Tracing the Phylogeography of Human Populations in Britain Based on 4th–11th Century mtDNA Genotypes

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Some of the transitional periods of Britain during the first millennium A.D. are traditionally associated with the movement of

Dover, Norton at Cleveland Market (Sherlock and Welch 1992), Market Lavington at Salisbury, and Castle Mall at Norwich. The excavations were all recent (between 1983 and 1993), and all samples have been kept in room temperature storage with minimal handling (only as needed for labeling and record keeping). Some teeth were from intact mandibles and are not known to have been handled at all. This is important as studies have found a high proportion of contaminants on museum material (Malmstrom et al. 2005), but the contamination level varies (e.g., Noonan et al. 2005) presumably as a consequence of the extent of handling.

The site at Leicester was known to have been a Roman-British cemetery. At least 21 graves contained coffins (detected from the presence of nails), and their position was east to west. Although there were no grave goods, other deposited objects (e.g., coins) indicated a date of fourth century. The cemetery at Norton seems from its size and grave

Authenticated ancient samples showed appropriate aDNA molecular behavior (such as signs of depurination and deamination lesions, arising due to oxidation and hydrolysis) including chimeric sequences with a preponderance of C/T heteroplasmic positions (Hofreiter et al. 2001; Gilbert et al. 2003). This is observed as double or "heterozygous" peaks in the trace sequence and as differences among clones. Ancient template is also indicated by their failure to amplify an alternative 400-bp PCR fragment (using conserved human primers for the mtDNA control region, Vigilant et al. 1989). No sequences showed obvious conflict with haplogroup-defining segregating sites, and all were consistent with modern European lineages. Cloned sequences also behaved as expected in aDNA work. In one case where the sequence was cloned in two overlapping fragments, all five clones showed two polymorphic sites while three clones showed a third site. These clones also showed some unique nonreproducible sites, presumably due to lesions in the DNA. In another case, 2 out of 21 clones had all five polymorphic sites that had been replicated from two other tooth extracts, while 19 were laboratory member sequences or derivations of the expected sequence. Four samples were replicated among independent laboratories. The full sequences of two haplotypes were confirmed at Oxford and Arizona, and a 150-bp subsequence (representing all polymorphic sites but one) was confirmed for two haplotypes at the Smithsonian (all four of these haplotypes were unique to the ancient sample set).

Phylogenetic Analyses

RMNs for the ancient populations are shown in figure 1. Despite the short sequence analyzed, most of the major Eurasian haplogroups and subhaplogroups can be identified in both networks. However, a marked difference between the early and late Saxon sites can also be seen. The early site shows a large proportion of sub-hg U5a1 and U5a1a, and also I, V, and W, while the later site at Norwich shows a large proportion of sub-hg T1. Sub-hg T1 is still relatively common in modern England, but absent from the fourth to seventh century sites (which present sub-hg T4 and T5). The RMN for the early site also seemed more

Genetic Distances

Genetic distances based on haplogroup and subhaplogroup frequencies were calculated, and the resulting matrix was reduced to two-dimensions by means of an MDS plot (fig. 3). This result is very consistent with our results for the rho distances. The ancient samples are well separated from the cluster of modern populations; however, the late Saxon site shows closer genetic distances to populations situated to the north (Scotland, Estonia, Norway, Finland, and Western Isles), while the early Saxon site is closer to more southern populations (Germany and Spain, as well as the Near Eastern populations of Armenia and Palestine). The MDS data are also consistent with the RMNs shown in figure 2, as the early and late ancient samples show differing representations of modern haplogroups in the network analysis (also seen in table 2).

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We compared ancient mtDNA sequences from two different periods and different locations in southern and central England with modern samples from Europe and the Middle East. We found a different representation of haplogroups in the two ancient samples grouped by period. Sub-hg U5a1, part of hg U5, which is believed to have originated or spread in Europe during the Upper Paleolithic, is well represented in the early ancient sample. On the other hand, the late ancient site shows a large proportion of sub-hg T1, which is associated with the Neolithic expansion (Richards et al. 2002). This suggests a different composition of the two temporal samples, which may be associated with different geographic origins. Although our sample sizes did not permit a test of geographic differentiation for a given time period, the geographic distances are small, and the time frame reflects a period when there are known to have been major demographic changes. We therefore emphasize the comparison between early and late Saxon sites.

The sample from northern Germany, representing the geographic region from which some Saxon groups originated, was not among the genetically closest founder populations for the early Saxon group (based on the rho data). This is in agreement with modern Y chromosome data that indicated a limited continental input from North Germany in southeast England, where most of the Anglo-Saxon settlements were located (Capelli et al. 2003). Our founder analyses showed a clear inversion of the genetically closer

Tab 9 1
R D ta c9 B9t 99 t 9 A c9 ta S9 9a M 9 P at

Ancient (48)		Early Ancient[a] (31)		Late Ancient[b] (17)		England[c] (20)		England (150)	
Pop[d]	Rho[e]	Pop	Rho	Pop	Rho	Pop	Rho ± SD	Pop	Rho
EN	0.729	**ES**	**0.581**	PA	0.588	ES	0.595 ± 0.168	ES	0.587
ES	0.771	EN	0.613	EN	0.941	SC	0.615 ± 0.158	SC	0.596
PA	0.771	**NO**	**0.731**	NG	0.941	NG	0.671 ± 0.184	NG	0.647
NO	0.889	**FI**	**0.742**	AR	1.000	SP	0.686 ± 0.201	SP	0.660
FI	0.896	**SC**	**0.812**	SP	1.059	NO	0.719 ± 0.203	NO	0.704
NG	0.896	SP	0.839	**ES**	**1.118**	PA	0.740 ± 0.196	PA	0.713
SP	0.917	NG	0.871	**SC**	**1.157**	FI	0.740 ± 0.221	FI	0.720
SC	0.934	PA	0.871	**FI**	**1.176**	WI	0.817 ± 0.251	WI	0.807
AR	1.083	WI	0.935	**NO**	**1.176**	NF	0.838 ± 0.292	AR	0.807
WI	1.167	IC	0.989	WI			6 0.158		

putative source populations for the early and late Saxon British sites. Northern populations were closest to the early ancient sites, while most southern populations were closer to the late Saxon site (northern France showed unexpectedly high rho values, even against modern England). Although resampling analyses showed potential bias using the rho statistic both for varying source population size and for small putative founder population size, we demonstrate the magnitude of these biases and find a well-supported difference even after taking these factors into account. The difference was reinforced in the MDS analysis where the early and late samples did not cluster together but clustered closer to northern and southern samples, respectively. The geographic relationship of these populations (as opposed to historical connection due to early medieval migration/invasion) seems to suggest deeper common ancestry among northern populations across Europe. This supports new findings as well as some new interpretations of earlier archaeological data, suggesting a common Mesolithic culture from Britain to Estonia (Clark 1936; Coles 1998; Pettitt 2003).

According to some archaeological interpretations based on the scarcity of archaeological findings, northern Europe was abandoned as people moved to southern refuges during the peak of the last glacial maximum (Mellars 1974; Evans 1975), from where they reexpanded north when the climate improved (Housley et al. 1997). However, others have proposed that some people could have stayed in the north, especially in areas that are now submerged under the North Sea (Coles 1998). When the Scandinavian and British ice sheets reached their maximum extent and the North Sea as a consequence receded to its lowest level (Fairbanks 1989) Britain was connected to the continent by a land bridge. This dry land, referred to as Doggerland, is now believed to have lasted longer and been larger than previously thought and may have been inhabited (Wymer 1991; Coles 1999). After the ice receded, they could have radiated out from those regions into communities further north (illustrated in fig. 4). Some of those now in the north of Europe (e.g., northern Germany) may represent people who migrated from the south, while some people in that region historically moved further north.

Coles (1999) proposed that the occupation of this land bridge may have played a role in delaying the onset of the Neolithic in Britain and Scandinavia, as a consequence of encounters between the Mesolithic northern populations and the agriculturist newcomers. Case (1969), in reference

The different apparent source populations at the Norwich site may reflect a greater influence of postglacial migrations from the south and Near East in this region, or this particular site, or during this time period. This site was also likely under Danelaw at this time and under the influence of the Danish Vikings. Modern England appears to continue to reflect these influences, though further Viking invasions and later immigration and emigration would have influenced the modern genotypic pattern as well. Modern data on Y chromosome analysis has also suggested that the Danes had a great impact in this area (Capelli et al. 2003). Immigration of women to Norwich at this time is suggested by the fact that modern England retains hg T1 (at 2.5%), which is found in Norwich (23%) but absent from the early Saxon sites. Although the site at Norwich is likely to have been larger and more cosmopolitan than the other sites in our analysis, there is nothing about the cemetery at Castle Mall to indicate that it represented anyone other than people local to the Norwich area at that time.

Our analyses of mtDNA sequences from ancient and modern Europe show a distinct pattern for the different time periods sampled. Unfortunately, the late ancient sample size is relatively small, but the resulting pattern is nonrandom and seems to support archaeological findings. The prevailing theory for numerous species is that populations in Europe expanded from southern refugia after the last glacial maximum (see Hewitt 2000), though evidence exists for various northern refugia as well (see review in Stewart and Lister 2001). For humans, modern mtDNA data has indicated the importance of the Franco-Cantabrian refuge during the glacial period (Achilli et al. 2004; Pereira et al. 2005). However, this is not necessarily in conflict with the interpretation of our data. We present data that for the first time analyses genotypes from the pre-Saxon/early Saxon period, and these are uniquely able to assess signatures for relatedness from this period of demographic change. A similar study using aDNA provided new information on the source of founding populations in the Canary Islands (Maca-Meyer et al. 2004). Even if the majority of the human population of northern Europe emigrated south during the LGM, a proportion remaining behind could be responsible for the genetic signature we have identified.

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Achilli, A., C. Rengo, C. Magri et al. (21 co-authors). 2004. The molecular dissection of mtDNA haplogroup H confirms that the Franco-Cantabrian glacial refuge was a major source for the European gene pool. Am. J. Hum. Genet. **75**:910–918.

Ammerman, A. J., and L. L. Cavalli-Sforza. 1971. Measuring the rate of spread of early farming in Europe. Man **6**:674–688.

Anderson, S., A. Bankier, B. Barrel et al. (13 co-authors). 1981. Sequence organisation of the human mitochondrial genome. Nature **290**:457–465.

Bandelt, H. J., P. Forster, B. C. Sykes, and M. B. Richards. 1995. Mitochondrial portraits of human populations using median networks. Genetics **141**:743–753.

Capelli, C., N. Redhead, J. K. Abernethy et al. (15 co-authors). 2003. A Y-chromosome census of the British Isles. Curr. Biol. **13**:979–984.

Case, H. 1969. Neolithic explanations. Antiquity **43**:176–186.

-259.4(and)-260.13i3g8farmiDb—427(and)-344278TDopolus42Phyp polymorphisms in five French groups: importance of regional sampling. Eur. J. Hum. Genet. **12**:293–300.

Dyer, J. 1990. Ancient Britain. Batsford, London.

Evans, B. 1975. The environment of early man in the British Isles. University of California Press, Berkeley, Calif.

Fairbanks, R. G. 1989. A 17,000-year glacio-eustatic sea level record: influence of glacial melting rates on the Younger Dryas event and deep-ocean circulation. Nature **342**:637–642.

Falsetti, A. B., and R. R. Sokal. 1993. Genetic structure of human populations in the British Isles. Ann. Hum. Biol. **20**:215–229.

Fischer, A. 1991. Pioneers in deglaciated landscapes: the expanhairs from southern African population. Proc. Natl. Acad. Sci. USA **86**:9350–9354.

Weale, M. E., D. A. Weiss, R. F. Jager, N. Bradman, and M. G. Thomas. 2002. Y chromosome evidence for Anglo-Saxon mass migration. Mol. Biol. Evol. **19**:1008–1021.

Wilson, J. F., D. A. Weiss, M. Richards, M. G. Thomas, N. Bradman, and D. B. Goldstein. 2001. Genetic evidence for different male and female roles during cultural transitions in the British Isles. Proc. Natl. Acad. Sci. USA **98**:5078–5083.

Wymer, J. 1991. Mesolithic Britain. Shire, Princes Risborough, UK.

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