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Landscape genetics of red deer (Cervus elaphus L., 1758) in Killarney National Park, Co. Kerry

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In recent years, the emergence of the discipline of ‘landscape genetics’ has proved to be a vital tool in quantifying the effects of landscape features (both natural and man-made) on gene flow in animal species. We applied this methodology to red deer in Killarney National Park (KNP), Co. Kerry, to infer possible barriers to gene flow. Bayesian analysis revealed the presence of two distinct genetic clusters, present on either side of Lough Leane. We discuss possible barriers to gene flow and the management implications of this for red deer in KNP and the wider implications of using landscape genetics in Irish mammalian research.

Keywords: Bayesian, gene flow, management, microsatellites, mitochondrial DNA

**INTRODUCTION**

Gene flow between populations or groups of animals is dictated by a multitude of internal (i.e. vagility/dispersal) and external (i.e. landscape and environmental) factors (Pérez-Espona et al. 2008). Understanding the interplay between these factors is central to maintaining viable, genetically diverse populations. It has been well established that landscape features affect the dispersal ability of animals and therefore impact upon gene flow and genetic structure of populations (Manel et al. 2003). These include natural barriers such as mountains and lakes (Pérez-Espona et al. 2008) and rivers (Cullingham et al. 2009, Franzø et al. 2010) and man-made barriers such as roads and developed areas (Epps et al. 2005, Riley et al. 2006, Franzø et al. 2010).

In recent years, a new discipline has emerged called ‘landscape genetics’ (Manel et al. 2003). This combination of population genetics and landscape ecology has allowed us to assess quantitatively the effects of landscape features on genetic structure. With the emergence of this new field of study, numerous methodologies have been applied, from pairwise relationships across putative barriers (e.g. Pérez-Espona et al. 2008, Franzø et al. 2010) to the application of Bayesian methods (e.g. Cullingham et al. 2009, McDevitt et al. 2009a) to infer genetic structure. Furthermore, accurate geographic information systems (GIS) have greatly aided visualization and quantification of the effects of landscape features (Pérez-Espona et al. 2008).

Given that landscape genetics is very much an emerging discipline, it is perhaps not surprising that there is a lack of such studies in Ireland. Of the terrestrial animals, mammals have undoubtedly received the most attention in terms of population genetics studies (McDevitt et al. 2009b). However, of those that applied the use of microsatellite markers (the genetic markers traditionally used for studies of inter- and intra-population variability and differentiation), none of these investigated the role of barriers to gene flow at the local or fine-scale level (Hamill et al. 2007, McDevitt et al. 2009a, 2009b). As the human population in Ireland has hit its highest levels since the Great Famine (1845-1852) such studies are both natural and human-mediated causes of fragmentation in Irish mammalian populations.

In this study, we investigate fine-scale genetic structure in red deer (Cervus elaphus L., 1758) in Killarney National Park (KNP), Co. Kerry, using a landscape genetics analysis. Red deer are sympatric with sika (C. nippon Temminck, 1838) in KNP (Carden et al. 2011) and both species are managed under the Killarney National Park Management Plan, which does not conflict with conservation efforts of their associated habitats (National Parks and Wildlife Service 2005). Red deer in KNP are genetically isolated from populations in the rest of the country and display
low levels of genetic diversity in comparison to other Irish populations (McDevitt et al. 2009b). Therefore, KNP may act as an island system, although illegal translocations and escapes cannot be ruled out. The aim of this study was to investigate if natural or human-mediated features disrupt gene flow in red deer using polymorphic microsatellite loci and mitochondrial DNA (mtDNA) in a landscape genetics analysis. The results of this study may have important implications not just for red deer but also other, less vague, Irish mammalian species of conservation value or concern within KNPs and across the island of Ireland as a whole.

**Methods**

**Study Area**

Killarney National Park (V72897) comprises an area of 10,289 hectares that is administered and maintained by the National Parks and Wildlife Service (NPWS) of the Department of the Environment, Heritage and Local Government. It lies to the south-west of Killarney town, Co. Kerry, within 25 km of the sea to the north and south. Continuous tracts of mountain, lake, bog and native forests exist in KNPs and these natural features support a rich flora and fauna (Carruthers 1998). The estimated population of red deer within KNP in 2009 was approximately 600 (O’Toole 2009 pers. comm.). Previous estimates were of the order of between 800 and 1000 individuals (National Parks and Wildlife Service 2005). Small numbers of red deer are managed, primarily in order to reduce conflict with human land-use objectives (e.g. damage to agriculture crops on neighbouring lands) and conservation efforts in woodland regeneration within KNPs. Numbers of red deer within KNPs in particular in the mountainous regions, have been historically low since at least the early part of the 19th century (see Whitehead 1960, 1964, Ryan 1998, 2001). The establishment of two deer forests during the 1840s around Lough Leane in KNPs gave some protection to the red deer forests during the 1840s around Lough Leane; this is reflected in the sampling (Fig. 1). Coordinates of the culling sites were determined by using the centre point of the field in which the animal was culled. DNA was extracted from ethanol-preserved tissue using the ZR Genomic DNA II Kit (Zymo Research) according to the manufacturer’s protocol or a simple salting out procedure (Miller et al. 1988).

**Culling Site Location Information**

Culling site location information was used in this study (Fig. 1). Relatively small numbers of animals are mostly culled on the east side of Lough Leane due to conflict with human land-use objectives along the border areas of KNPs, the ongoing conservation efforts with regards to woodland habitats within KNPs and the occurrence of much smaller densities of red deer (>600) on the west side of the Lough; this is reflected in the sampling (Fig. 1). Coordinates of the culling sites were determined by using the centre point of the field in which the animal was culled. DNA was extracted from ethanol-preserved tissue using the ZR Genomic DNA II Kit (Zymo Research) according to the manufacturer’s protocol or a simple salting out procedure (Miller et al. 1988).

**Genetic Analysis**

Nine polymorphic microsatellite loci, RT1, RT7, RT13 (Wilson et al. 1997), BL42, BM203, BM757, BM4513 (Bishop et al. 1994), IDVG55 (Mezzalani et al. 1995) and OaFCB193 (Buchanan and Crawford 1993) were used to genotype red deer according to the protocol described in McDevitt et al. (2009b).

In order to infer contemporary fine scale genetic structure in red deer within KNPs we conducted a Bayesian clustering analysis using the software GENELAND v. 3.1.5 (Guillot et al. 2005a, 2005b, 2008). GENELAND incorporates geographical information to produce accurate inferences about population structure and spatial barriers/borders between inferred genetic clusters. The software uses a Monte Carlo Markov Chain (MCMC) method to maximize Hardy-Weinberg and linkage equilibrium within inferred clusters (Guillot et al. 2005b). Geographical localities were set to the centre points of fields in which the animals were culled. Twenty independent runs were performed with 1,000,000 iterations and a burn-in period of 200,000 iterations. The number of genetic clusters (K) was set from 1-5. The number of K was obtained from the runs with the highest posterior probability. As a comparison, we also conducted clustering analysis in STRUCTURE v. 2.3.3 (Pritchard et al. 2000) without spatial information. Ten independent runs were performed with 500,000 iterations and a burn-in of 100,000 in Co. Kerry in the early 1950s. Accurate counts of red deer in KNPs began in 1970; estimates at this time approached 600. Whitehead (1964) reported a count of 100 red deer in Co. Kerry in the early 1950s. Accurate counts of red deer in KNPs began in 1970; estimates at this time approached 600. We tested for isolation by distance (IBD) patterns by conducting spatial autocorrelation analysis (the analysis of genetic relatedness of microsatellite evolution (Di Rienzo et al. 1994) with 10 per cent of the infinite allele model and 90 per cent of the stepwise mutation model. mtDNA sequences were obtained from all 39 samples. The entire control region was amplified using primers CE-CR-FOR and CE-CR-REV (McDevitt et al. 2009b) according to the protocol described in McDevitt et al. (2009b) and, in total, 757 base pairs (bp) were analyzed for each individual. Individuals were grouped into the clusters identified by GENELAND (see Results) to determine if there was significant differentiation in haplotype frequencies. This was implemented in Arlequin v. 3.11 (Excoffier et al. 2005).

**Results**

GENELAND identified two distinct genetic clusters in red deer (average log posterior probability: -815.014) within KNPs (Fig. 2). STRUCTURE also identified two distinct clusters (average log posterior probability: -775.9) but for the purposes of this study we focus on the spatially explicit results obtained from GENELAND. Cluster 1 (west of the Lough, Fig. 2) consisted of 7 individuals and Cluster 2 (east of the Lough, Fig. 2) consisted of 32 individuals. Cluster 1 had higher measures of genetic diversity (AR = 3.52; HE = 0.704) than Cluster 2 (AR = 2.84; HE = 0.512). Differentiation between clusters was assessed using the software BOTTLENECK v. 1.2.11 (Cornuet and Luikart 1996) and has been previously on a study of fine scale genetic structure in red deer (Frantz et al. 2008).

Conventional FST analysis (Weir and Cockerham 1984) was performed on clusters identified by GENELAND using the software FSTAT v. 2.9.3 (Goudet 1995) to test if identified clusters were significantly differentiated. Allelic richness (AR) and expected heterozygosity (HE) were also calculated for each cluster. These measures are suitable for groups with different and small sample sizes. The Killarney population as a whole and each cluster separately were tested for heterozygosity excess in order to detect recent population bottlenecks. BOTTLENECK v. 1.2 (Cornuet and Luikart 1996, Piry et al. 1999) was run under the two-phase model of microsatellite evolution (Di Rienzo et al. 1994) with 10 per cent of the infinite allele model and 90 per cent of the stepwise mutation model.
identified by GENELAND was significant (FST = 0.072; P < 0.05). Recent bottlenecks were not detected in either KNP as a whole (Wilcoxon test: P = 0.997) or within clusters identified by GENELAND (Cluster 1: P = 0.248; Cluster 2: P = 0.997). Significant (P < 0.05) negative slopes were found in spatial autocorrelation analysis, conforming to IBD patterns. However, this association was weak (slope b: -0.035).

Only two control region haplotypes (separated by a single bp) were found: haplotypes KN1 and KN2 (McDevitt et al. 2009b). Haplotype KN2 was not found in Cluster 1. Differentiation in haplotype frequencies between clusters was of the same order of magnitude as those found in microsatellites but was not significant (FST = 0.074; P = 0.509).

**DISCUSSION**

Bayesian analysis of population structure in combination with spatial information revealed the presence of two distinct genetic clusters in red deer in KNP (Fig. 2). Indeed, all independent runs returned the same result. IBD analysis revealed that geographic distance was a factor in differentiation but this association was weak. IBD can affect Bayesian inference of genetic structure (Frantz et al. 2010) but this generally has to be a strong association to influence results (Guillot and Santos 2009). Neither is the inference affected by family groups within the inferred clusters, only full sib/parent-offspring pairs were identified in the dataset (data not shown). Differentiation between the clusters was significant; stronger than has been reported in other ungulate species across putative barriers (Coulom et al. 2006, Pérez-Esposa et al. 2008). Coulom et al. (2006) found weak (yet still significant) differentiation in roe deer (Capreolus capreolus L. 1758) between areas separated by roads, rivers and canals. From a visual inspection of the geography and topography of the region (Fig. 1), it appears as though Lough Leane may act as a barrier to gene flow between the inferred clusters. Pérez-Esposa et al. (2008) found that although sea lochs/loughs, mountain slopes, roads and forests were barriers to gene flow in Scottish red deer, inland lochs/loughs and rivers might in fact facilitate gene flow because the cost of circumnavigating them was greater than swimming across. In addition, red deer in KNP have been observed swimming from various islands of Lough Leane (Fig. 1) to the eastern shores on several occasions, particularly males (O’Toole 2009 pers. comm.). Therefore, it seems likely that there are multiple factors restricting gene flow in KNP other than Lough Leane.

Red deer are a vagile species and can disperse considerable distances (150 km; Clutton-Brock et al. 1982; Catt and Staines 1987). Human development and infrastructure do not appear to impede gene flow on the eastern side of the Lough (Figs 1 and 2). In North America, C. elaphus is known to use areas of human activity as a means of refuge from wolves (Mably et al. 2010), but this is an unlikely reason in Ireland as the last Kerry grey wolf (Canis lupus L. 1758) was shot in 1720 (Ryan 2001). However, it does indicate that the species is perhaps not perturbed by humans in the way other mammals might be. We know that red deer are capable swimmers (see above) so the Lough is unlikely to act as an absolute barrier to gene flow. Instead, it is probable that several distinct features restrict gene flow. Although development and infrastructure do not impair gene flow on the east side of the Lough, it may impede individuals from circumnavigating it. Topography also changes on either side of the Lough and a combination of mountains and major roads may impede gene flow occurring south of the Lough (Fig. 1) as has been noted in Scottish red deer (Pérez-Esposa et al. 2008). Habitat selection may also play a role in isolating the two clusters. Females in particular may select for similar habitats to where they were born and fine-scale genetic structure is more evident in female red deer than males (Nussey et al. 2005, Frantz et al. 2008). Unfortunately this dataset is too small to test for such associations.

A possible mechanism by which the two clusters are retained and gene flow restricted is that the species is managed in KNP. Red deer numbers have remained historically low in KNP. Historical documents and reports indicate decreases in numbers after the Great Famine, World War I, the War of Independence, the Civil War and World War II due to increased hunting pressures (food resource) and before the Wildlife Act (1976), not only in KNP but to a greater extent throughout the country (Whitehead 1960, 1964). This is supported by a lack of evidence for a recent bottlenekevent within KNP analyzed as a whole or for each cluster separately. If red deer are maintained at relatively low densities, this promotes divergence between the inferred clusters. Historically, red deer numbers have been relatively low (<20-60) on the west side of Lough Leane (Tories, Shehy, and Purple mountains etc.; Whitehead 1960, 1964). The lack of significant structuring in mtDNA suggests that this divergence is a more recent phenomenon. Because of their polygynous mating system, we should expect mitochondrial divergence to be four times higher than that of autosomal microsatellite loci (Peit et al. 2002) but we found divergence to be almost identical for both. However, it is difficult to make such associations given that there are only two control region haplotypes in the sampled individuals. High density and high dispersal promotes gene flow between putative populations (Gauffre et al. 2008) and this appears to be lacking for red deer within KNP. Measures of genetic diversity were higher west of the Lough than in the east. Furthermore, genetic diversity is low in Killarney overall in comparison to the rest of the country (see Table 1 in McDevitt et al. 2009b) so it is important to promote healthy levels of diversity and gene flow within KNP. This is obviously an important consideration for the ongoing and future management of red deer in KNP as the current measures consider the species as a single management unit/population. However, caution needs to be exercised when drawing conclusions based on this limited dataset. There is no doubt that a great deal of further, more evenly and widely distributed, sampling is warranted, in addition to analyzing the effects of individual, putative barriers quantitatively.

The methodology presented herein represents a novel and useful tool in detecting fine-scale genetic structure in Irish mammals and will, therefore, aid conservation decisions. With the availability of sophisticated GIS tools and data we can now measure the effects of natural and human-mediated features on genetic structure at both individual and population levels. Genetic data can be combined with extensive telemetry data from GPS/VHF/satellite collars to infer structure (e.g. McDevitt et al. 2009a) and has the potential to be used with non-invasive approaches such as utilizing scat and hair samples (Mullins et al. 2010).

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References


Deer management requirements in Irish forestry

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A major challenge is presented to Irish foresters in attempting to balance the demands of society for ever more diverse forests with the need to ensure that our deer populations are maintained in accordance with the range of management objectives and activities which these forests are expected to provide and support. Deer can exert considerable influence, not simply on woodland development and timber quality, but also on a range of other ecological variables that may impact on priority conservation species and habitats. Since forest management is by default also habitat management, sustainably managing deer populations will require foresters and forest owners to equip themselves with the knowledge to plan and manage forests with deer in mind across multiple rotations and at a range of scales from ‘leaf to landscape’.

Keywords: Forest management, Deer management, National strategy

INTRODUCTION

The importance of Ireland’s national forest estate to our wildlife populations cannot be overstated. Our native woodlands and exotic plantations support a wide range of mammal, invertebrate and bird life (O’Halloran et al. 2002, Rooney and Hayden 2002). Since independence, Ireland has come a long way towards reversing historical deforestation and Ireland’s current afforestation rate is now among the highest in Europe - forest cover now stands at almost 11 per cent with over 700,000 hectares planted since 1922 (Neeson 1991, O’Carroll et al. 2009). In addition to providing vital rural employment, the expansion of our forest resources has facilitated a concurrent revival of key mammalian species such as the pine marten (Martes martes (L., 1758)), lesser horseshoe bat (Rhinolophus hipposideros (Bechstein, 1800)) and the red squirrel (Sciurus vulgaris (L., 1758)) (Carey et al. 2007, Marnell et al. 2009). Until recently this benefit to wildlife was not an intended objective of Irish forest policy, but rather a welcome by-product (Clear 1970). Irish forests are now managed in accordance with the principles of Sustainable Forest Management (SFM) with multifunctional objectives in mind, and are no longer confined simply to commercial timber production (Forest Service 2000). In recent years there has been particular emphasis on recreation and biodiversity enhancement (Forest Service 2000, O’Carroll et al. 2009).

Within this context, our expanding and increasingly diverse national forest estate has provided an ideal habitat for thriving populations of red deer (Cervus elaphus L., 1758), sika (Cervus nippon Temminck, 1838) and fallow deer (Dama dama (L., 1758)); populations of each of these species have expanded accordingly (Rooney and Hayden 2002, O’Carroll 2005). Deer are essentially woodland animals, adapted and evolved to live and thrive in such habitat (Prior 1994). Where abundant populations exist, it is well established that deer can impact adversely on commercial timber production (Mooney 1952, Clear 1970, Mayle 1999, Reimoser 1999, Rooney and Hayden 2002, COFORD 2005). High deer population densities can also exert adverse influence over forest development and associated biodiversity values (Prior 1994, Putman 1996, Dilson et al. 2009, Joys et al. 2004, Vavra et al. 2007). Thus, a major challenge is presented to Irish foresters in balancing the demands of society for ever more diverse forests with the need to ensure that deer populations are maintained in accordance with the range of objectives and activities which these forests are expected to provide and support.

DEER SPECIES IN IRELAND

There are four currently known species of deer present in Ireland and, with the exception of red deer, all have been introduced.

Red deer are the least numerous of our deer species and are largely accepted as Ireland’s only native deer species. The species is strongly represented in Cos Donegal, Wicklow, Galway and Kerry. The Kerry population is thought to