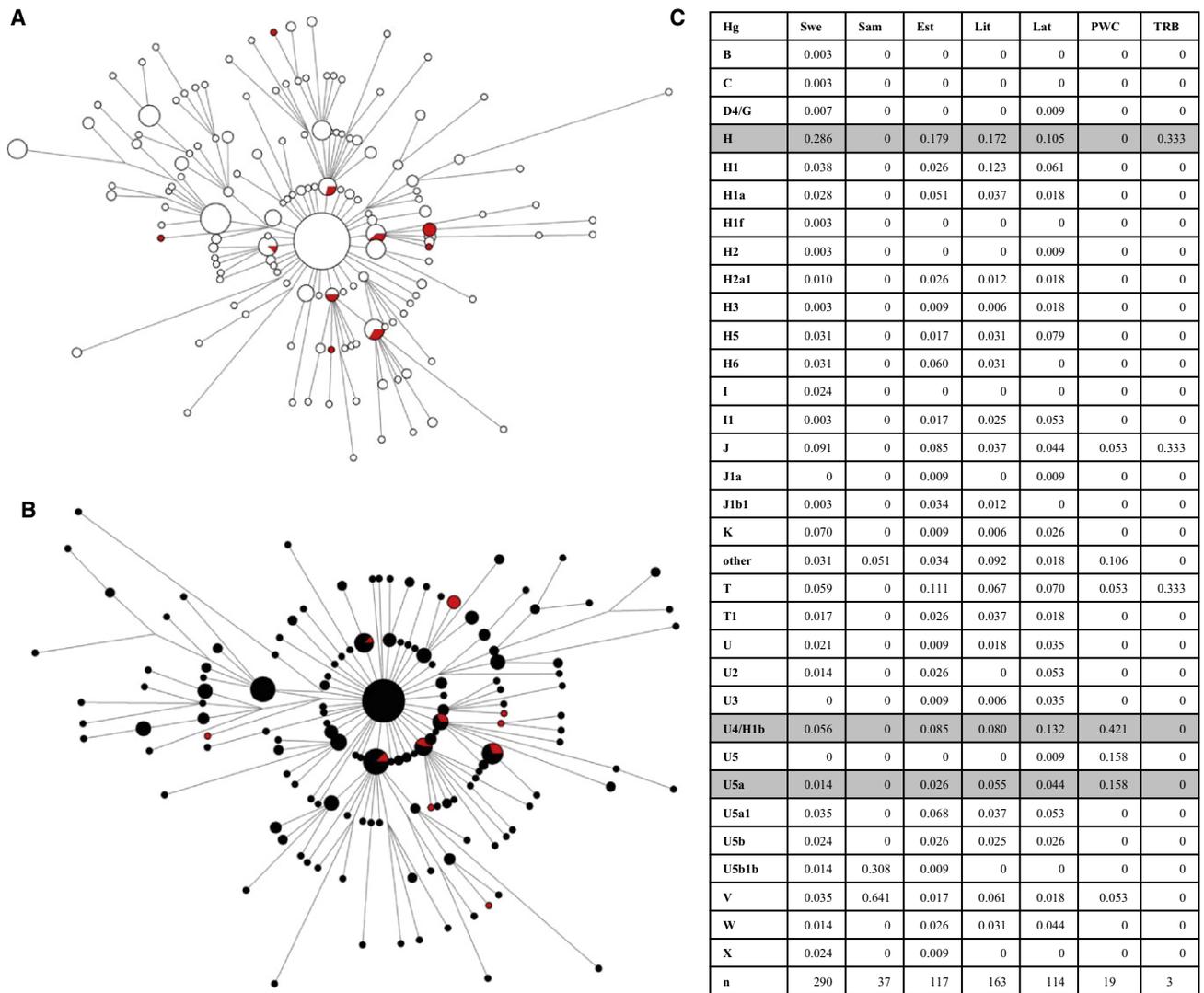


Figure 2. Haplogroup Distributions in Investigated Populations

Reduced-median networks were constructed with 1 as threshold.

(A) PWC (red) shares five haplotypes (Hts) with the 323 Norwegian sequences (white) and keeps five private.

(B) PWC (red) shares four Hts with the 290 Swedish sequences (black) and keeps six private.

(C) The most common Hgs in the PWC sample are rare in the Swedish sample, whereas the frequency is somewhat elevated in the samples from the Baltic peoples.

The following abbreviations are used: Hg, haplogroup; Swe, Swedes; Sam, Saami; Est, Estonians; Lit, Lithuanians; Lat, Latvians; PWC, Pitted Ware culture; and TRB, Funnel Beaker culture.

[26], and modern [4, 5] genetic data suggest that this is probably the case. Thus, theories favoring a Neolithization process that involved population continuity and was mediated by culture exchange only appear increasingly unlikely. Interestingly, however, the data analyses are consistent with a view that the eastern Baltic area remained a genetic refugia for some of the European hunter-gatherer populations. This is in agreement with findings of Mesolithic to Neolithic continuity among Latvian cemeteries [27]. Although the hunter-gatherer lifestyle was culturally replaced here, as in Scandinavia, the populations of the eastern Baltic area may have kept a certain level of population continuity.

Experimental Procedures

Skeletal remains from 74 individuals of eight middle-Neolithic sites were initially selected. Of these, 41 yielded sequence data, but only 22 (19 PWC

and 3 TRB) met all requirements demanded for authenticity (Supplemental Data). A set of nonhuman samples, mainly harp seals (*Phoca groenlandica*, $n = 31$), of the same age and from the same sites as the human remains were used as controls and screened for human DNA (contamination) as well as for putative animal DNA (preservation). The material was extracted in duplicates via a silica spin-column method including chemical decontamination [14] (Supplemental Data).

Quantitative real-time PCR of an 80 bp and a 136 bp coding-region fragment was used to assess the total human mtDNA content in all samples and to screen for appropriate molecular behavior (degradation [14]) (Supplemental Data, Table S1). We amplified the D-loop in seven overlapping fragments of varying size and sequenced them on the Roche Genome Sequencer FLX System to retrieve synthetic clones. Tagged primers were used to provide for individual identification after sequencing [15] (Supplemental Data).

For analyses, we used 316 bp of the D-loop, spanning 16,051–16,383 (16 positions were removed, Supplemental Data). Analysis of molecular variance and F_{st} calculations was carried out with the Arlequin 3.1 software [19]. Networks were constructed with the NETWORK software and the reduced median algorithm [16], with the threshold set for 1. Coalescent

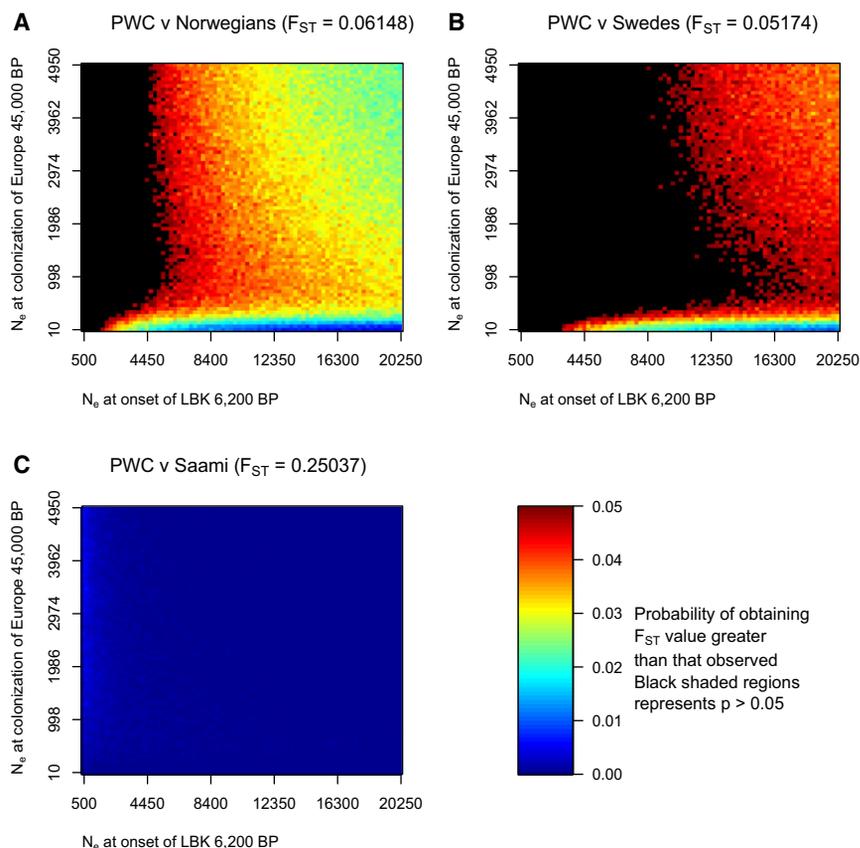


Figure 3. Probabilities of Obtaining the Observed Genetic Differences, as Measured by F_{ST} , between Ancient PWC and Modern Population Samples under a Model of Population Continuity. Population sample comparisons are (A) PWC hunter-gatherers versus modern Norwegians, (B) PWC hunter-gatherers versus modern Swedes, and (C) PWC hunter-gatherers versus modern Saami. Probabilities were calculated by comparing observed F_{ST} values to those obtained by coalescent simulation across a range of combinations of ancestral effective female population sizes at the start of farming in Scandinavia 6,200 years ago (x axis) and the initial colonization of Europe 45,000 years ago (y axis). Observed F_{ST} values are indicated, and black shaded areas indicate p values > 0.05 .

simulations were performed assuming a wide range of combinations of ancestral population size at the Upper Palaeolithic colonization of Europe, 45,000 years ago ($N_e = 10-4,959$, and time of arrival of farming in Scandinavia 6,200 years ago, $N_e = 500-20,350$). Sequences were sampled from each simulation according to the numbers and dates of the data considered, and the proportion of simulated F_{ST} values that were greater than those observed were calculated (details on the evolutionary models are provided in Supplemental Data).

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, five tables, and four figures and can be found with this article online at [http://www.cell.com/current-biology/supplemental/S0960-9822\(09\)01694-7](http://www.cell.com/current-biology/supplemental/S0960-9822(09)01694-7).

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References

1. Armelagos, G.J., and Harper, K.N. (2005). Genomics at the origins of agriculture: Part II. *Evol. Anthropol.* 14, 109–121.
2. Sampietro, M.L., Lao, O., Caramelli, D., Lari, M., Pou, R., Martí, M., Bertranpetit, J., and Lalueza-Fox, C. (2007). Palaeogenetic evidence supports a dual model of Neolithic spreading into Europe. *Proc. Biol. Sci.* 274, 2161–2167.
3. Zvelebil, M. (2004). In *Traces of Ancestry: Studies in Honour of Colin Renfrew*, M. Jones, ed. (Cambridge: McDonald Institute Monographs).
4. Richards, M.B., Macaulay, V.A., Bandelt, H.J., and Sykes, B.C. (1998). Phylogeography of mitochondrial DNA in western Europe. *Ann. Hum. Genet.* 62, 241–260.
5. Semino, O., Passarino, G., Oefner, P.J., Lin, A.A., Arbuzova, S., Beckman, L.E., De Benedictis, G., Francalacci, P., Kouvatsi, A., Limborska, S., et al. (2000). The genetic legacy of paleolithic *Homo sapiens sapiens* in extant europeans: A Y chromosome perspective. *Science* 290, 1155–1159.
6. Stenbäck, N. (2003). Människorna vid havet. Platser och keramik på ålandsöarna perioden 3500–2000 f.Kr. PhD thesis, Stockholm University. In *Stockholm Studies in Archaeology*, Volume 28 (Stockholm: Stockholm University).
7. Richards, M.P., Schulting, R.J., and Hedges, R.E. (2003). Sharp shift in diet at onset of Neolithic. *Nature* 425, 366.
8. Lidén, K., Eriksson, G., Nordqvist, B., Götherström, A., and Bendixen, E. (2004). "The wet and the wild followed by the dry and the tame" – or did they occur at the same time? Diet in Mesolithic - Neolithic southern Sweden. *Antiquity* 78, 23–33.
9. Fischer, A. (2002). Food for Feasting? An evaluation of explanations of the neolithisation of Denmark and southern Sweden. In *The Neolithisation of Denmark – 150 Years of Debate*. Sheffield Archaeological Monographs 12, A. Fischer and K. Kristensen, eds. (Sheffield: J.R. Collis Publications).
10. Kossinna, G. (1909). Der Ursprung der Urfinnen und der Urindogermanen und ihre Ausbreitung nach dem Osten. *Mannus* 7, 17–52 and 225–245.

11. Malmer, M.P. (1969). Gropkeramikplatsen Jonstorp. *Antikvariskt Arkiv*, Volume 36 (Stockholm: Kungl. Vitterhets Historie och Antikvitets Akademien).
12. Browall, H. (1991). In *Västsvenska Stenåldersstudier*, Volume 8, H. Browall, P. Persson, and K.G. Sjögren, eds. (Göteborg: GOTARC), pp. 111–142.
13. Almgren, O. (1907). Nordiska stenåldersskulpturer. *Fornvännen* 2, 113–125.
14. Malmström, H., Svensson, E.M., Gilbert, M.T., Willerslev, E., Götherström, A., and Holmlund, G. (2007). More on contamination: The use of asymmetric molecular behavior to identify authentic ancient human DNA. *Mol. Biol. Evol.* 24, 998–1004.
15. Binladen, J., Gilbert, M.T., Bollback, J.P., Panitz, F., Bendixen, C., Nielsen, R., and Willerslev, E. (2007). The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing. *PLoS ONE* 2, e197.
16. Bandelt, H.J., Forster, P.B., Sykes, C., and Richards, M.B. (1995). Mitochondrial portraits of human populations. *Genetics* 141, 743–753.
17. Kivisild, T., Tolk, H.V., Parik, J., Wang, Y., Papiha, S.S., Bandelt, H.J., and Villems, R. (2002). The emerging limbs and twigs of the East Asian mtDNA tree. *Mol. Biol. Evol.* 19, 1737–1751.
18. Richards, M., Macaulay, V., Hickey, E., Vega, E., Sykes, B., Guida, V., Rengo, C., Sellitto, D., Cruciani, F., Kivisild, T., et al. (2000). Tracing European founder lineages in the Near Eastern mtDNA pool. *Am. J. Hum. Genet.* 67, 1251–1276.
19. Excoffier, L., Laval, G., and Schneider, S. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol. Bioinform. Online* 1, 47–50.
20. Tillmar, A.O., Coble, M.D., Wallerström, T., and Holmlund, G. (2009). Homogeneity in mitochondrial DNA control region sequences in Swedish subpopulations. *Int. J. Leg. Med.*, in press. Published online July 10 2009. 10.1007/s00414-009-0354-7.
21. Helgason, A., Hickey, E., Goodacre, S., Bosnes, V., Stefánsson, K., Ward, R., and Sykes, B. (2001). mtDNA and the islands of the North Atlantic: Estimating the proportions of Norse and Gaelic ancestry. *Am. J. Hum. Genet.* 68, 723–737.
22. Lappalainen, T., Laitinen, V., Salmela, E., Andersen, P., Huoponen, K., Savontaus, M.L., and Lahermo, P. (2008). Migration waves to the Baltic Sea region. *Ann. Hum. Genet.* 72, 337–348.
23. Ho, S.Y., and Endicott, P. (2008). The crucial role of calibration in molecular date estimates for the peopling of the Americas. *Am. J. Hum. Genet.* 83, 142–146.
24. Brace, C.L., Nelson, A.R., Seguchi, N., Oe, H., Sering, L., Qifeng, P., Yongyi, L., and Tumen, D. (2001). Old World sources of the first New World human inhabitants: A comparative craniofacial view. *Proc. Natl. Acad. Sci. USA* 98, 10017–10022.
25. Brace, C.L., Seguchi, N., Quintyn, C.B., Fox, S.C., Nelson, A.R., Manolis, S.K., and Qifeng, P. (2006). The questionable contribution of the Neolithic and the Bronze Age to European craniofacial form. *Proc. Natl. Acad. Sci. USA* 103, 242–247.
26. Haak, W., Forster, P., Bramanti, B., Matsumura, S., Brandt, G., Tänzer, M., Villems, R., Renfrew, C., Gronenborn, D., Alt, K.W., and Burger, J. (2005). Ancient DNA from the first European farmers in 7500-year-old Neolithic sites. *Science* 310, 1016–1018.
27. Larsson, L., and Zagorska, I. (2006). Back to the origin: research in the Mesolithic-Neolithic Zvejnieki cemetery and environment, northern Latvia. *Acta Archaeologica Lundensia*, Volume 52 (Stockholm: Almqvist & Wiksell International).