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ORIGINAL ARTICLE

Genetic diversity and population structure of Scottish Highland red deer (*Cervus elaphus*) populations: a mitochondrial survey

S Pérez-Espona[1,2], FJ Pérez-Barbería[2], WP Goodall-Copestake[3], CD Jiggins[1,4], IJ Gordon[5] and JM Pemberton[1]

[1]*Institute of Evolutionary Biology, School of Biological Sciences, The University of Edinburgh, Edinburgh, UK;* [2]*The Macaulay Institute, Craigiebuckler, Aberdeen, UK;* [3]*British Antarctic Survey, Natural Environment Research Council, Cambridge, UK;* [4]*Department of Zoology, The University of Cambridge, Cambridge, UK and* [5]*Sustainable Ecosystems, CSIRO Davies Laboratory, Aitkenvale, Australia*

The largest population of red deer (*Cervus elaphus*) in Europe is found in Scotland. However, human impacts through hunting and introduction of foreign deer stock have disturbed the population's genetics to an unknown extent. In this study, we analysed mitochondrial control region sequences of 625 individuals to assess signatures of human and natural historical influence on the genetic diversity and population structure of red deer in the Scottish Highlands. Genetic diversity was high with 74 haplotypes found in our study area (115 × 87 km). Phylogenetic analyses revealed that none of the individuals had introgressed mtDNA from foreign species or subspecies of deer and only suggested a very few localized red deer translocations among British localities. A haplotype network and population analyses indicated significant genetic structure ($\Phi_{ST} = 0.3452$, $F_{ST} = 0.2478$), largely concordant with the geographical location of the populations. Mismatch distribution analysis and neutrality tests indicated a significant population expansion for one of the main haplogroups found in the study area, approximately dated c. 8200 or 16 400 years ago when applying a fast or slow mutation rate, respectively. Contrary to general belief, our results strongly suggest that native Scottish red deer mtDNA haplotypes have persisted in the Scottish Highlands and that the population retains a largely natural haplotype diversity and structure in our study area.
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Introduction

The red deer (*Cervus elaphus*) is one of the largest and most widely distributed mammal game species in Europe. The largest population of wild red deer in Europe occurs in the British Isles, with the majority of the population found in the Scottish mainland (Clutton-Brock and Albon, 1989). In Scotland, red deer have been continuously present since the end of the last glaciations (c.11 000 years BP; Lister, 1984) when forests interspersed with open areas were abundant throughout the country (Clutton-Brock and Albon, 1989). However, due to hunting and deforestation associated with the development of farming cultures (c. 5000 years BP), red deer were gradually displaced northwards into the mountainous Highlands (Whitehead, 1964; Lister, 1984). Continued deforestation and hunting during the 16th–18th Centuries caused a steep decline in Scottish red deer populations with the numbers of red deer at their lowest by the second half of the 18th Century (Clutton-Brock and Albon, 1989). However, quite large populations of red deer are thought to have survived in some areas in the Highlands, namely Atholl, Black Mount, Glenartney, Glen Fiddich, Invercauld, Mar, Abergeldie, Badenoch, Birkall, Glen Isla and Rannoch (Sinclair, 1814; Black and Black, 1861; Whitehead, 1960, 1964; Clutton-Brock and Albon, 1989). In the nineteenth century, the range and abundance of red deer rose again due to a growing interest in deer hunting coupled with a decrease in profits from sheep rearing, which allowed large areas of land to be re-colonized by deer (Lowe and Gardiner, 1974; Clutton-Brock and Albon, 1989). During the nineteenth and twentieth centuries, introductions of foreign stock also took place to improve hunting trophy quality (Whitehead, 1960, 1964; Lowe and Gardiner, 1974). Although introductions and translocations were aimed to improve trophy hunting and therefore might have involved movement of males, introduction and translocation of females is also well documented. Furthermore, because of the polygynous mating system of red deer and competition among males to hold a harem, reproductive success of introduced females is likely to have been higher than of introduced males. Severe past population declines coupled with the introductions and translocations that occurred during the nineteenth and twentieth centuries have led some to believe that the Scottish red deer population contains introgressed non-native genes and that population genetic structure may have been blurred by human intervention. More recently, protection of red deer from indiscriminate culling,

Correspondence: Dr S Pérez-Espona, School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1US, UK.
E-mail: silvia.perezespona@bristol.ac.uk
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coupled with a series of mild winters and lower competition with sheep, has led to increased red deer numbers such that populations are thought to have doubled in the past 40–50 years (Clutton-Brock *et al*., 2004). Nowadays, red deer are distributed across the Highlands, islands and southwest of Scotland; the highest densities are found in the Highlands where c. 400 000 individuals are thought to occupy an area covering 300 000 km² of the Scottish mainland (Clutton-Brock and Albon, 1989; Clutton-Brock *et al*., 2004).

Despite the fact that the largest numbers of European red deer are found in Scotland, studies assessing Scottish red deer genetic diversity are few and concentrated on deer on islands or peninsulas and have highlighted the influence of human activities on these populations. Studies on the Kintyre peninsula (Argyll) using nuclear microsatellite and mtDNA data revealed that sika deer introduced from Japan have successfully interbred with red deer (Abernethy, 1994; Goodman *et al*., 1999). A survey of the long-term study area on the island of Rum using mtDNA markers showed that red deer haplotype diversity was low and a divergent haplotype closely related to Corsican red deer (*Cervus elaphus corsicanus*) was found in some individuals, both features presumably a result of the entire population having originated from documented introduction events (Nussey *et al*., 2006). Low mtDNA variability was also found in the island of Arran, where the current population also originated from introduced individuals, and the island of Islay where documented introductions are known to have occurred in one studied locality (Hmwe *et al*., 2006).

Genetic diversity and population genetic structure for red deer in the mainland of Scotland has not been widely assessed but has shown a higher diversity and less disturbed population structure than on the islands (Hmwe *et al*., 2006; Pérez-Espona *et al*., 2008). In a study comparing data from 11 microsatellites and mtDNA sequences obtained from 69 individuals sampled in four Scottish mainland populations, two island populations (Arran and Islay) and one English population, Hmwe *et al*. (2006) found that overall there was a lack of congruence between geographical and genetic structure, which they attributed to the impact of past red deer management practices on some of the populations studied. However, this study included some island populations and one small English population of red deer for which extensive introductions have been documented. The two of the mainland Scottish populations studied by Hmwe *et al*. (2006), Dunarchy and Achnacarry, for which there are no documented introductions, presented higher genetic diversity and a population structure concordant with geography. A recent study which included 695 red deer sampled in 14 estates of the Scottish mainland and genotyped for 21 microsatellites found high genetic diversity and significant population structure in the study area, with isolation by distance and landscape features playing an important role in population structure (Pérez-Espona *et al*., 2008).

In this study, mtDNA control region sequences of 625 red deer individuals sampled in the Scottish Highlands were analysed. On account of the extensive variation revealed, we first assessed whether there was any evidence for exotic haplotypes as a result of past introductions of females. Second, we assessed the genetic diversity and population structure of mtDNA diversity in the study area and the likely historical processes responsible for the observed pattern.

Materials and methods

Study area and sample collection

The study area comprised 14 open hill estates distributed across a 115 × 87 km area in the Scottish Highlands (Figure 1). Samples consisted of an ear tip or a sample of

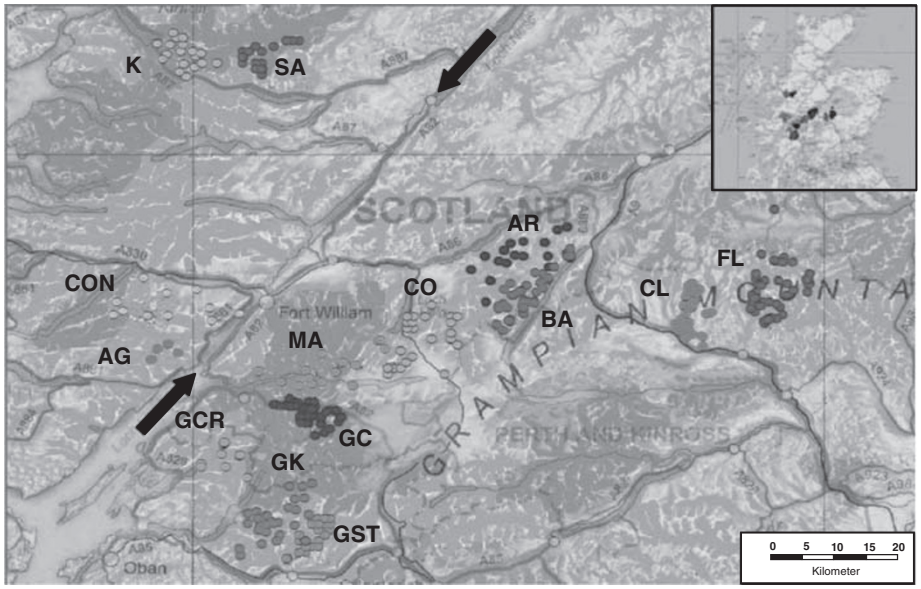

Figure 1 Map of the study area showing sampling sites. Dots on the map do not correspond to the total of individuals sampled but to the culling areas (several individuals were sampled in the same location). Black arrows indicate location of the Great Glen. FL = Forest Lodge ($n = 41$), CL = Clunes ($n = 60$), BA = Ben Alder ($n = 42$), AR = Ardverikie ($n = 55$), CO = Corrour ($n = 27$), MA = Mamore ($n = 51$), GC = Glencoe ($n = 50$), GCR = Glencreran ($n = 30$), GK = Glenkinglass ($n = 49$), GST = Glenstrae ($n = 34$), CON = Conaglen ($n = 48$), AG = Ardgour ($n = 25$), SA = South Glen Affric ($n = 58$), K = Kintail ($n = 55$). See online version for colour figure.

jaw muscle from a total of 625 legally shot red deer (345 males and 280 females). Samples were collected from different localities within the estates during the male shooting season (1 July–20 October; Clutton-Brock and Albon, 1989) with most of the samples obtained at the end of summer. Female samples were collected during the female shooting season (21 October–15 February; Clutton-Brock and Albon, 1989) with most of the samples collected in winter. Tissue samples were stored either at −20°C or in tubes containing 100% ethanol.

DNA extraction and sequencing of the mitochondrial control region

Genomic DNA was extracted from ear or jaw muscle using the DNAace Spin Tissue Mini Kit (Bioline, London, UK) or with the DNEasy Tissue Kit (QIAGEN, GmbH, Hilden, Germany), following the manufacturer's instructions. The mitochondrial control region (mtDNA CR) and the partial flanking region of tRNA genes were amplified using the primers CST2 and CST39 (Polziehn *et al*., 1998). Amplification of the mtDNA CR was conducted in 50 μl PCRs using 10–15 ng of template DNA, 1 × NH4 Buffer, 1.5 mM $MgCl_2$, 0.6 μM of each primer, 1 unit of BIOTAQ polymerase (Bioline, London, UK) and double processed tissue culture distilled H_2O (Sigma-Aldrich, Buchs, Switzerland) to bring the volume up to 50 μl. The PCR cycling protocol for the amplification of the mtDNA CR involved an initial denaturation step of 94 °C for 3 min, a three step-cycling consisting of a denaturing step of 94 °C for 30 s, annealing at 56 °C for 30 s and ramping at 0.3 °C/s to an extension step of 72 °C of 1 min. The cycle was repeated 29 times and was followed by a final extension of 72 °C for 10 min. PCR products were run in a 1% agarose gel and visualized using ethidium bromide staining to check if amplification was successful. Successful amplifications were purified using the Sigma-Genosys Genelute PCR Clean-up Kit (Sigma-Aldrich) following the manufacturer's instructions. A fragment of approximately 1000 bp of the mtDNA CR was sequenced in two reactions using 4 μl of purified PCR product, 2 μl of the reaction mix DYEnamic ET Terminator Cycle Sequence Kit (Amersham BioSciences, Piscataway, NJ, USA) and 3 μl of primer CST2 in the first reaction and primer CST29 (Polziehn and Strobeck, 2002) in the second reaction. Sequencing cycling consisted of 25 cycles including a denaturation step of 95 °C for 20 s, an annealing step of 50 °C for 15 s and an extension step of 60 °C for 1 min. Sequences were run on a capillary ABI 3730 DNA Analyzer sequencer (Applied Biosystems, Foster City, CA, USA), manually edited using SEQUENCE NAVIGATOR v.1 (Applied Biosystems) and unambiguously aligned by eye.

Data analysis

Assessment of introgression of mtDNA from foreign deer stock into Scottish Highland red deer and translocations of individuals among British localities

Phylogenetic analyses were conducted to assess if any of the individuals included in this study had introgressed mtDNA from other species or subspecies of deer. For these analyses we used a single accession of each haplotype found in this study and 47 additional mtDNA CR sequences from red deer and other species within the Cervinae subfamily downloaded from GenBank (See Supplementary Table S1 in Supplementary Information). Sequence alignment was conducted with the web-based software MUSCLE (Edgar, 2004). Gaps introduced by the automatic alignment, corresponding to inferred insertion and deletion events, were manually altered in MEGALIGN v. 5 (distributed by DNAstar Inc., Madison, WI, USA) to maximize DNA character positional homology. Ambiguously aligned regions, microsatellite repeats and positions for which the majority of samples contained a gap were excluded from subsequent analyses.

Phylogenetic analyses were conducted using Bayesian inference and maximum parsimony. MRBAYES v. 3.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) was used for the Bayesian inference. Three simultaneous independent analyses were run, each starting from a random tree with the default option of one cold and three incrementally heated chains. The chains were run for 1 500 000 generations and sampled every 100 generations, with the first 500 000 generations discarded as a burn-in which appeared to be sufficient based on plots of posterior parameter estimates against sample number visualized using TRACER (Rambaut and Drummond, 2004). Default values were left for the other parameters, except for the model of DNA evolution that was set to a General Time Reversible Model (Rodríguez *et al*., 1990) with γ distributed rate heterogeneity (Yang, 1994). This Model was selected as the best supported Model identified using MODELTEST v. 3.06 (Posada and Crandall, 1998) which did not appear to be over-parameterized in Bayesian phylogenetic analyses based upon inspection of posterior parameter values using TRACER (Rambaut and Drummond, 2004). A majority rule phylogram was generated from the 30 003 post-burn-in samples from all three independent analyses.

Maximum parsimony tree searches were conducted using PHYLIP v. 3.65 (Felsenstein, 2005). Gaps were treated as missing data and the search options were left at default values (equal weights, thorough tree search and 10 000 saved trees). To generate support values, invariant characters were removed and 1000 bootstrap pseudo-replicates were generated saving 10 trees per replicate.

Mitochondrial genetic diversity and population structure of Scottish Highland red deer

Estimates of genetic diversity and population structure were conducted in ARLEQUIN v. 3.1 (Schneider *et al*., 2000). Genetic diversity indices consisted of haplotype diversity (h), nucleotide diversity (π) and number of polymorphic sites (s). Relationships between haplotypes were assessed by constructing a haplotype minimum spanning network using the program TCS v. 1.21 (Clement *et al*., 2000) which uses statistical parsimony to connect haplotypes at the 95% confidence level. A median-joining network was also constructed with the program NETWORK v. 4.2.0.1 (http://www.fluxus-engineering.com; Bandelt *et al*., 1999). Population structure across the study area and between all sampling sites was assessed by calculating Φ_{ST} which takes into account haplotype frequencies and the genetic distance between haplotypes (Excoffier *et al*., 1992). For the genetic model we used Tamura and Nei's TN93 genetic distance model (Tamura and Nei, 1993). Population structure was also calculated by means of

F-statistics (Wright, 1951) using only haplotype frequencies. Significance values for the two methods of computation of population structure were obtained after 1000 permutations. Pairwise population differentiation was calculated using Φ-analogues of Wright's F-statistics (Wright, 1951). Significance values were obtained after 1000 permutations among populations using Fisher's exact tests (Ryman and Jorde, 2001), with a Bonferroni correction applied for multiple comparisons (Rice, 1989). To assess the distribution of genetic variation among regions (Φ_{CT}) and populations within regions (Φ_{SC}) without having to define groups *a priori* (as in hierarchical AMOVA) we conducted spatial analyses of the molecular variance using the program SAMOVA v. 1.0 (Dupanloup *et al*., 2002). This approach uses the geographical location of populations and aims to define groups of populations (K) which maximize the value of Φ_{CT} by assigning populations into groups on the basis of geographical proximity and genetic homogeneity. Analyses were conducted for values of $K = 2$–11 with 10 000 simulated annealing steps each starting from 500 sets of initial conditions.

To test if the pattern of population differentiation followed an isolation by distance model (Wright, 1943; Slatkin, 1993), we conducted Mantel tests (Mantel, 1967) in FSTAT v. 2.9.3.2 (updated from Goudet, 1995) to test the significance of the regression between pairwise genetic distances expressed as $F_{ST}/(1-F_{ST})$ against the natural log-transformed geographical distance (Rousset, 1997). Statistical significance was estimated after 10 000 permutation tests.

Inferences regarding past demographic effects on the genetic variation in current Scottish Highland red deer were conducted by comparing mismatch distributions of pairwise nucleotide differences between haplotypes to those expected under a sudden population expansion model (Slatkin and Hudson, 1991; Rogers and Harpending, 1992; Rogers, 1995). Unimodal distributions are expected for populations that recently expanded or experienced a bottleneck, as individuals within a population will present similar haplotype divergence (in terms of nucleotide differences) (Slatkin and Hudson, 1991; Rogers and Harpending, 1992). In contrast, a multimodal or 'ragged' distribution is expected for a stable or slowly declining population (Slatkin and Hudson, 1991). Statistical significance for the mismatch distributions was obtained using a goodness-of-fit test based on the sum of squared deviations between the observed and expected distributions (Schneider and Excoffier, 1999) and the Harpending's raggedness index *rg* (Harpending, 1994) after 1000 simulations using the estimated parameters of the expected distribution for a population expansion. Population expansions were further assessed using the neutrality test *Fs* (Fu, 1997) as this has been shown to be a powerful test to detect population growth when large sample sizes are available (Ramos-Onsins and Rozas, 2002). Large and negative significant values of *Fs* indicate an excess of recent mutations (haplotypes at low frequency) compared to those expected for a stable population, which can be interpreted as a signature of recent population growth or genetic hitchhiking (Fu, 1997). Large significant positive values of *Fs* indicate a greater deficit of rare haplotypes compared with those expected for a stable population indicating that the population has probably experienced a bottleneck (Fu, 1997). Mismatch distribution analyses and neutrality tests (*Fs*) were conducted in ARLEQUIN v 3.1 and statistical significance obtained using 1000 simulated samples.

Results

Assessment of introgression of mtDNA from foreign deer stock into Scottish Highland red deer and translocations of individuals among British localities

The alignment of the 625 sequences (821 bp long) resulted in 74 haplotypes, with 57 polymorphic sites of which 28 were parsimony informative. The average number of substitutions between haplotypes was low ($k = 5.456$). The complete sequences for the 74 haplotypes found in this study have been deposited in GenBank (accession nos. EF636727-EF636800).

Parsimony and Bayesian phylogenetic analyses generated similar tree topologies; however, only the phylogeny generated with Bayesian inference is shown as it presented a more resolved topology (Figure 2). The phylogenetic relationships inferred here largely agreed with those found in previous phylogenetic studies (Randi *et al*., 2001; Polziehn and Strobeck, 2002; Ludt *et al*., 2004; Pitra *et al*., 2004). There were no well supported (>80% maximum parsimony bootstrap, MPB) differences between the results of this and the above-mentioned previous phylogenetic studies. However, there were weakly supported conflicts (66% MPB) for the order of relationships among Sika deer, Thorold's or white-lipped deer and Wapiti lineages between this study and those of Ludt *et al*. (2004) and Pitra *et al*. (2004).

The red deer accessions included in this study all formed part of a distinct Western European red deer mitochondrial lineage associated with other distinct lineages corresponding to Corsican–North African deer and Central European red deer. These three lineages together comprised a strongly supported monophyletic group clearly differentiated from other deer lineages (Central Asian red deer, Sika deer, Thorold's or white-lipped deer and Wapiti). As previously found by Nussey *et al*. (2006), the haplotype Rum A clustered with Corsican red deer with very high support, 100 Bayesian Posterior Probability and 98.5 MPB. The 74 haplotypes found in this study, haplotypes found in other studies of British red deer (Hmwe *et al*., 2006), the Scandinavian red deer (*C. e. atlanticus*) haplotype and the Spanish red deer (*C. e. hispanicus*) haplotype all fell within the clade containing Western European red deer.

Despite the lack of strong statistical support for clades within the Western European red deer polytomy, visual inspection of the tree suggested some levels of geographical structure as particular clusters of haplotypes were found in particular areas of the Scottish Highlands (Figure 2). For example, a haplotype found in Achnacarry by Hmwe *et al*. (2006) clustered together with haplotypes found in populations SA and K in this study, populations which are geographically close to Achnacarry. Only a limited number of possible translocation events among British localities were suggested by the phylogenetic analyses. The haplotype H18 (found in one individual in CON and one individual in AG) clustered with haplotypes found on the island of Arran (SW of Scotland) and Euston (Suffolk, England). Another translocation or introduction event was also suggested by H16

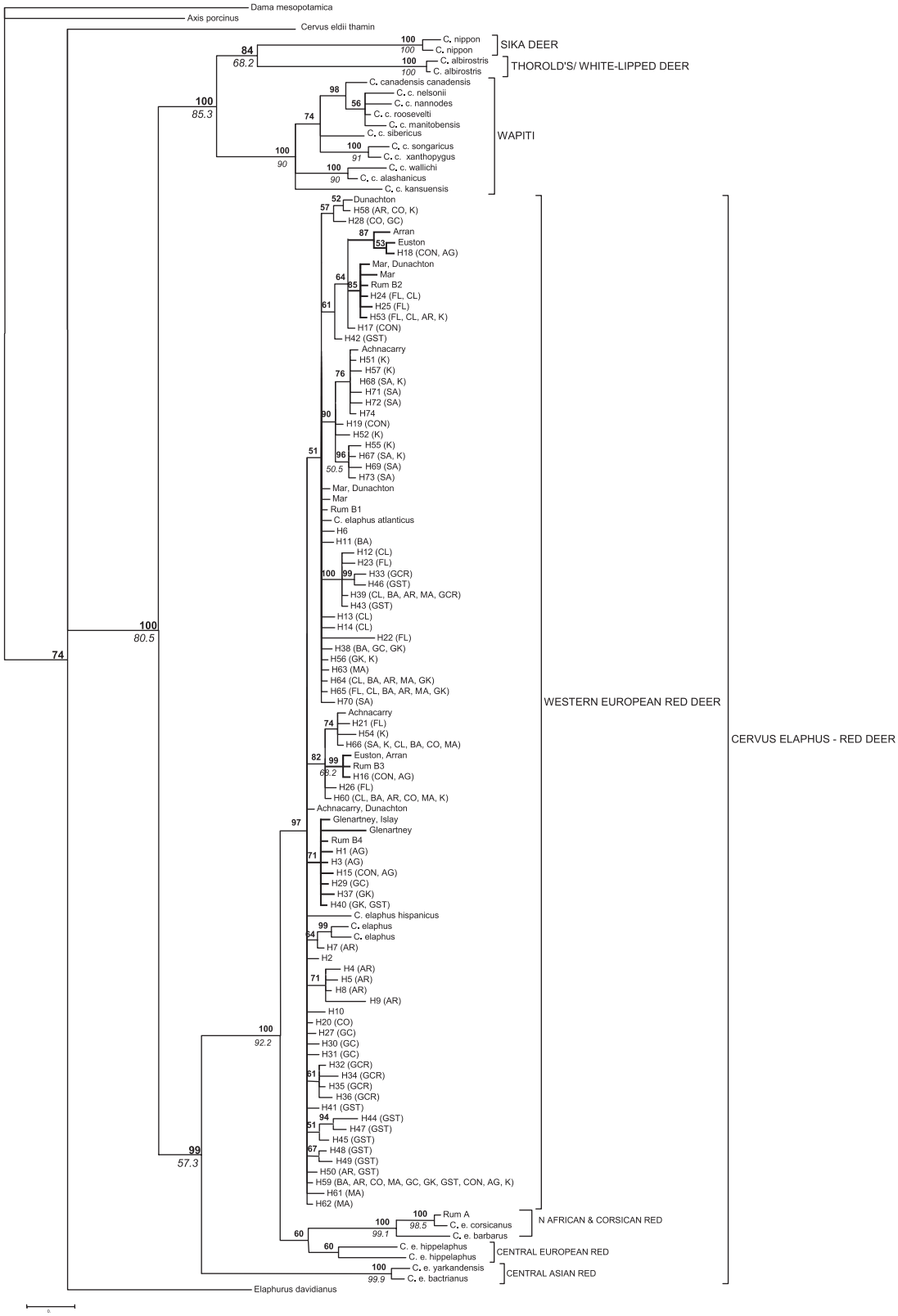

Figure 2 Unrooted phylogram illustrating phylogenetic relationships of Scottish Highland red deer and the 47 sequences of Cervinae download from GenBank. The phylogram was constructed using Bayesian inference with a General Time Reversible Model + γ model (Rodríguez *et al*., 1990) for mtDNA control region sequences. Bayesian posterior probability values are shown in bold and above the branches. MPB support values (Felsenstein, 1985) are shown in italics and below branches. Branch length units are expected substitutions per site. Haplotypes are labelled as species or locations of haplotypes found in previous studies or as labelled in this study (see Supplementary Information). For those haplotypes found in this study, the sampling sites where they were found are shown in brackets (see Figure 1 for population name abbreviations). Branches with thick black lines highlight possible deer translocation events.

Table 1 Genetic diversity indices derived from the 74 haplotypes found in the 14 populations of Scottish Highland red deer studied ($n = 625$). Abbreviations of populations are as in Figure 1

Population	*No. of individuals*	*No. of haplotypes*	*Haplotype diversity*	*Nucleotide diversity*
			$h \pm s.d.$	$\pi \pm s.d.$
FL	41	8	0.7049 ± 0.0663	0.0036 ± 0.0021
CL	60	10	0.8328 ± 0.0256	0.0055 ± 0.0030
BA	42	9	0.7875 ± 0.0422	0.0033 ± 0.0020
AR	55	13	0.8364 ± 0.0262	0.0036 ± 0.0020
CO	27	7	0.5983 ± 0.1048	0.0027 ± 0.0017
MA	51	8	0.5090 ± 0.0779	0.0018 ± 0.0012
GC	50	8	0.6420 ± 0.0602	0.0025 ± 0.0016
GCR	30	5	0.2529 ± 0.1037	0.0006 ± 0.0006
GK	49	7	0.6998 ± 0.0418	0.0031 ± 0.0019
GST	34	12	0.7576 ± 0.0570	0.0034 ± 0.0021
CON	48	6	0.5621 ± 0.0682	0.0037 ± 0.0022
AG	25	7	0.6967 ± 0.0647	0.0037 ± 0.0022
SA	58	9	0.7417 ± 0.0255	0.0056 ± 0.0031
K	55	13	0.8061 ± 0.0335	0.0057 ± 0.0031

(found in two individuals in CON and two individuals in AG) that clustered with haplotypes found in Euston, Arran and Rum.

Mitochondrial genetic diversity and population structure of Scottish Highland red deer

Genetic diversity indices for the Scottish Highland red deer populations analysed here are summarized in Table 1. AR presented the highest haplotype diversity (0.8364 ± 0.0262) and GCR the lowest (0.2529 ± 0.1037). The highest nucleotide diversity was found in *K* (0.0057 ± 0.0031) and the lowest in GCR (0.0006 ± 0.0006). Some haplotypes were common and shared between populations, with H59 being the most common haplotype, found in 25.92% of individuals. H59 was found at a relatively high frequency in most of the populations except in FL, CL, GCR and SA where it was absent and in K where only one individual presented H59. Other haplotypes were common in some adjacent populations of the study area (for example, H15 in CON and AG, H66, 67, 68 in SA and K) or confined to individual populations (for example, H32 in GCR). Haplotypes unique to one individual represented 7.2% of the sample. Relationships between the haplotypes were almost identical when using both programs, TCS and NETWORK (Figure 3). Haplotype H59, the most common haplotype, appeared at one extreme of the network giving rise to the rest of haplotypes. Haplotype structuring was evident because of the fact that some star-like phylogenies were found in different parts of the network. As already suggested by the phylogenetic tree, some of the star-like groups were only found in a particular population or in adjacent populations, with related rarer haplotypes radiating from a single common one (for example, the cluster around H32, found only in GCR and the clusters around H67 and H68, found only in SA-K).

Significant population genetic structure was found in the study area with a global $\Phi_{ST} = 0.3452$ ($P < 0.001$) and a global $F_{ST} = 0.2478$ ($P < 0.001$). Pairwise population differentiation tests showed that most of the populations were significantly differentiated from each other except for some adjacent or nearby populations (Table 2). The highest differentiation value was found between FL and GCR ($\Phi_{ST} = 0.\ 7476$, $P < 0.05$). The pairwise population analyses also showed that AG was not significantly differentiated from GK and GST, populations located on the other side of the Great Glen. This analysis also indicated that the populations SA and K, located west of the Great Glen, were more similar to FL and CL (located east of the Great Glen) than to the two similarly distant populations located west of the Great Glen, CON and AG.

Isolation by distance analyses showed a significant correlation between geographical distance and genetic differentiation ($r = 0.4181$, $P = 0.0003$) with 17.48% of the genetic differentiation explained by the model. SAMOVA analyses indicated that although the Φ_{CT} value was maximal when $K = 11$, the major increase on the Φ_{CT} was for $K = 3$, with values only increasing slightly thereafter (Table 3). The spatial analyses did not indicate an effect of the Great Glen as a major partition of the data as CON and AG were grouped with populations sampled east of the Great Glen when $K = 3$. With $K > 3$ further differentiation of populations was attained, with GCR forming a separate group for $K \geqslant 4$ due to the set of unique related haplotypes found in this estate. The grouping of populations obtained with $K = 5$ was in agreement with the results obtained from the F_{ST} pairwise analysis reflecting the lack of significant differentiation of AG with the populations GK and GST. However, the grouping obtained with $K = 6$ reflected a pattern of differentiation which clearly agreed with the geographical position of the populations (Table 4, see Figure 1), with the exception of GCR being clearly differentiated from any of the nearby populations.

Mismatch distribution analyses and Fu's *Fs* neutrality test were conducted for each of the main genetic groups found in the study area ($K = 3$ in the SAMOVA analyses). Significant population growth (or genetic hitchhiking) was detected for the main group containing the populations (AR-BA-CO-MA-GC-GCR-GK-GST-CON-AG), which showed a significant negative *Fs* value and a unimodal pattern of mismatch distribution (Figure 4, Table 4). When analyses were conducted grouping populations according to $K = 4$, similar results were obtained with significant

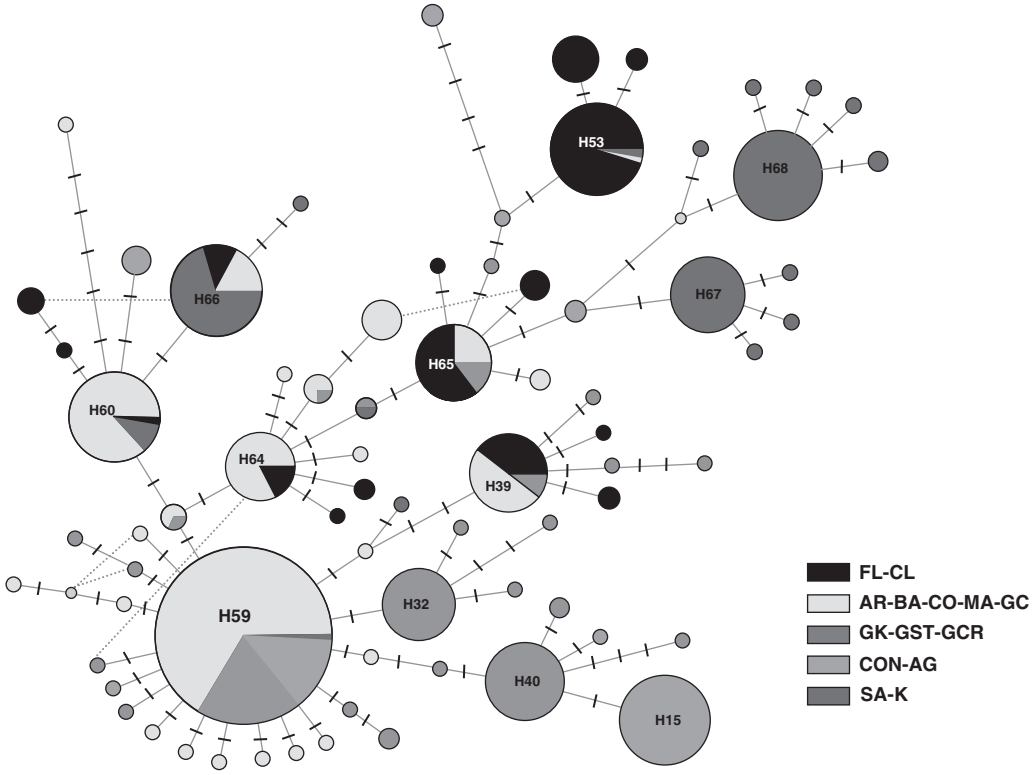

Figure 3 Haplotype network based on the number of substitution differences among Scottish Highland red deer haplotypes. Circle size is scaled to the number of individuals presenting that particular haplotype. Colours indicate the proportion of individuals sampled in different geographical regions within the study area. Branches are not scaled to the number of nucleotide substitutions; substitutions among haplotypes are indicated by vertical bars along the branches. Small grey circles represent missing intermediate haplotypes. Grey dotted lines indicate alternative links among haplotypes. See online version for colour figure.

Table 2 Pairwise population differentiation estimates of mtDNA Φ_{ST} for 14 red deer populations of Scottish Highland red deer ($n = 625$)

Population	*FL*	*CL*	*BA*	*AR*	*CO*	*MA*	*GC*	*GCR*	*GK*	*GST*	*CON*	*AG*	*SA*	*K*
FL														
CL	**0.1107**													
BA	0.5276	0.2627												
AR	0.4896	0.2371	**0.0081**											
CO	0.5737	0.3062	**0.0296**	**0.0696**										
MA	0.6629	0.4081	**0.0750**	0.1266	**−0.0051**									
GC	0.5947	0.3157	0.1340	0.1725	**0.0729**	0.1470								
GCR	0.7476	0.5234	0.4606	0.4579	0.4666	0.5057	0.4451							
GK	0.5796	0.3449	0.1769	0.1751	0.1754	0.2331	0.2190	0.4916						
GST	0.5866	0.3499	0.1417	0.1595	**0.0953**	0.1356	0.1399	0.4299	**0.0202**					
CON	0.5997	0.4198	0.3192	0.3091	0.3345	0.3950	0.4006	0.5631	0.1459	0.1933				
AG	0.5931	0.3723	0.2051	0.2111	0.1828	0.2400	0.2567	0.5096	**0.0524**	**0.0498**	**0.0393**			
SA	0.3762	0.2391	0.2879	0.2842	0.3245	0.4090	0.3822	0.5322	0.38256	0.3815	0.4455	0.3982		
K	0.3605	0.2146	0.2572	0.2528	0.2938	0.3793	0.3520	0.5128	0.3574	0.3545	0.4271	0.3739	**−0.0112**	

Bold indicates population pairs that were not significantly differentiated after applying strict Bonferroni correction (critical $P < 0.000549$).

population expansion indicated for the main genetic group and for GCR (See Supplementary Table S2 and Figure S1 in Supplementary Information). Mismatch distribution analyses indicated one peak of expansion with $\tau = 4.312$ for the main genetic group (AR-BA-CO-MA-GC-GCR-GK-GST-CON-AG). An approximate timing of the population expansion in years (t) can be estimated from $\tau = 2\,\mathrm{ut}$, where $u = \mu k$, $\mu =$ mutation rate and $k =$ length of the sequence. Assuming a mutation rate of 0.04–0.08 substitutions per site per million years, as applied in other studies of deer (for example, Randi *et al*., 2004; Royo *et al*., 2007) and a generation time of 4 years (based on the average age at which most Scottish female red deer first reproduce, Moyes *et al*., 2006) and the fact that mitochondrial DNA is maternally inherited, the population expansion took place c. 8200 (2900–16 400) or 16 400 (5800–32 700) when considering a fast or slow mutation rate, respectively.

Table 3 Results from the spatial analysis of molecular variance (SAMOVA), showing values for variation among groups (Φ_{CT}) and within populations (Φ_{SC})

K	*SITES*	Φ_{CT}	Φ_{SC}
2	[FL], [K, SA, CON, AG, GCR, GK, GST, GC, MA, CO, BA, AR, CL]	0.2962	0.3119
3	[FL, CL], [K, SA], [CON, AG, GCR, GK, GST, GC, MA, CO, BA, AR]	0.3344	0.1678
4	[K, SA], [FL, CL], [GCR], [CON, AG, GK, GST, GC, MA, CO, BA, AR]	0.3439	0.1384
5	[K, SA], [FL, CL], [GCR], [CON] [AG, GK, GST, GC, MA, CO, BA, AR]	0.3512	0.1002
6	[K, SA], [FL], [CL], [GCR], [CON, AG], [GK, GST, GC, MA, CO, BA, AR]	0.3527	0.0809
7	[SA, K], [FL], [CL], [CON], [GCR], [GK, GST, AG], [GC, MA, CO, BA, AR]	0.3514	0.0487
8	[SA, K], [FL], [CL], [CON], [GCR], [GK, GST, AG], [GC], [MA, CO, BA, AR]	0.3529	0.0288
9	[SA, K], [FL], [CL], [CON], [GCR], [GK, GST, AG], [GC], [MA, CO], [BA, AR]	0.3571	0.0076
10	[SA, K], [FL], [CL], [CON], [GCR], [GK, GST], [AG], [GC],[MA, CO], [BA, AR]	0.3596	−0.0031
11	[SA, K], [FL], [CL], [CON], [GCR], [GK], [GST], [AG], [GC],[MA, CO], [BA, AR]	0.3602	−0.0046

All values were statistically significant ($P<0.001$) except those from $K=2$ ($P>0.05$).

Table 4 Mismatch distribution analyses and *Fs* neutrality test to detect population expansions for the different main genetic groups found in the study area ($K=3$ in the SAMOVA analyses)

Groups of populations	Fs	P	SSD	P	rg	P	τ
[FL-CL]	−0.414	0.5	0.24	0.37	0.06	0.26	8.311 (0.016–14.613)
[K-SA]	−1.61	0.36	0.062	0.082	**0.115**	0.03	9.28 (0.098–15.199)
[AR-BA-CO-MA-GC-GCR-GK-GST-CON-AG]	**−25.83**	0.001	0.02	0.184	0.046	0.23	4.312 (0.928–7.301)

Numerical values in bold indicate statistical significance.
Fs: Fu's neutrality test (Fu, 1997); SSD: sum of square deviations between the observed and the expected mismatch; *rg*: raggedness index of the observed distribution (Harpending, 1994), τ: time since expansion in mutational units.

Discussion

Assessment of introgression of mtDNA from foreign deer stock into Scottish Highland red deer and translocations of individuals among British localities

As for many other populations of red deer in Europe, Scottish red deer populations have been directly or indirectly influenced by human activities over centuries (see Introduction). Despite the documented introductions and interbreeding of Scottish red deer with foreign deer stock (Whitehead, 1960, 1964), none of the individuals included in this study showed introgression of mtDNA haplotypes from other subspecies or species of deer. Owing to the large number of sequences included in this study, the lack of individuals with introgressed mtDNA suggests that in our study area successful introduction and hybridization between red deer and non-native deer was limited. The results from our study contrast to those previously obtained in other studies were signatures of hybridization or strong effects of past human intervention on Scottish red deer populations has been detected (Abernethy, 1994; Goodman *et al*., 1999; Hmwe *et al*., 2006, Nussey *et al*., 2006). One reason for the discrepancy between our results and those from previous studies is that some of them included island red deer populations or English red deer populations for which the entire or part of the population is known to have originated from different introduction events, some of them involved animals from deer parks where intentional hybridization was carried out to improve trophy hunting. In addition, because of the low number of samples included in Hmwe *et al*. (2006)'s study inferences regarding the genetic status of red deer in the Scottish mainland are not robust. Another reason is that, although individual Sika sightings have been reported in some parts of our study area, numbers of Sika are much lower than those found in the Kintyre Peninsula (Argyll) where the range of Sika and red deer populations overlap and hybridization has been detected (Abernethy, 1994, Goodman *et al*., 1999).

Although possible hybridization events in our study area involving non-native male deer will remain undetected because of the maternal inheritance of mtDNA, we would expect that owing to the strongly polygynous mating system characteristic of red deer, breeding success of introduced females must have been higher to that of introduced males. Considering the large number of samples included in our study, if past introductions had been extensive and successful in our study area this would have been reflected in the mtDNA diversity, structure and phylogenetic analyses. Furthermore, a previous study of these populations using nuclear microsatellite markers (bi-parentally inherited) indicated that patterns of population structure in our study area were strongly concordant with geography (Pérez-Espona *et al*., 2008) which would further suggest that the effect of introductions was limited. Nonetheless, to thoroughly assess possible introgression as a result of introductions of foreign male deer stock, a variable male-specific marker in the Y-chromosome or/and admixture studies using microsatellite markers comparing Scottish Highland red deer populations and individuals from potential source populations are required.

Phylogenetic analyses suggested very few possible translocation events among British localities. Some haplotypes found only in a total of six individuals from CON and AG clustered with haplotypes previously found

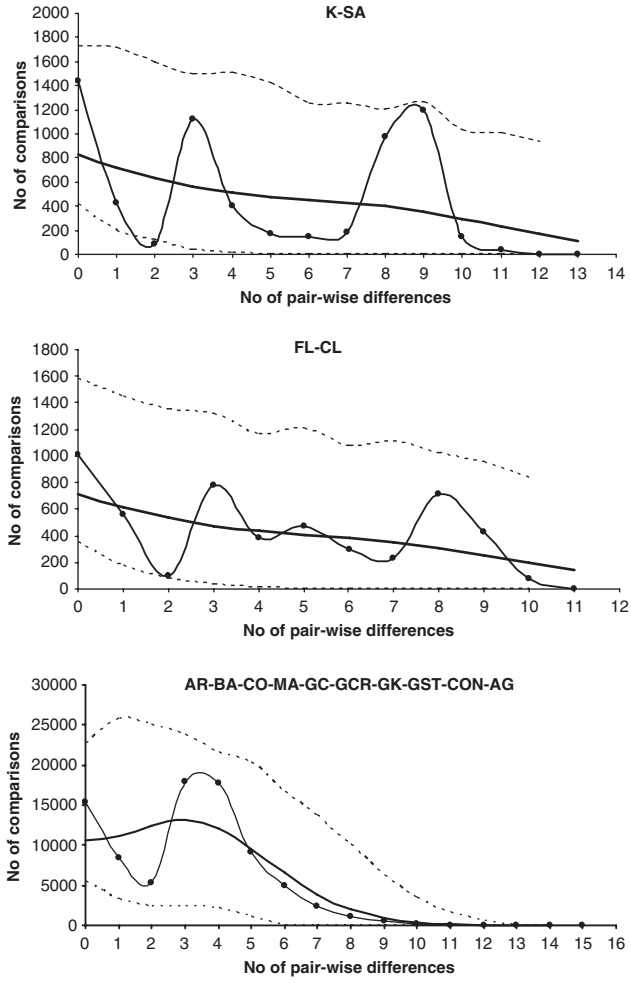

Figure 4 Mismatch distributions of pairwise nucleotide differences for each of the red deer main genetic groups ($K = 3$ in the SAMOVA analyses) found in the study area. Observed distribution of pairwise nucleotide differences (thin line). Distribution of pairwise nucleotide differences expected under a model of population expansion (thick line). Upper and lower bound of the 95% confidence intervals for the observed values (dashed lines).

on the islands of Arran, Islay and Rum and at Euston (England) (see Figure 2), locations where current populations of red deer have been strongly influenced by human intervention (Hmwe *et al*., 2006; Nussey *et al*., 2006). The clustering of haplotypes found in these distant locations could suggest that these haplotypes are native to the area of CON and AG and that their presence in the deer parks and in the islands is due to translocations in these localities. Alternatively, the presence of these haplotypes in CON and AG could be due to introductions of individuals from the same source populations used to stock the islands and Euston. Setting aside these very limited and localized translocations, the main clustering of haplotypes was largely concordant with geography, strongly suggesting that human activities have not completely blurred red deer population boundaries in our study area. Therefore, some of the haplotypes found in this study probably represent a variety of mtDNA haplotypes of the native populations of Scottish red deer that survived in some areas of the Highlands after the severe population declines (Sinclair, 1814; Black and Black, 1861; Whitehead, 1960, 1964; Clutton-Brock and Albon, 1989).

Mitochondrial genetic diversity and population structure of Scottish Highland red deer

With the exception of GCR, Scottish Highland red deer populations showed moderate to high levels of haplotype diversity, similar to those in other Scottish mainland populations by Hmwe *et al*. (2006) but much higher than those found in the Scottish islands (Hmwe *et al*., 2006; Nussey *et al*., 2006). Differences in genetic diversity among sampling sites did not correlate with population sizes of red deer currently found on the estates, except for GCR. The population of red deer at GCR showed the lowest haplotype diversity ($h = 0.2529$), a similar value to that found for red deer in the island of Arran (Hmwe *et al*., 2006).The low genetic diversity in GCR can be explained by the smaller population of red deer that is found in this estate (Whitehead, 1960, 1964). This population is likely to have undergone through a severe bottleneck, as suggested by the low haplotype and nucleotide diversity found and by historical records reporting a much larger population of deer in the area before the 18th Century (Sinclair, 1814). The lack of haplotype sharing between GCR and any of the other studied populations suggests that female gene flow in this area is very limited, likely owing to landscape features surrounding the estate acting as gene flow barriers (Pérez-Espona *et al*., 2008).

Comparisons of the average genetic diversity estimates found in our study ($h = 0.67$) with those found in other studies of deer are difficult because of methodological differences between studies. Nonetheless, comparisons with results from studies conducted at a similar geographical scale indicate that Scottish Highland red deer are more diverse than Japanese Sika deer in Kantoh (average $h = 0.37$; Yuasa *et al*., 2007) and white-tailed deer (*Odocoileus virginianus*) from the coastal plain of Georgia and South Carolina (average $h = 0.41$; Purdue *et al*., 2000). The low genetic diversity found in these two studies in comparison to our study can be attributed to the impact of severe habitat fragmentation in Sika deer in the Kantoh (Yuasa *et al*., 2007) and to overharvesting of white-tailed deer from the coastal plain of Georgia and South Carolina in the early nineteenth century (Purdue *et al*., 2000). Genetic diversity found in Scottish Highland red deer was within the range of that found in other genetic surveys of European populations of deer conducted at larger geographical scales (for example, Hartl *et al*., 2003 and Feulner *et al*., 2004 for red deer; Vernesi *et al*., 2002 and Royo *et al*., 2007 for roe deer).

The low nucleotide diversity (average $\pi = 0.0035$) coupled with the high haplotype diversity found in Scottish Highland red deer indicate that several mtDNA lineages evolved in the area yielding a relatively large number of haplotypes differing by a small number of nucleotides. The wide distribution and high frequency of haplotype H59 in the minimum spanning network (see Figure 3), giving rise to all other haplotypes suggest that this haplotype could reflect an ancestral population that subsequently expanded (Donnelly and Tavaré, 1986; Crandall and Templeton, 1993; Takahata, 1998). Limited historical and contemporary gene flow between regions, together with gene flow barriers and the

effects of genetic drift, might have resulted in new derived haplotypes restricted to particular geographical areas (Ibrahim *et al*., 1996) such as those found for example in K-SA and in GCR (see Figure 3).

Population structure analyses also revealed significant mtDNA differentiation across the study area ($\Phi_{ST} = 0.3452$; $F_{ST} = 0.2478$). The fact that a higher differentiation value was attained when the genetic distance among haplotypes was considered (Φ_{ST}) indicates that on average haplotypes found within a particular area were more similar to each other than to haplotypes found in other areas. Isolation by distance explained 17.48% of the genetic variation, a similar amount to that found in white-tailed deer from the coastal plain of Georgia and South Carolina (16.81%, $Z = 0.41$, Purdue *et al*., 2000). A previous microsatellite-based study showed that landscape features, in particular those located along the Great Glen, significantly influenced the population genetic structure of Scottish Highland red deer (Pérez-Espona *et al*., 2008). However, this large differentiation between populations either side of the Great Glen was only prevalent for the K and SA populations in the mtDNA analyses as haplotypes found in CON and AG were more closely related to those found in some populations east of the Great Glen. This suggests a common colonization event for the origin of the CON and AG populations and some populations collected east of the Great Glen and a higher female gene flow between these populations in the past, before contemporary landscape features became a hindrance to red deer movement (Pérez-Espona *et al*., 2008).

Population structure estimates obtained with mtDNA were higher than those previously obtained with microsatellite data in our study area ($F_{STmicrosatellites} = 0.019$; Pérez-Espona *et al*., 2008). Discrepancies in the population structure patterns obtained with the two different genetic markers could be attributed to dispersal differences between the sexes. As a strongly polygynous mammal, the red deer is characterized by a strong female philopatry and a male-biased dispersal (Clutton-Brock and Albon, 1989). The higher level of structure found with the mtDNA in contrast to that found with microsatellite data therefore could be explained by males dispersing at a higher rate and/or longer distances than females. In addition, discrepancies between the two kinds of genetic marker might be because of mtDNA data reflecting patterns owing to earlier red deer history. Furthermore, because of the generally non-recombining nature of mtDNA, selective sweeps (the fixation of one haplotype because of high fitness) or background selection (reductions in effective population sizes because of the elimination of low fitness haplotypes) could also affect the levels of population structure observed (Ballard and Whitlock, 2004). Finally, the F_{ST} value obtained with the microsatellite data might be an underestimate because of the high polymorphism of the 21 markers used in the previous study (Hedrick, 1999; Balloux and Lugon-Moulin, 2002).

Mismatch distribution analyses and Fu's *Fs* neutrality test indicated population expansion or genetic hitchhiking in the main genetic group found in the study area (AR-BA-CO-MA-GC-GCR-GK-GST-CON-AG). Owing to the similar genetic signature that both population growth and genetic hitchhiking might leave, it is impossible to determine which is responsible for the negative value of *Fs* found in our study (Fu, 1997). However, as similar general patterns of population differentiation (with the exception of CON and AG being more similar to K-SA than to populations east of the Great Glen) were found in a previous study using 21 microsatellite markers on the same individuals (Pérez-Espona *et al*., 2008), it is unlikely that the signal obtained with the neutrality test is due to genetic hitchhiking. Dating of the population expansion from mismatch distribution analyses indicated that the expansion occurred c. 8200 (2900–16 400) or c. 16 400 (5800–32 700) years ago, depending on the mutation rate applied. However, these dates should be interpreted cautiously due to the concerns associated with the use of mismatch distribution analyses for detecting population expansions (Ramos-Onsins and Rozas, 2002) and the fact that substitution rates derived from phylogenetic analyses might not be appropriate for population-level analyses (see Ho *et al*., 2005 and references therein). Despite the abovementioned concerns, the dates obtained indicate that the population expansion was not a consequence of recent influence of man on red deer populations.

Implications for management and conservation of Scottish Highland red deer

An understanding of the genetic diversity and population structure of red deer is important to provide a sustainable management plan for red deer on the Scottish Highlands. Management plans for red deer populations in Scotland are made at the level of Deer Management Groups which include several estates or other landholdings sharing a population of deer (Clutton-Brock and Albon, 1989). The geographical scale at which this study was conducted provides useful information on the genetic diversity and population boundaries, which can inform future management and conservation strategies.

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References

Abernethy K (1994). The establishment of a hybrid zone between red and sika deer (genus *Cervus*). *Mol Ecol* **3**: 551–562.

Balloux F, Lugon-Moulin N (2002). The estimation of population differentiation with microsatellite markers. *Mol Ecol* **11**: 155–165.

Bandelt HJ, Forster P, Rohl A (1999). Median-joining network for inferring intraspecific phylogenies. *Mol Biol Evol* **16**: 37–48.

Ballard JWO, Whitlock MC (2004). The incomplete natural history of mitochondria. *Mol Ecol* **13**: 729–744.
Black A, Black C (1861). *Black's Picturesque Tourist of Scotland* 15th edn. Adam & Charles Black: Edinburgh.
Clement M, Posada D, Crandall KA (2000). TCS: a computer program to estimate gene genealogies. *Mol Ecol* **9**: 1657–1659.
Clutton-Brock TH, Albon SD (1989). *Red Deer in the Highlands*. BSP Professional Books: Oxford.
Clutton-Brock TH, Coulson T, Milner JM (2004). Red deer stocks in the Highlands of Scotland. *Nature* **429**: 261–262.
Crandall KA, Templeton AR (1993). Empirical tests of some predictions from coalescent theory with application to intraspecific phylogeny reconstruction. *Genetics* **134**: 959–969.
Donnelly P, Tavaré S (1986). The ages of alleles and a coalescent. *Adv Appl Prob* **18**: 1–19.
Dupanloup I, Schneider S, Excoffier L (2002). A simulated annealing approach to define the genetic structure of populations. *Mol Ecol* **11**: 2571–2581.
Edgar RC (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucl Acids Res* **32**: 1792–1797.
Excoffier L, Smouse PE, Quattro JM (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.
Felsenstein J (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **4**: 783–791.
Felsenstein J (2005). *PHYLIP (Phylogeny Inference Package) Version 3.65*.. Department of Genome Sciences UoW: Seattle.
Feulner PGD, Bielfeldt W, Zachos FE (2004). Mitochondrial DNA and microsatellite analyses of the genetic status of the presumed subspecies *Cervus elaphus montanus* (Carpathian red deer). *Heredity* **93**: 299–306.
Fu Y-X (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**: 915–925.
Goodman SJ, Barton NH, Swanson GM, Abernethy K, Pemberton JM (1999). Introgression through rare hybridization: A genetic study of a hybrid zone between red and sika deer (Genus *Cervus*) in Argyll, Scotland. *Genetics* **152**: 355–371.
Goudet J (1995). FSTAT (version 1.2): a computer program to calculate *F*-statistics. *J Hered* **86**: 485–486.
Harpending RC (1994). Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Hum Biol* **66**: 591–600.
Hartl G, Zachos F, Nadlinger K (2003). Genetic diversity in European red deer (*Cervus elaphus* L.): anthropogenic influences on natural populations. *C R Biol* **326**: 37–42.
Hedrick PW (1999). Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution* **53**: 313–318.
Hmwe SS, Zachos FE, Sale JB, Rose HR, Hartl GB (2006). Genetic variability and differentiation in red deer (*Cervus elaphus*) from Scotland and England. *J Zool* **270**: 479–487.
Ho SYW, Phillips MJ, Cooper A, Drummond AJ (2005). Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. *Mol Biol Evol* **22**: 1561–1568.
Huelsenbeck JP, Ronquist F (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**: 754–755.
Ibrahim KM, Nichols RA, Hewitt GM (1996). Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. *Heredity* **77**: 282–291.
Lister AM (1984). Evolutionary and ecological origins of British deer. *P Royal Soc Edinb B* **82**: 205–299.
Lowe VPW, Gardiner AS (1974). A re-examination of the subspecies of red deer (*Cervus elaphus*) with particular reference to the stocks of Britain. *J Zool* **174**: 185–201.
Ludt CJ, Schroeder W, Rottman O, Kuehn R (2004). Mitochondrial DNA phylogeography of red deer (*Cervus elaphus*). *Mol Phylogenet Evol* **3**: 1064–1083.
Mantel N (1967). The detection of disease clustering and a generalized regression approach. *Cancer Res* **27**: 209–220.
Moyes K, Coulson T, Morgan BJT, Donald A, Morris SJ, Clutton-Brock TH (2006). Cumulative reproduction and survival costs in female red deer. *Oikos* **115**: 241–252.
Nussey DH, Pemberton J, Donald A, Kruuk LEB (2006). Genetic consequences of human management in an introduced island population of red deer (*Cervus elaphus*). *Heredity* **97**: 56–65.
Pérez-Espona S, Pérez-Barbería FJ, McLeod JE, Jiggins CD, Gordon IJ, Pemberton JM (2008). Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). *Mol Ecol* **17**: 981–996.
Pitra C, Fickel J, Meijaard E, Groves PC (2004). Evolution and phylogeny of old world deer. *Mol Phylogenet Evol* **33**: 880–895.
Polziehn RO, Hamr J, Mallory FF, Strobeck CC (1998). Phylogenetic status of North American wapiti (*Cervus elaphus*) subspecies. *Can J Zool* **76**: 998–1010.
Polziehn RO, Strobeck C (2002). A phylogenetic comparison of red deer and wapiti using mitochondrial DNA. *Mol Phylogenet Evol* **22**: 342–356.
Posada D, Crandall KA (1998). MODELTEST: Testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
Purdue JR, Smith MH, Patton JC (2000). Female philopatry and extreme spatial heterogeneity in white-tailed deer. *J Mammal* **81**: 179–185.
Rambaut A, Drummond AJ (2004). TRACER (computer program). Available at http://evolve.zoo.ox.ac.uk/software.html.
Ramos-Onsins SE, Rozas J (2002). Statistical properties of new neutrality tests against population growth. *Mol Biol Evol* **19**: 2092–2100.
Randi E, Mucci N, Claro-Hergueta F, Bonnet A, Douzery EJP (2001). A mitochondrial DNA control region phylogeny of the Cervinae: speciation in *Cervus* and implications for conservation. *Anim Conserv* **4**: 1–11.
Randi E, Alves PC, Carranza J, Milošević-Zlatonović S, Sfougaris A, Mucci N (2004). Phylogeography of roe deer (*Capreolus capreolus*) populations: the effects of historical genetic subdivisions and recent nonequilibrium dynamics. *Mol Ecol* **13**: 3071–3083.
Rice WR (1989). Analyzing tables of statistical tests. *Evolution* **43**: 223–225.
Rodríguez F, Oliver JF, Marín A, Medina JR (1990). The general stochastic model of nucleotide substitution. *J Theor Biol* **142**: 485–501.
Rogers AR, Harpending HC (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Mol Biol Evol* **9**: 552–569.
Rogers AR (1995). Genetic evidence for a Pleistocene population explosion. *Evolution* **49**: 608–615.
Ronquist F, Huelsenbeck JP (2003). MRBAYES3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
Rousset F (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* **145**: 1219–1228.
Royo LJ, Pajares G, Alvarez I, Fernández I, Goyache F (2007). Genetic variability and differentiation in Spanish roe deer (*Capreolus capreolus*): A phylogeographic reassessment within the European framework. *Mol Phylogenet Evol* **42**: 47–61.
Ryman N, Jorde PE (2001). Statistical power when testing for genetic differentiation. *Mol Ecol* **10**: 2361–2374.
Schneider S, Excoffier L (1999). Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites. Application to human mitochondrial DNA. *Genetics* **152**: 1079–1089.

Schneider S, Roessli D, Excoffier L (2000). *Arlequin Version 2.000: A Software for Population Genetic Analysis*. Distributed by Genetics and Biometry Laboratory, University of Geneva: Switzerland.
Sinclair J (1814). *The General Report of the Agricultural State, and Political Circumstances, of Scotland* Vol. I. Arch Constable & Co.: Edinburgh, and Longman, Hurst, Rees, Orme & Brown: London.
Slatkin M, Hudson RR (1991). Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics* **129**: 555–562.
Slatkin M (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**: 264–279.
Takahata N (1998). The coalescent in two partially isolated diffusion populations. *Genet Res* **52**: 213–222.
Tamura K, Nei M (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* **10**: 512–526.
Vernesi C, Pecchioli E, Caramelli D, Tiedemann R, Randi E, Bertorelle G (2002). The genetic structure of natural and reintroduced roe deer (*Capreolus capreolus*) populations in the Alps and central Italy, with reference to the mitochondrial DNA phylogeography of Europe. *Mol Ecol* **11**: 1285–1297.
Whitehead GK (1960). *The deer stalking grounds of Great Britain and Ireland*. Hollis and Carter: London.
Whitehead GK (1964). *The deer of Great Britain and Ireland*. Routledge & Kegan Paul: London.
Wright S (1943). Isolation by distance. *Genetics* **28**: 114–138.
Wright S (1951). The genetical structure of populations. *Ann Eugenics* **15**: 323–354.
Yang Z (1994). Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *J Mol Evol* **39**: 306–314.
Yuasa T, Nagata J, Hamasaki S, Tsuruga H, Furubayashi K (2007). The impact of habitat fragmentation on genetic structure of the Japanese sika deer (*Cervus nippon*) in southern Kantoh, revealed by mitochondrial D-loop sequences. *Ecol Res* **22**: 97–106.

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