Feasibility and utility of microsatellite markers in archaeological cattle remains from a Viking Age settlement in Dublin

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Summary

Nineteen cattle bones from the Viking 10th and early 11th century levels in Dublin were assessed for presence of reliable genotypes from three autosomal markers. Due to the good preservational condition of the samples, it was possible to amplify and type at least two out of three of the microsatellite markers (*CSRM60*, *HEL1* and *ILSTS001*) in 11 specimens. Full three-loci genotypes were obtained from a subset of seven of these samples. A comparative analysis was performed using data from the same three markers in 11 extant British, Irish and Nordic cattle breeds. Although the medieval remains displayed lower levels of diversity than the modern European breeds, the results fit within the ranges obtained from the extant populations. The results indicate a probable origin for the ancient Irish cattle as the remains group significantly more closely with breeds from the British Isles than with those from Scandinavia. The data collected indicate that microsatellites may be useful for the further study of ancient cattle.

Keywords ancient DNA, biomolecular archaeology, breed relationships, European cattle, medieval Dublin, microsatellite.

Introduction

Ancient DNA (anDNA) research in livestock has focused primarily on the analysis of mitochondrial DNA (mtDNA) variation (for example Bailey *et al.* 1996; MacHugh *et al.* 1999; Watanobe *et al.* 1999; Troy *et al.* 2001; Vila *et al.* 2001; Bar-Gal *et al.* 2002). Ancient cattle studies have been highly informative in tracing underlying patterns of domestication, although the information content of cattle mtDNA haplotypes imposes limitations on their use, particularly among Western European cattle populations that are recognized as displaying little or no mtDNA population genetic structure (Troy *et al.* 2001). However, microsatellite loci, also known as short tandem repeat (STR) polymorphisms, have been shown to provide a breed-level genetic resolution within cattle (MacHugh *et al.* 1997, 1998), and it is therefore desirable to extend microsatellite-based studies from extant populations to archaeological specimens. Although microsatellites have been successfully amplified in ancient human remains (Ramos *et al.* 1995; Burger *et al.* 1999), this application to remains from domestic animals is novel.

During the 840s AD, Scandinavian longphorts or shipping ports became established in Dublin and Annagassan, County Louth, serving as both permanent trading stations and shelter for the Norse invaders. In 902 AD, the Dublin Vikings were banished to northern England by a coalition of Irish kings, but returned in 917 AD to establish a real town (termed as dún) at Dublin. This town, called Dubh Linn (the black pool), was situated near the natural harbour formed by the boundaries of the River Liffey to the north and the River Poddle to the east, downstream from the original longphort. This resettled site evolved into an enclosed town with an extensive network of streets, buildings and plots

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Accepted for publication 5 July 2003

(Wallace 1992). Although Scandinavian and English evidence places the end of the Viking Age in the middle of the 11th century, the Scandinavians in Ireland blended with the indigenous population to produce the Hiberno-Norse culture, and these people remained unchallenged until the arrival of the Anglo-Normans in 1169 AD (Wallace 1981).

One of the best-preserved areas of the Viking settlement was uncovered in Fishamble Street, during excavations commenced in 1962 by The National Museum of Ireland. Due to the damp climate and waterlogged state of the site, most of the material had been conserved under anoxic conditions and the quality of preservation was exceptional (Wallace 1984). Although it is more widely accepted that domesticated cattle remains present in and around medieval Dublin were indigenous animals taken from the surrounding areas (Wallace 1987), it is possible that they may have included imported cattle from England or Scandinavia and their status is unclear. In order to uncover the origins of these cattle remains, microsatellite analysis was performed on a panel of 19 cattle bones recovered from plots 1 and 2 in Fishamble Street. Previous examination of mtDNA variation did not determine the origins of the domesticated animals (MacHugh *et al.* 1999), but it was anticipated that the increased information content offered by microsatellite markers could unravel breed associations between the ancient cattle and relevant extant populations.

Materials and methods

Archaeological material collection from the Fishamble Street excavations

Nineteen cattle calcaneus (ankle) bones were retrieved from the Fishamble Street site (Table 1). Using morphometric criteria these skeletal elements were ascertained to include a minimum number of 16 individuals. The preservation environment was essentially anoxic, as demonstrated by the excellent preservation of timber used for construction of dwellings on the site (Wallace 1992). Coins and dendrochronological evidence indicates that the material dates to between about 920 and 1020 AD.

Previous studies of ancient DNA survival have found a good correlation between relatively subjective measures of bone preservation and the presence of amplifiable endogenous DNA (Richards *et al.* 1995; Colson *et al.* 1997). The Fishamble Street bovine bone material was dense, with

Table 1 Cattle calcaneus bones analysed from the Fishamble Street excavations, with associated laboratory codes and PCR amplification results for each of the three microsatellites in base pairs (bp).

			Microsatellite locus											
			CSRM60				*HEL1*				*ILSTS001*			
Laboratory code	Extractions[1]	Number of PCRs	AA[2]	AB[3]	BB[4]	Haplotype (bp)	AA[2]	AB[3]	BB[4]	Haplotype (bp)	AA[2]	AB[3]	BB[4]	Haplotype (bp)
WQ01a	2/2	12	n/a	n/a	n/a	None	10	0	0	(111, 111)	12	0	0	(93, 93)
WQ01b	0/2	n/a	–	–	–	n/a	–	–	–	n/a	–	–	–	n/a
WQ02	0/2	n/a	–	–	–	n/a	–	–	–	n/a	–	–	–	n/a
WQ03	2/5	16	10	0	0	(101, 101)	12	0	0	(113, 113)	15	0	0	(93, 93)
WQ04a	2/3	16	n/a	n/a	n/a	none	10	0	0	(113, 113)	10	0	0	(93, 93)
WQ04b	3/5	20	1	11	0	(103, 105)	20	0	0	(113, 113)	3	17	0	(93, 95)
WQ05	0/2	n/a	–	–	–	n/a	–	–	–	n/a	–	–	–	n/a
WQ06	0/2	n/a	–	–	–	n/a	–	–	–	n/a	–	–	–	n/a
WQ07a	0/2	n/a	–	–	–	n/a	–	–	–	n/a	–	–	–	n/a
WQ07b	2/4	16	1	9	0	(103, 105)	3	12	0	(103, 113)	13	0	0	(93, 93)
WQ08	0/3	n/a	–	–	–	n/a	–	–	–	n/a	–	–	–	n/a
WQ09	2/4	16	0	10	1	(103, 105)	1	11	0	(105, 113)	12	0	0	(93, 93)
WQ10	0/2	n/a	–	–	–	n/a	–	–	–	n/a	–	–	–	n/a
WQ11	2/4	12	0	12	0	(103, 105)	0	12	0	(113, 113)	8	0	0	(93, 93)
WQ12	3/4	22	n/a	n/a	n/a	None	4	10	1	(105, 113)	21	0	0	(93, 93)
WQ13	2/4	17	1	12	0	(103, 105)	17	0	0	(113, 113)	16	0	0	(93, 93)
WQ14	0/2	n/a	–	–	–	n/a	–	–	–	n/a	–	–	–	n/a
WQ15	2/4	12	n/a	n/a	n/a	None	12	0	0	(113, 113)	12	0	0	(93, 93)
WQ16	2/4	10	10	0	0	(103, 103)	10	0	0	(113, 113)	10	0	0	(93, 93)

n/a, not applicable.

[1]Number of extractions yielding amplification products/total number of extractions.

[2]In repeated amplifications, AA, total number of allele 1 homozygotes observed.

[3]AB, total number of heterozygotes observed.

[4]BB, total number of allele 2 homozygotes observed.

undamaged external surfaces, and could therefore be considered well preserved using the gross morphological criteria outlined by Hagelberg *et al.* (1991). These samples included the subset of bones used in a previous mitochondrial study conducted by MacHugh *et al.* (1999).

DNA extraction and amplification of archaeological specimens

Bone samples were prepared using the modified Yang *et al.* (1998) procedure described by MacHugh *et al.* (2000). The number of DNA extractions performed for each specimen are indicated in Table 1. Polymerase chain reaction (PCR) set-up was conducted in a laboratory dedicated solely to pre-amplification ancient work. As it has been demonstrated that ancient DNA is inevitably fragmented and degraded (Handt *et al.* 1994), the three microsatellites were chosen primarily for their small allele size ranges: *CSRM60* (*D10S5*), 83–111 base pairs (bp); *HEL1* (*D15S10*), 101–117 bp; and *ILSTS001* (*D7S13*), 77–97 bp. Primer details were taken from Kaukinen and Varvio (1993); Barendse *et al.* (1994), and Kemp *et al.* (1995), respectively.

A first-round of PCR was carried out in a dedicated PCR machine, and PCR amplifications were in 20 μl reaction volumes containing 10× PCR buffer [10 mM Tris HCl (pH 8.8); 50 mM KCl; 0.1% Triton X-100], 2.5 mM $MgCl_2$, 200 μM dNTPs, 4 μM of both the forward and the reverse primers, and 0.5 units of PLATINUM® *Taq* polymerase (Life Technologies, Carlsbad, CA, USA). A 2 min denaturation step at 94 °C was followed by 20 cycles of 1 min denaturation at 94 °C, 1 min of annealing at 55 °C, and 1 min of extension at 72 °C, followed by a final 4 min extension step at 72 °C. These faint products were then visualized on 1.5% agarose minigels. Several extraction and PCR blanks were always included and remained negative throughout.

Positive samples, obtained from 11 of the 19 bones (Table 1), were cleaned using a Concert™ rapid purification kit (GIBCO BRL®; used according to the manufacturers instructions with DNA eluted in 50 μl of TE). A second-round, radioactively-labelled PCR was then performed in the main analytical laboratory. Reactions took place in 96-well microtitre plates with 5.5 μl of cleaned DNA in 10 μl reaction volumes. The reaction conditions were as before, except that the concentration of dCTP in the dNTP mix was reduced to 10 μM, and the concentration of magnesium chloride was varied depending upon the marker used (*CSRM60* at 1.5 mM; *HEL1* and *ILSTS001* at 1.0 mM). ^{32}P radionucleotide was randomly incorporated during the PCR amplification by the addition of 0.5 μCi [α-^{32}P] dCTP at 3000 Ci $mmol^{-1}$ per reaction. The reactions were carried out with the following cycling parameters: 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 54–58 °C for 30 s (*CSRM60* at 55 °C; *HEL1* at 54 °C; *ILSTS001* at 58 °C) and 72 °C for 30 s. A final extension of 4 min at 72 °C was also carried out. The radiolabelled products were then assessed for length variation using standard 6% polyacrylamide sequencing gel electrophoresis and autoradiography. M13mp18 phage vector was used to provide sequence ladders for allele size calibration. This technique was precise enough to reproducibly resolve single repeat unit differences.

The criteria for authenticating microsatellite genotypes were those described by Burger *et al.* (1999). In brief, DNA was extracted from each sample at least twice (Table 1), and each extract was amplified by PCR at least five times. To counteract the possibility of null alleles or the generation of false chimeric alleles, a modification of the approach advocated by Taberlet *et al.* (1996) was also used. If highly ambiguous results were obtained, an allele was only recorded if it was observed at least three times from the five positive PCR products, and an individual was only scored as homozygous if the allele was seen in all five independent amplifications. These results also had to be duplicated in a second extract. The analysis of five products from each sample-locus combination reduces the probability of false-homozygous typing to 0.008 (cited in Schmerer *et al.* 1999).

Amplification of modern specimens

For the purpose of this study, the positive ancient results have been collated into a single group, although the medieval samples are not strictly a single panmictic population as they came from deposits ranging in age from 920 to 1020 AD, covering approximately 20 cattle generations. The ancient microsatellite data obtained (Table 1) was compared with 11 extant breeds (Tables 2 & 3, Fig. 1). The 12 populations studied comprise the ancient group from Fishamble Street and 11 European breeds: five British Isles, one indigenous Irish, four Scandinavian and one Continental (Table 2). Data for these populations were taken partially from previously analysed results (MacHugh *et al.* 1997, 1998; Loftus *et al.* 1999; Cymbron *et al.* in preparation) and new data were also collected.

Statistical and phylogenetic analysis

To assess the levels of genetic variability in the 12 populations, both the mean number of alleles per locus (MNA) and gene diversity (average expected heterozygosity) values were estimated (Table 2). Unbiased estimates of gene diversity and observed heterozygosity values, with associated standard errors, were calculated according to Nei (1987). Allele frequencies were determined by direct counting. The MNA values have been shown to be inadequate estimates of genetic variation if the sample sizes for each population are notably different (Valsecchi *et al.*

Table 2 Populations included in the analysis and their country of origin, including the total number of individuals sampled (in parentheses) and the associated two letter codes. The average heterozygosities and mean number of alleles (MNA) are also included, with associated standard deviations.

Breed	Code	Origin of breed (no. of animals)	Average heterozygosity		MNA (full sample)[1]	MNA (uniform sample)[2]
			Expected	Observed		
Ancient group						
Fishamble Street	WQ	920–1020 AD Dublin, Ireland (11)	0.38 ± 0.16	0.36 ± 0.11	3.0 ± 0.58	2.7 ± 0.26
Britain						
Aberdeen Angus	AN	Northeast Scotland (33)	0.41 ± 0.17	0.41 ± 0.09	3.7 ± 1.20	2.7 ± 0.40
Galloway	GA	Galloway, Scotland (23)	0.43 ± 0.22	0.50 ± 0.15	4.0 ± 1.53	2.9 ± 0.38
Hereford	HE	Western England (34)	0.42 ± 0.14	0.43 ± 0.09	4.3 ± 0.88	2.8 ± 0.39
Highland	HI	West Highlands, Scotland (35)	0.42 ± 0.20	0.46 ± 0.14	4.3 ± 1.45	2.9 ± 0.37
Jersey	JE	Jersey (34)	0.49 ± 0.24	0.48 ± 0.14	4.0 ± 1.15	3.2 ± 0.31
Ireland						
Kerry	KE	Southwest Ireland (40)	0.48 ± 0.10	0.49 ± 0.07	2.7 ± 0.33	2.5 ± 0.18
Scandinavia						
Iceland	IC	Iceland (15)	0.68 ± 0.02	0.58 ± 0.06	3.7 ± 0.33	3.5 ± 0.20
Norwegian Red	NO	Norway (15)	0.50 ± 0.18	0.46 ± 0.09	3.7 ± 0.88	3.1 ± 0.30
Telemark	TK	Telemark, Norway (15)	0.52 ± 0.11	0.43 ± 0.03	3.7 ± 0.67	3.2 ± 0.30
Western Fjord	WF	Southwest Norway (15)	0.65 ± 0.13	0.63 ± 0.11	4.3 ± 0.33	4.0 ± 0.28
Continental						
Charolais	CH	Southeast France (36)	0.63 ± 0.11	0.64 ± 0.05	4.7 ± 1.20	3.9 ± 0.37

[1]The MNA was calculated using all the animals from each population sampled.

[2]Uniform population sizes were used for the second column of MNA values. A random sample of seven animals was used for each population (see text for a more detailed explanation). Associated standard errors were negligible.

Table 3 Genetic distance (D_A) matrix obtained from the frequencies of three microsatellite markers among 12 breeds. Genetic distances between the 11 extant breeds and the Fishamble Street population are ranked in column 1, with the British Isles breeds underlined.

Population	Fishamble Street	Hereford	Jersey	Charolais	Galloway	Highland	Aberdeen Angus	Norwegian Red	Kerry	Western Fjord	Telemark
Hereford	0.0922	0.0000									
Jersey	0.1232	0.1231	0.0000								
Charolais	0.1258	0.1331	0.1357	0.0000							
Galloway	0.1284	0.1733	0.0821	0.1358	0.0000						
Highland	0.1353	0.1136	0.1107	0.1730	0.1079	0.0000					
Aberdeen Angus	0.1652	0.2121	0.0736	0.1984	0.1623	0.1440	0.0000				
Norwegian Red	0.1694	0.1622	0.0893	0.1179	0.0808	0.0821	0.1586	0.0000			
Kerry	0.2065	0.2079	0.2114	0.2457	0.2329	0.1926	0.2909	0.1783	0.0000		
Western Fjord	0.2367	0.1803	0.1487	0.0961	0.1457	0.1525	0.2180	0.0992	0.2767	0.0000	
Telemark	0.2970	0.3201	0.1905	0.1634	0.0974	0.2353	0.2802	0.0906	0.2789	0.1508	0.0000
Iceland	0.4745	0.4005	0.4030	0.2867	0.3198	0.4289	0.5329	0.3321	0.5667	0.2303	0.3253

1997). To compensate for the large differences between the sample sizes, the MNA values were compared between the populations using a resampling approach. The smallest number of alleles equalled 14 for marker *CSRM60* in the ancient Dublin population. Fourteen random alleles were selected without replacement from each of the breeds, and this approach was repeated 1000 times. This calculation was performed using a Microsoft Excel©-based microsatellite program (Park 2002). An exact test for deviations from Hardy–Weinberg equilibrium (HWE) was performed using the ARLEQUIN computer program (Version 2.000; Schneider *et al.* 1997) with a Markov chain set to 10 000 steps with 1000 steps of dememorization.

Interbreed genetic distances (D_A) were estimated as described in Nei *et al.* (1983) using the DISPAN computer program (T. Ota, Center for Human Genetics, Boston University). Multidimensional scaling (MDS; Kruskal 1964) was performed based on the D_A matrix obtained (Table 3) using the SPSS© programme (Standard Version, release 10.0.5). The two-dimensional MDS plot is shown in Fig. 1. The SPSS programme was also used to perform a Mann–Whitney rank-sum test (Mann & Whitney 1947).

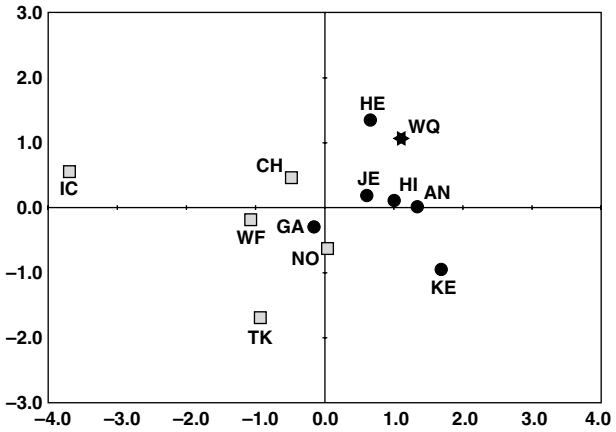

Figure 1 A two-dimensional multidimensional scaling plot, drawn using data from three microsatellites, summarizing D_A genetic distances among 11 extant European breeds and the medieval Fishamble Street population. Black circles represent the British and Irish breeds, grey squares denote the Scandinavian and Continental breeds and a black star corresponds to the Fishamble Street population. The two-letter codes are as in Table 2. The ancient population groups more closely with the British Isles breeds than those from Scandinavia.

Results

Authenticity of archaeological results

As detailed in Table 1, it was possible to generate positive amplification products from at least two of the three microsatellites in 11 of the 19 cattle bones from the Wood Quay site. This success rate of approximately 58% is in general agreement with Richards *et al.* (1995), who suggest that more than 50% of skeletal remains from the past 2000 years are likely to contain endogenous DNA. To evaluate the possibility of contamination, numerous extraction and PCR controls were used for all DNA isolation, purification and amplification steps, and were blank in all cases. A *Sus scrofa* (wild boar; CPC-98) vertebra from the 5000-year-old site of Carsington Pasture Cave in Derbyshire was extracted and amplified in tandem with the Fishamble Street samples, and no boar samples gave products with the cattle-specific primers. Seven of the 11 positive Fishamble Street samples gave amplification products with all three markers (Table 1).

Of the 11 samples that gave positive results, 24 of the 43 extractions were successful. In total, 169 amplification reactions were generated for each locus (Table 1), and 78, 145 and 149 of these gave PCR products for *CSRM60*, *HEL1* and *ILSTS001*, respectively. Five of the seven positive results from *CSRM60* were heterozygotes, with this value being three out of 11 for *HEL1*, and one out of 11 for *ILSTS001*. When all the haplotypes seen for these heterozygote individuals are taken into consideration, allelic drop out occurred 15.4% of the time (*CSRM60* = 6.9%; *HEL1* = 21.4%; *ILSTS001* = 15.0%). Chimeric alleles were not observed for any of the three loci. Apart from WQ11 and WQ13, which generated the same genotype with the three loci, the results differed for each fully-typed ancient individual.

Genetic diversity and phylogenetic analyses

Even when sample size is taken into account, the Fishamble Street population has a low MNA value (2.7 alleles; Table 2), a result that is more similar to the Island breeds sampled (Aberdeen Angus, Galloway, Hereford, Highland, Jersey and Kerry: mean = 2.8 alleles) than the Scandinavian group (Iceland, Norwegian Red, Telemark and Western Fjord: mean = 3.5 alleles). Although there is little variation among the observed heterozygosity values of the populations, the ancient samples have the lowest value (0.36; Table 2), which is closer to the British Isles group mean of 0.46 than the Scandinavian group mean of 0.53. Three populations significantly deviated from HWE at locus *CSRM60* with $P < 0.01$ for Galloway and Highland and $P < 0.05$ for Fishamble Street. The two-dimensional MDS plot constructed using the genetic distances among 12 cattle populations (Table 3; Fig. 1) places the Fishamble Street population among the British Isles breeds studied, nearest the Hereford breed.

Data from only three loci are insufficient to give confidence in the detailed structure of the MDS plot. However, we took a simple statistical approach to evaluate one aspect of the genetic distance data, by ordering the D_A values of each breed with respect to the Fishamble Street population. Four British breeds (Aberdeen Angus, Galloway, Hereford, Highland and Jersey) exhibit the closest affinity to the Fishamble Street samples (D_A ranging from 0.0922 to 0.1652; underlined values in Table 3). The Irish Kerry breed (0.2065) is separated from this grouping by the Norwegian Red (0.1694), and distances for the remaining Scandinavian breeds (Iceland, Telemark and Western Fjord) exceed this value in a range from 0.2367 to 0.4745. A Mann–Whitney rank-sum test indicated that the tendency for Fishamble Street versus British Isles genetic distances to be smaller than those versus Scandinavian samples was a significant one ($P < 0.02$).

When the seven anDNA specimens that gave reproducible results from all three markers (Table 1) were compared with modern genotypes, WQ11 and WQ13 generated the same genotype, WQ03 matched with one Scandinavian animal (NO), WQ07b corresponded to one Charolais animal, WQ09 matched with four British (1 AN, 1 HE, 1 HI and 1 JE) and one Charolais animal, and WQ16 had the same genotype as six British animals (1 GA, 3 HE, 1 HI and 1 JE). WQ04b had a novel haplotype not seen in any of the other 11 populations studied.

Discussion

In molecular studies using ancient specimens, the DNA available for genetic typing is often in the picogram range,

and authenticity of the genotypes obtained must be examined. When the template has a low-copy number or the sample quality is poor, selective amplification of one allele often occurs, leading to the incorrect typing of a heterozygous individual as a homozygote. Although it is possible that false alleles may have occurred from slippage artefacts generated during the first cycles of the first-round PCR, rigorous precautions were taken to ensure that each genotype was reliable and reproducible. In addition, all extraction and PCR controls used for all DNA isolation, purification and amplification steps were blank. A common contamination was unlikely as only 28.6% (two out of seven) of the Fishamble Street samples were identical (Table 1), whereas the average value for the modern breeds was 60.4%.

The success rate for amplification of nuclear markers in this study is high, at 57.9%. This is not wholly unexpected, as the Fishamble Street samples were pre-chosen using two basic criteria. First, the bones exhibited an excellent preservation condition; and secondly, the remains have been shown previously to amplify reliable mitochondrial haplotypes with a success rate of 84.2% (C. J. Edwards, unpublished data). This compares favourably to the average laboratory success rates of 28.1% for mitochondrial analyses of all *Bos* sp. remains, and 56.3% for European remains alone.

The absence of calf remains at the Fishamble Street site, coupled with the finding that 57% of the cattle present were over 4-years old (cited in Wallace 1985), suggests that mature cattle were being driven into the town from the surrounding countryside and were rarely kept within the settlement itself. This assumption that the Viking town cattle remains were Irish in origin is supported statistically with the ancient population exhibiting a significantly closer relationship to the British Isles breeds than the Scandinavian group ($P < 0.02$).

Microsatellite analysis of archaeological livestock remains could provide information concerning autosomal genetic variation, therefore allowing more general questions to be addressed than studies using mitochondrial DNA alone. For small populations, microsatellite markers allow a greater degree of structural analysis than mtDNA because of their increased level of polymorphism and high heterozygosity. The mtDNA analysis is of limited use in studies of cattle diversity in Europe as resolution is afforded only to the broad regional level. The positive results obtained here demonstrate the feasibility of using microsatellites for the study of ancient cattle, which could be very powerful given the existence of a large database containing genotypes from widely sampled extant cattle (http://www.projects.roslin.ac.uk/cdiv/). Although mixed results have been obtained from human skeletal remains (Ramos *et al.* 1995; Zierdt *et al.* 1996; Burger *et al.* 1999), STRs have been utilized successfully on studies of leather, parchment, pot contents and glues (Burger *et al.* 2000).

The Viking settlement at Dublin represents a centre where the three ethnic groupings of Irish, English and Scandinavian have, to varying degrees, played a role. When archaeological evidence is taken into account, this study suggests an Irish origin for these early medieval cattle.

Acknowledgements

We thank Ronan Loftus and Teresa Cymbron for assistance with sample collection and laboratory work, David Magee, Patricia Edwards, Brian McEvoy and Emma Finlay for helpful comments on earlier versions of the manuscript, and Andrew Chamberlain for the wild boar vertebra used as a control specimen. C. J. Edwards is supported by the Enterprise Ireland Basic Research Grants Scheme (project no. SC/1999/409). This work was part-funded by the Wellcome Trust (grant no. 047485/Z/96/Z).

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