

ORIGINAL INVESTIGATION

Genetic structure of, and hybridisation between, red (*Cervus elaphus*) and sika (*Cervus nippon*) deer in Ireland

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Abstract

This study investigated the levels of genetic diversity and variation exhibited by red and sika deer in Ireland, along with the extent and regional location of hybridisation between these two species. Bi-parental (microsatellites) and maternally-inherited (mitochondrial DNA) genetic markers were utilised that allowed comparisons between 85 red deer from six localities and 47 sika deer from 3 localities in Ireland. Population genetic structure was assessed using Bayesian analysis, indicating the existence of two genetic clusters in sika deer and three clusters in red deer. Levels of genetic diversity were low in both red and sika deer. These genetic data presented herein indicate a recent introduction of sika deer and subsequent translocations in agreement with historical data. The origins of the current red deer populations found in Ireland, based on genetic data presented in this study, still remain obscure. All hybrid deer (red/sika) found in this study were found in Wicklow, Galway and Mayo where the 'red-like' deer exhibited sika deer alleles/haplotypes, and *vice versa* in the case of Wicklow. Molecular methods proved invaluable in the identification of the hybrid deer because identification of hybrids based on phenotypic external appearances (pelage and body proportions) can be misleading. Areas where red and sika deer are sympatric need to be assessed for the level and extent of hybridisation occurring and thus need to be managed in order to protect the genetic integrity of 'pure' red deer populations.

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Introduction

Despite the fascinating mix of natural and human involvement in the colonisation of Ireland by terrestrial

mammals (Searle 2008), very few species have been subjected to detailed Ireland-wide genetic analysis. Previous work on a select number of mammals, including the mountain hare *Lepus timidus* (Hamill et al. 2006, 2007), the red squirrel *Sciurus vulgaris* (Finnegan et al. 2008) and the pygmy shrew *Sorex minutus* (McDevitt et al. in press), have provided detailed coverage of genetic variation of terrestrial mammalian species in Ireland. It is perhaps surprising

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then that Ireland's largest and most charismatic mammal, the red deer (*Cervus elaphus*), has not yet been subjected to a detailed genetic analysis. In contrast, population genetics and structure have been analysed in this species in numerous studies in Britain (e.g. Nussey et al. 2005; Hmwe et al. 2006; Pérez-Espona et al. 2008) and continental Europe (e.g. Zachos et al. 2003; Frantz et al. 2006; Nielsen et al. 2008).

It is still debated whether red deer in Ireland are native or introduced (Staines et al. 2008; Pérez-Espona et al. 2009a). According to radiocarbon dated skeletal remains, red deer are present in the fossil record in Ireland from $27\,730 \pm 380$ radio-carbon years before present (yrs BP) until $11\,790 \pm 120$ yrs BP, then it is absent until $4\,190 \pm 65$ yrs BP (Woodman et al. 1997). This 7 500 year gap suggests it is more probable that the current Irish population is derived from several human-mediated introductions (both ancient and modern) than it is native (Woodman et al. 1997; McCormick 1999). Several Irish red deer populations are known to be descended from red deer derived from Continental European and British stocks and were subsequently introduced to Ireland in the early 1990s to Screebe Estate in County (Co.) Galway (Banwell 1994) and in 1891 to Glenveagh in Co. Donegal (Whitehead 1964). The first documented introduction of red deer to Ireland was recorded in 1246 when an unknown number of deer were translocated from the Royal Forest, Chester, England to the then Royal Forest, Glencree, Co. Wicklow (Moffat 1938; Whitehead 1960, 1964). These introductions from various geographically isolated regions would have a large effect on the genetic structure of red deer within Ireland as a whole. The origin of red deer in the Killarney Valley, Co. Kerry is still unknown. There is currently no evidence that the Killarney population is derived from a glacial relict population, although they may be the only remnant stock of Ireland's ancient population (Staines et al. 2008). Red deer from Co. Kerry have been exported to Cos. Fermanagh and Tyrone in the north and to Scotland in the 18th and 19th Centuries (Whitehead 1964) and in the early 1980s, with the purpose to protect the pure genetic integrity of this believed 'native' population, to Cos. Galway (Connemara National Park), Cork (Doneraile Wildlife Park) and Kerry (the island of Inis Mhic Uileáin).

Another species of deer whose range is sympatric with that of the red deer in Ireland is the sika deer (*Cervus nippon*; Hayden and Harrington 2000). Sika deer were introduced into Ireland in 1860 by Lord Powerscourt onto his estate in Co. Wicklow (Powerscourt 1884). Two females and one male were translocated to Co. Kerry in 1864 (Fehily and Shipman 1967) and sika deer are now fully established in this region (Hayden and Harrington 2000). Red and sika deer can interbreed in enclosures (Harrington 1982) and in the wild (Goodman et al. 1999;

Diaz et al. 2006; Senn and Pemberton 2009) and this is known to have occurred in Ireland as early as 1884, primarily in Co. Wicklow (Powerscourt 1884), with additional reports from Cos. Kerry (Moffat 1938) and Fermanagh (Brooke 1898). Harrington (1979) applied morphometrics and a qualitative genetic method using serum proteins to reveal that no deer in Co. Wicklow were 'pure', essentially forming a hybrid swarm in the region (Pérez-Espona et al. 2009a). However, it is not certain to what extent, or indeed if, hybridisation has occurred in Co. Kerry (Long et al. 1998) and in the north of the country where the two species are sympatric (Hayden and Harrington 2000).

The purpose of this study is to conduct an investigation into the overall genetic diversity and population structure of red and sika deer in Ireland and to determine if and where hybridisation has occurred using control region sequences from the mitochondrial genome (mtDNA; which is inherited maternally) and microsatellite data (bi-parentally inherited) from all individuals.

Material and methods

Sample collection and DNA extraction

Tissue samples from males and females were collected throughout 2007 and 2008 from legally culled deer. A total of 85 red deer and 47 sika deer were collected from 6 and 3 loosely defined populations/regions (Table 1; Figs. 1A and B), respectively. DNA was extracted from ethanol-preserved tissue using the ZR Genomic DNA II Kit (Zymo Research) according to the manufacturer's protocol.

Mitochondrial DNA analyses

MtDNA sequences were obtained from all red and sika deer samples. The entire control region was amplified using primers CE-CR-FOR (5'-CAATACAC-TGGTCTTGTAACC-3') and CE-CR-REV (5'-TAA-TAGGAA GGCTGGGACC-3'). Polymerase chain reactions (PCR) consisted of 1 µL (20–100 ng/µL) of DNA extract, 1.5 µL (0.375 µM) of both forward and reverse primers and 46 µL of the PCR mix MegaMix~Blue (Microzone Ltd.). PCR amplification was carried out using an initial denaturation of 95°C for 3 min followed by 30 cycles of 95°C for 45 s, annealing temperature of 55°C for 45 s and extension of 72°C for 90 s. This was followed by a final extension step of 10 min. PCR products were purified and sequenced using both forward and reverse primers by Macrogen Inc. (Seoul, Korea).

Sequences were edited and assembled into contigs in Sequencer v. 4.5 (GeneCodes). Sequences were aligned

Table 1. Locality of sample origin and number of individuals (*n*) in each species sampled in each locality.

Map No.	Locality name	Red deer (<i>C. elaphus</i>)					Sika deer (<i>C. nippon</i>)						
		<i>n</i>	H_O	H_E	A_R	π	<i>h</i>	<i>n</i>	H_O	H_E	A_R	π	<i>h</i>
1	Co. Down	2	–	–	–	–	–	2	–	–	–	–	–
2	Co. Wicklow	10	0.687	0.846	6.490	0.01639	0.643	33	0.569	0.591	3.640	0	0
3	Killarney, Co. Kerry	46	0.500	0.550	3.650	0.00157	0.492	12	0.229	0.192	1.630	0.00087	0.182
4	Co. Galway	13	0.598	0.702	3.990	0.00800	0.782	–	–	–	–	–	–
5	Co. Mayo	3	–	–	–	–	–	–	–	–	–	–	–
6	Co. Donegal	11	0.633	0.775	5.790	0.00726	0.808	–	–	–	–	–	–

See Fig. 1A for approximate geographic locations. Measures of observed (H_O) and expected heterozygosities (H_E), allelic richness (A_R) for microsatellite loci, and nucleotide (π) and haplotype (*h*) diversities for mtDNA are given for each locality with more than 3 individuals.

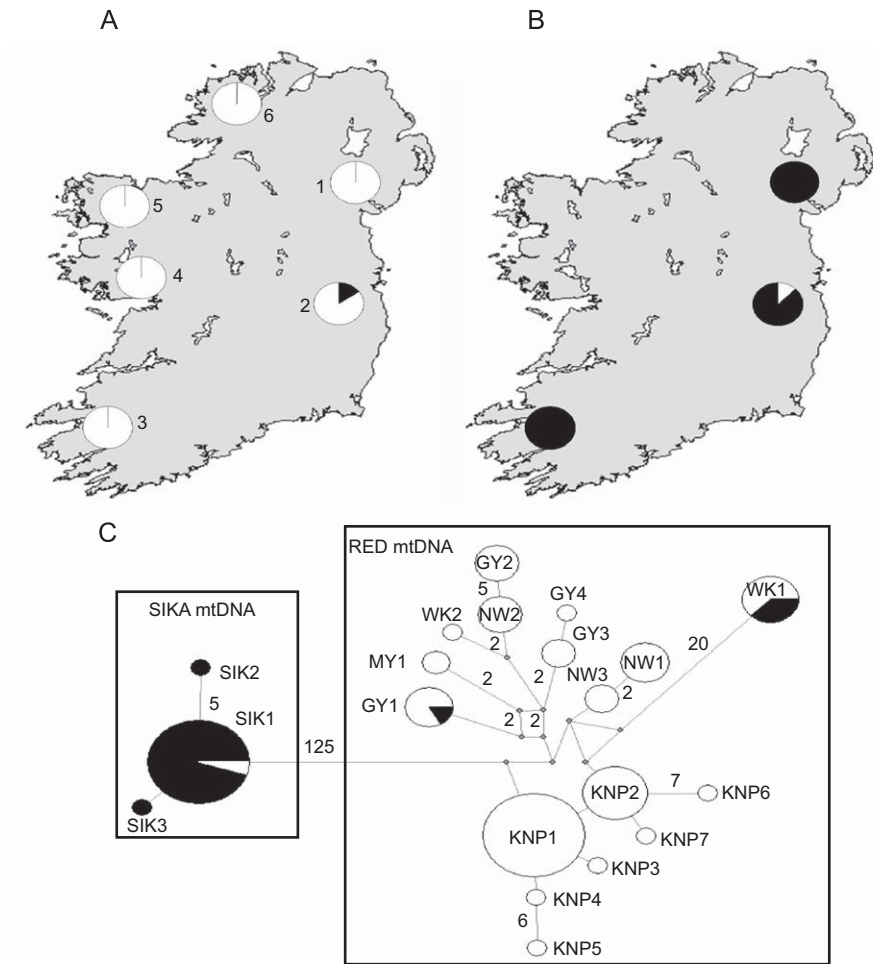


Fig. 1. Distribution of red deer mtDNA (white) among individuals identified as red deer in Ireland (A). Distribution of sika deer mtDNA (black) among individuals identified as sika deer (B). Two red deer possessed sika deer mtDNA (A) and four sika deer possessed red deer mtDNA (B). Refer to Table 1 for details on localities for each population (1, 2, 3, 4, 5 and 6). Median-joining network of individuals identified as red deer (white) and sika deer (black; C). Circle sizes are proportional to frequency (see Table 2). Small grey dots indicate missing haplotypes. Numbers on branches indicate more than one mutation event.

manually using MEGA v. 4.0 (Tamura et al. 2007). Sequences were trimmed to exclude inconsistencies and unreliable sequence data at either end of the control

region, a total of 757 base pairs (bp) were analysed for red deer and 836 bp for sika deer. The difference in the amount of sequence data between species is due to a

39 bp tandem repeat being present in multiple repeats in sika deer whereas red deer have only a single copy (Senn and Pemberton 2009).

A web BLAST search revealed some deer determined morphologically as red deer (*C. elaphus*) by stalkers possessed sika deer (*C. nippon*) control region haplotypes and *vice versa*. Control region haplotypes were identified using DAMBE v. 4.5.50 (Xia and Xie 2001). Relationships among haplotypes from all sequences were examined using a median-joining network (Fig. 1C) implemented in Network v. 4.5 (Bandelt et al. 1999). Analyses of control region data within each species excluded individuals possessing control region haplotypes from the other species. Nucleotide and haplotype diversity of both red and sika deer within each locality and Ireland as a whole were calculated using DnaSp v. 4.10.9 (Rozas et al. 2003).

Microsatellite DNA analyses

In total, 9 polymorphic microsatellite loci RT1, RT7, RT13 (Wilson et al. 1997), BL42, BM203, BM757, BM4513 (Bishop et al. 1994), IDVGA55 (Mezzalani et al. 1995), and OarFCB193 (Buchanan and Crawford 1993) that have shown polymorphism in both species in previous studies (Slate et al. 1998; Bonnet et al. 2002) were tested on red and sika deer in this study. Locus BM4513 gave inconsistent results for sika deer so was excluded from further analyses involving sika deer. A total of 132 individuals were genotyped in 10- μ L multiplex reactions containing 0.5 μ L of DNA extract (10–50 ng/ μ L) and 1 \times Multiplex PCR MasterMix (Qiagen Ltd., UK). Primer concentrations ranged between 0.1 and 0.4 μ M. Amplification conditions were as follows: 95 °C for 15 min; 35 cycles of 94 °C for 45 s, 54 °C for 90 s, 72 °C for 1 min and a final extension at 72 °C for 45 min. All PCR products were run on an ABI PRISM 3130xl Genetic Analyser 16 Capillary system (Applied Biosystems) and sized with internal lane standard (600 LIZ; Applied Biosystems) using the program Genemapper v. 4.0 (Applied Biosystems).

With the exception of samples obtained from Killarney National Park (Co. Kerry) and Screebe Estate (12/13 individuals from Co. Galway), samples were collected randomly by deer stalkers throughout the island of Ireland. Therefore, for analysis of genetic structure of both species in Ireland, we made no assumptions as to the spatial localisation of samples. We tested for population substructure within each of the two species using the Bayesian clustering algorithm implemented in STRUCTURE v. 2.2 (Pritchard et al. 2000; Falush et al. 2003). Ten red and six sika deer, identified as hybrids using either mtDNA or microsatellites (see Results), were excluded from these analyses. Ten independent runs were performed for

each K (number of inferred genetic clusters) value (1–10) in each species using 500 000 iterations, with a burn-in period of 100 000 iterations. STRUCTURE was run using the admixture model and correlated frequencies (Pritchard et al. 2000; Falush et al. 2003). The number of clusters (K) was calculated by obtaining the mean posterior probability of these data ($\ln \text{Pr } X | K$) over the 10 independent runs. Individuals were assigned to clusters based on their highest membership coefficient (Q) to a particular genetic cluster averaged over the 10 independent runs.

Tests for linkage disequilibrium between all pairs of loci within each population of each species and overall within each species were implemented in GENEPOP v. 3.4 (Raymond and Rousset 1995) using 10 000 permutations. Measures of observed (H_O) and expected heterozygosity (H_E) and allelic richness (A_R) for each locality and clusters identified by STRUCTURE were calculated in FSTAT v. 2.9.3 (Goudet 1995). Localities with small sample sizes (Cos. Mayo and Down) were excluded from these analyses.

Hybridisation

Hybridisation was tested between species using a Bayesian admixture analysis approach implemented in STRUCTURE to obtain individual genetic assignment to either *C. elaphus* or *C. nippon* based on the 8 microsatellite loci that amplified in both species. We assumed the presence of two genetic clusters/species ($K = 2$; Sanz et al. 2009; Senn and Pemberton 2009). STRUCTURE was run with 10 independent runs using 500 000 iterations, with a burn-in period of 100 000 iterations. Thus, each of the 132 individuals could be assigned to either the *C. elaphus* cluster (when membership probability is >0.99), to the *C. nippon* cluster (<0.01) or to the interspecific hybrid group ($0.01 < Q < 0.99$). The use of a less stringent 0.01 as a cut-off point increases the chance of identifying hybrids, although it also potentially increases the risk of misidentifying ‘pure’ individuals as hybrids due to ancestral polymorphism (Senn and Pemberton 2009). Three classes of individuals were considered: ‘pure’ red deer (*C. elaphus*), ‘pure’ sika deer (*C. nippon*) and hybrids.

Results

Mitochondrial DNA

Analyses of the control region sequences revealed that two individuals identified as red deer possessed sika mtDNA and four individuals identified as sika deer possessed red deer mtDNA (Fig. 1C). All of these individuals were collected in Co. Wicklow (Figs. 1A and B).

Table 2. Number and distribution of red deer haplotypes in Ireland (see Fig. 1C).

Haplotype	Localities found	No. of individuals
KNP1	Killarney, Donegal	33
KNP2	Killarney	10
KNP3	Killarney	1
KNP4	Killarney	1
KNP5	Killarney	1
KNP6	Killarney	1
KNP7	Killarney	1
NW1	Donegal, Mayo, Galway, Down, Wicklow	6
NW2	Donegal	5
NW3	Donegal	3
GY1	Galway, Wicklow, 1 sika deer	6
GY2	Galway	4
GY3	Galway, Mayo	3
GY4	Galway	1
MY1	Mayo	2
WK1	Wicklow, 3 sika deer	8
WK2	Wicklow	1

Excluding the above individuals, a total of 51 polymorphic sites were found in the control region of Irish red deer, giving 17 haplotypes (Fig. 1C). These have been deposited in Genbank (Accession nos.: FJ743483–FJ743499). Overall nucleotide diversity (π) and haplotype diversity (h) was 0.00834 ± 0.00118 and 0.808 ± 0.037 , respectively. Nucleotide and haplotype diversity was lowest in the Killarney red deer, with measures of up to ten times higher in other regions (Table 1). Haplotype KNP1 was found in the majority of individuals from Killarney and two individuals from Donegal (Table 2). Haplotype NW1 was found in individuals from Cos. Donegal, Mayo, Down, Galway and Wicklow (Table 2).

Only 5 polymorphic sites were found in the control region of the sika deer, giving three haplotypes (Accession nos.: FJ743500–FJ743502; Fig. 1C). Nucleotide and haplotype diversity were very low ($\pi = 0.00025 \pm 0.00023$ and $h = 0.051 \pm 0.048$). Nucleotide and haplotype diversities were zero in Wicklow and low in Co. Kerry (Table 1). A single haplotype was found in the Wicklow region with a further two haplotypes found in Co. Kerry.

Microsatellite DNA

A total of 122 alleles were found in red deer using 9 microsatellites and 61 alleles were found in sika deer using 8 microsatellites. Tests for linkage disequilibrium over all populations in red deer revealed that locus IDVGA55 showed evidence of linkage with loci BL42

and RT13 ($P < 0.001$). However, this differed between populations and all loci were retained in the analyses. No locus showed evidence of linkage in sika deer. Clustering analyses in STRUCTURE defined 3 distinct genetic clusters in the red deer (Ln Pr X | K: –2027.94). Red deer in Killarney National Park formed one distinct cluster (Red Cluster 1; Fig. 2A) while red deer from Co. Galway (and one individual from Co. Mayo) formed another distinct cluster (Red Cluster 2; Fig. 2A) and all other individuals were grouped together in a third cluster (Red Cluster 3; Fig. 2A). Measures of H_O , H_E and A_R in ‘Red Cluster 1’ were 0.500, 0.550 and 4.31, respectively; 0.604, 0.708 and 4.80, respectively, in ‘Red Cluster 2’; and 0.588, 0.801 and 7.95, respectively, in ‘Red Cluster 3’.

Sika deer formed two distinct clusters in Ireland (Ln Pr X | K: –554.23). Individuals from Killarney National Park formed their own distinct genetic cluster (‘Sika Cluster 1’) while individuals from Cos. Wicklow and Down were a mixture of ‘Sika Cluster 1’ and ‘Sika Cluster 2’ (Fig. 2A). Measures of H_O , H_E and A_R in ‘Sika Cluster 1’ were 0.242, 0.210 and 1.97, respectively and 0.563, 0.556 and 3.80, respectively, in ‘Sika Cluster 2’.

Hybridisation between red and sika deer

Bayesian admixture analyses of microsatellite data assigned 88.2% (75/85) of red deer (*C. elaphus*) samples to the ‘pure’ red deer genetic cluster at a probability > 0.99 . Two individuals (2.4%) were identified as belonging to the ‘pure’ sika deer genetic cluster and these same individuals possessed a sika deer mtDNA haplotype. These were both from the Wicklow region. A total of eight red deer individuals (9.4%) were identified as interspecific hybrids ($0.011 < Q < 0.989$). Six red deer identified as hybrids were from the Wicklow region (membership to red Q = 1: 0.654; 0.0.823; 0.655; 0.859; 0.989 and 0.664) with a further two individuals from Cos. Mayo and Galway (red Q: 0.977 and 0.970; Fig. 2B). A large number of sika deer (42/47; 89.4%) were ‘pure’ sika deer. Five sika deer (10.6%) were classified as an interspecific hybrid ($0.016 < Q < 0.984$). All these individuals were from Co. Wicklow (sika Q = 1: 0.035; 0.886; 0.055; 0.984 and 0.981; Fig. 2B). Two of these individuals possessed a red deer mtDNA haplotype. Interestingly, one further sika deer which possessed red deer mtDNA was classified as ‘pure’ sika using microsatellites.

Discussion

Genetic diversity and structure of red deer

Overall genetic diversity within Ireland using mtDNA was equivalent to other red deer populations in

Scotland, Bulgaria and Romania and others (Hajji et al. 2007). This is likely due to the fact that the Irish population is derived from multiple introductions rather than maintaining genetic diversity over a long period of time (Whitehead 1960, 1964; Staines et al. 2008). However, red deer within Killarney National Park had

a very low level of nucleotide diversity (Table 1), much lower than that observed in other regions of Ireland and populations in Scotland and England (Hmwe et al. 2006; Pérez-Espona et al. 2009b). Control region haplotypes were generally shared throughout the island with the exception of the Killarney red deer. Two Donegal red

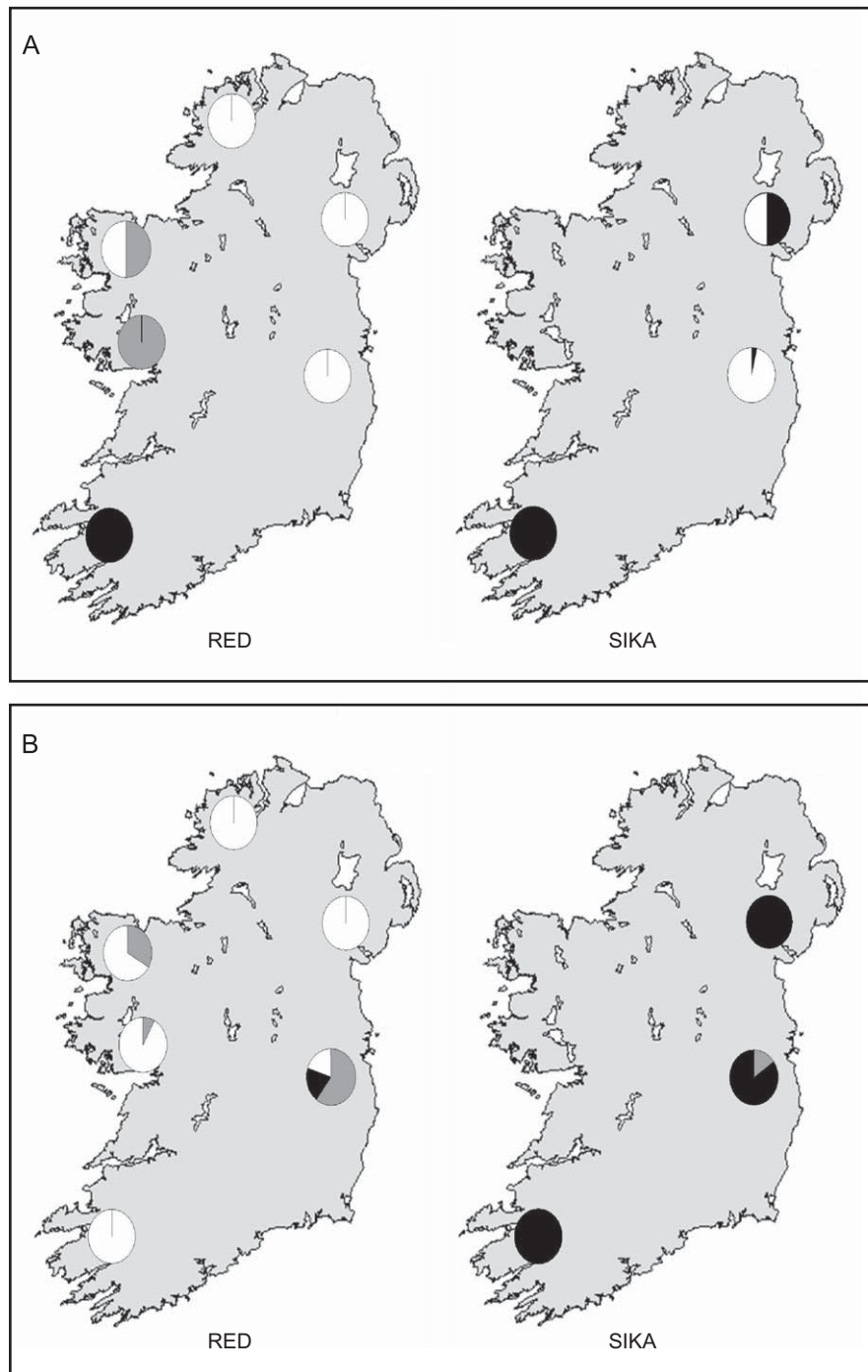


Fig. 2. Distribution of genetic clusters identified by STRUCTURE (A) of red deer and sika deer in Ireland. The ‘Red Cluster 1’ is represented by black, the ‘Red Cluster 2’ by grey and the ‘Red Cluster 3’ by white for red deer. ‘Sika Cluster 1’ is represented by black and the ‘Sika Cluster 2’ by white in sika deer. Distribution of ‘pure’ red deer (white), hybrids (grey) and ‘pure’ sika deer (black) found in red and sika deer (B).

deer in the north shared a haplotype with the southern Killarney red deer but this is likely due to deer being translocated in the past from the southern region of Ireland to the northern regions (Whitehead 1960, 1964). Haplotype NW1 is spread throughout the country, occurring in Cos. Donegal, Mayo, Down, Galway and Wicklow (Table 2) and three other haplotypes were shared between regions. Clearly the most divergent group according to mtDNA is the red deer occupying the Wicklow region (haplotype WK1; Fig. 1C). Although two individuals share haplotypes with red deer from other parts of the country, the majority of red deer from the region are distinct from the rest of the country. This may be due to a unique introduction. Lord Powerscourt is known to have imported red deer from the Island of Islay, Scotland to his estate in Wicklow at some stage in the late 1800s (Whitehead 1960, 1964) and red deer were translocated in 1246 from England to the Glencree valley in Co. Wicklow which is nearby to Powerscourt's Estate (Moffat 1938). The origins of the Wicklow red deer, in addition to the other red deer populations in Ireland, are being explored within a concurrent red deer phylogeographic study (R.F. Carden, A.D. McDevitt and C.J. Edwards, pers. comm.).

Bayesian clustering analysis revealed the presence of three distinct genetic clusters within Ireland (Fig. 2A). All individuals from Killarney grouped in a single cluster (Red Cluster 1), individuals from Co. Galway and one individual from Co. Mayo formed another cluster (Red Cluster 2) and finally, individuals from the rest of the country formed the third genetic cluster (Red Cluster 3). The clustering of Killarney red deer is not surprising given its isolation from other red deer populations. The founding stock of the Screebe Estate herd in Co. Galway (where 12/13 individuals were from) originates from Britain (Warnham deer park) and western European stocks (Banwell 1994). The third genetic cluster is composed of what appears to be the relatively isolated (from one another) populations/regions of Cos. Donegal, Down, Mayo, Wicklow (Fig. 2A). STRUCTURE identifies the minimum number of genetic clusters (Pritchard et al. 2000) so this may be a somewhat over-simplified overview. However, the results obtained from microsatellites concur with the results obtained from mtDNA (see above). Killarney red deer are isolated from the rest of the country using both types of molecular markers. Like the mtDNA data, the microsatellite data indicates gene flow between the populations in the rest of the country. Currently, red deer populations (with the exception of Killarney) in Ireland may be more connected to each other than the distribution range of the species suggests (Hayden and Harrington 2000; Staines et al. 2008), at least the eastern, northern and north-western red deer populations may be interconnected (Gammell et al.,

pers. comm.). Alternatively, it is highly likely that these results may be confounded by unreported translocation of live deer from one geographical area to another in order to establish and/or add to a local population of deer (Frantz et al. 2006). Further investigations are required to quantify absolute ranges of each population in Ireland.

Measures of genetic diversity using microsatellites were very low in Killarney compared to Galway and the rest of Ireland. This may be an artefact of the populations occurring in the rest of the country representing several populations rather than two genetic clusters. However, it is clear that the Killarney population has levels of diversity similar to those present in threatened red deer populations in Tunisia for example (Hajji et al. 2007). Historical records pertaining to the Killarney population indicate a bottleneck or population crash around 1860 (post Famine) and prior to or around 1970 (Ryan 2001), which was prior to the Irish Wildlife Act 1976, where red deer were protected by law. Furthermore, these records from the 18th Century onwards indicate that the red deer numbers continued to remain low, even after the last wolf was shot in Co. Kerry in 1720 (Ryan 2001). There are no reports of red deer numbers increasing after the only natural mammalian predator was eliminated from the island. These factors, coupled with the population's isolation from the rest of Ireland, may have caused levels of genetic diversity to remain low.

Genetic diversity and structure of sika deer

Measures of genetic diversity of Irish sika deer were typically low, as expected for a recently introduced species. Only three mtDNA haplotypes were found in 47 animals (Fig. 1C). This species has only been present in Ireland for approximately 150 years and levels of diversity were similar to those found in Scotland (Senn and Pemberton 2009). Two distinctive genetic clusters were identified using microsatellites (Fig. 2A) and this can be simplified into two clusters-Co. Kerry (Sika Cluster 1) and Cos. Down and Wicklow (Sika Cluster 2; Fig. 2A). These regions had low levels of diversity; Kerry being particularly low which is not surprising given that it was founded by only 3 individuals from Wicklow in 1864 (Fehily and Shipman 1967).

Hybridisation between red and sika deer

It has been known from observations of hybrid phenotypes in the Wicklow region, direct cross-breeding experiments and protein testing that hybridisation has been occurring between red and sika deer in Ireland for some time (Powerscourt 1884; Harrington 1973, 1979, 1982). What is unknown though is whether this has

spread to other parts of the country where the species' ranges are sympatric, and the full extent to which this occurs in wild-ranging red and sika deer.

Two red deer possessed sika deer mtDNA haplotypes but microsatellite analysis revealed that these two individuals belonged to the 'pure' sika genetic cluster. Phenotypically, it is difficult to identify hybrid deer in Wicklow based solely on their respective external appearances (pelage characteristics). Body proportions (head, neck, body ratios) vary considerably depending on the dominant phenotype displayed and assignment of such characteristics can be highly subjective to the observer. These characters may be confounded by successive generations of hybrid deer that may favour assignment towards either red or sika deer based on either head or body exhibited characteristics that are similar to the pure form (Senn and Pemberton 2009; R.F. Carden, pers. comm.).

Six interspecific hybrids were found in individuals identified as red deer and five individuals identified as sika deer in the Wicklow region (Fig. 2B). A further two red deer individuals from Cos. Mayo and Galway were identified as hybrids. We used a less stringent method to identify hybrid individuals than in previous studies of red-sika hybridisation in Britain (Diaz et al. 2006; Senn and Pemberton 2009) but our method is justified in this study given that three sika deer individuals carrying red deer mtDNA would not have been identified as hybrids if the $Q=0.05$ cut-off point used initially by Senn and Pemberton (2009) was applied. This is also confounded by the fact that only three of the loci used by Senn and Pemberton (2009) were used in this study, thus making the use of a less stringent method necessary to identify hybrid individuals that are not 1st or 2nd generation hybrids (Senn and Pemberton 2009). The majority of 'red deer' in Wicklow were identified as hybrids with five of these individuals being either 1st or 2nd generation hybrids (sika $Q=0.141-0.346$). This implies that the breeding hind of these individuals was a red deer and the breeding stag a sika deer (because individuals possessed red deer mtDNA). Harrington (1982) failed to show that male sika deer produced offspring with female red deer in enclosures but it appears as though this is the case in wild populations in Ireland (this study) and Scotland (Senn and Pemberton 2009). Interestingly, Harrington (1979, 1982) suggested that most, if not all, of the deer occurring in the Wicklow region were hybrids and that deer in the region have now formed a hybrid swarm (Pérez-Espona et al. 2009a). The results presented here show that the majority of supposedly 'red deer' individuals were hybrids (Fig. 2B) but we might expect to see the same situation in sika deer if most of the deer in the region are hybrids. Only five individuals were identified as hybrids with another individual having red deer mtDNA and 'pure' sika deer microsatellites. Therefore, only a small minority of sika deer are hybrids

(12.8%), the majority of sika deer in Wicklow appear to be 'pure' sika deer. This is possibly due to the direction of hybridisation being determined by colonising (or 'pioneering') sika males (Pérez-Espona et al. 2009a; Senn and Pemberton 2009), and the knock-on effect of this will be a greater dilution of red deer DNA, rather than sika deer DNA. Hybridisation in wild, free-ranging red and sika deer still appears to be a rare event and based on the results of this study and contrary to popular belief, it is doubtful that all, or even most, of the deer in Wicklow are hybrids (Harrington 1979; Pérez-Espona et al. 2009a).

The majority of hybrids found in this study were confined to the Wicklow region (Fig. 2B). However, hybrids were also found in Cos. Mayo and Galway. Unfortunately, we had no sika deer from this region as sika deer are not known to be sympatric with red deer in this area (Hayden and Harrington 2000). There is a population of the large bodied Manchurian sika deer (*C. n. mantchuricus*) in the south Mayo region, escaped from an enclosed area (P. Wood, pers. comm.). Hybridisation has been documented in Co. Fermanagh in a Park (Brooke 1898) and these hybrids may have dispersed westwards over time. It is known among local deer stalkers that large-sized sika deer have been illegally translocated to Cos. Mayo and Galway over the years and with the free-roaming escaped Manchurian sika deer in the area (P. Wood, pers. comm.), suggests a possible mechanism by which hybridisation occurred. This could have occurred either by 'pure' sika deer breeding with 'pure' red deer or the translocation of hybrid individuals. The presence of hybrids in the West of Ireland is potentially a threat to the red deer of Screebe Estate, particularly as this herd is important commercially from a hunting perspective and the translocated Killarney red deer within Connemara National Park.

It has been suggested that hybridisation mostly arises in either enclosures or Parks (Pérez-Espona et al. 2009a) but it can and does occur in wild populations (Kintyre, Scotland; Senn and Pemberton 2009). Importantly, despite the range of sika deer completely overlapping that of red deer in Killarney National Park, it appears that no hybridisation has occurred between the species (Harrington 1979; Long et al. 1998). It is important to note, however, that this was based on a limited number of samples in the area, particularly sika deer. However, a large amount of red deer were analysed in Killarney (compared to the other regions) and judging by the number of hybrids found in Wicklow, we would expect to record them in Killarney also. There must be some explanation as to why hybridisation has not occurred in Co. Kerry and is supposedly widespread in Wicklow, as both species' ranges have been sympatric for almost the same period of time. The lack of hybrids in Kerry may be due to behavioural and/or morphological differences.

Thus, morphological and behavioural differences may exist. Several possible reasons could be put forward tentatively at this point; however, further in-depth investigations are warranted. One reason could be due to overall body size differences—Wicklow sika deer are of a larger body size than Kerry sika deer, and both Wicklow and Kerry red deer are relatively similar in overall body sizes (Carden 2006). There are large body and skeletal size differences between both sexes of adult sika and red deer (Carden 2006). Thus, mating may be attained, if size is a precursor to successful mating in a hybridisation event, between the larger Wicklow sika and the Wicklow red deer.

Management implications

It is important to understand the underlying mechanisms as to why hybridisation occurs between these two species and what can be done to prevent it. Given the short period of time that the two species have been in contact in Ireland (~150 years), hybridisation is still relatively rare and is not as widespread in areas as was previously thought (Harrington 1979; Pérez-Espona et al. 2009a). However, it seems inevitable that sympatric populations will become hybridised over time (Pérez-Espona et al. 2009a; Senn and Pemberton 2009) and expanding sika deer populations represent a very real threat to the genetic integrity of ‘pure’ red deer populations. While hybridisation can be beneficial to a species, as new adaptive diversity can be generated (Seehausen 2004; Reyer 2008; McDevitt et al. 2009), the persistence of ‘pure’ populations that are genetically unique and isolated, such as the Killarney red deer within an Irish context, must remain paramount. Selective shooting of obvious phenotypically hybrid individuals as well as selective culling of pioneering sika stags entering red deer areas should be encouraged (Pérez-Espona et al. 2009a), particularly in the West of Ireland and Killarney. This study represents a preliminary investigation into hybridisation between red and sika deer in Ireland and it is vital that ongoing research be expanded upon and conducted on identifying regions where hybridisation occurs in Ireland and where it could potentially be a threat to red deer populations.

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