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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 features) factors (Pérez-Espona *et al.* 2008). Understanding the interplay between these factors is central to maintaining viable and 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, Frantz *et al.* 2010) and man-made barriers such as roads and developed areas (Epps *et al.* 2005, Riley *et al.* 2006, Frantz *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, Frantz *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.* 2009b, 2009c). As the human population in Ireland has hit its highest levels since the Great Famine (1845-1852) such studies are warranted to investigate 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

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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 escapees 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 vagile, Irish mammalian species of conservation value or concern both within KNP and across the island of Ireland as a whole.

Methods

Study Area

Killarney National Park (V972897) 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 KNP 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 KNP. Numbers of red deer within KNP, 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 KNP gave some protection to the red deer herd dwelling on these lands although these were subjected to hunting (and poaching) on these estates (Ryan 2001). Whitehead (1964) reported between 200-500 red deer in Co. Kerry in the early 1950s. Accurate counts of red deer in KNP began in 1970; estimates at this time approximated 100 individuals. In 1994, some 640 red deer were counted in KNP (Ryan 1998).

Sampling

Tissue samples from a total of 51 red deer were collected in KNP during 2007-2009 following legal culling exercises. Of these, 39 (10 males, 18 females and 11 of unknown sex) with associated culling site location information were 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 KNP, the ongoing conservation efforts with regards to woodland habitats within KNP and the occurrence of much smaller densities of red deer (>60) 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).

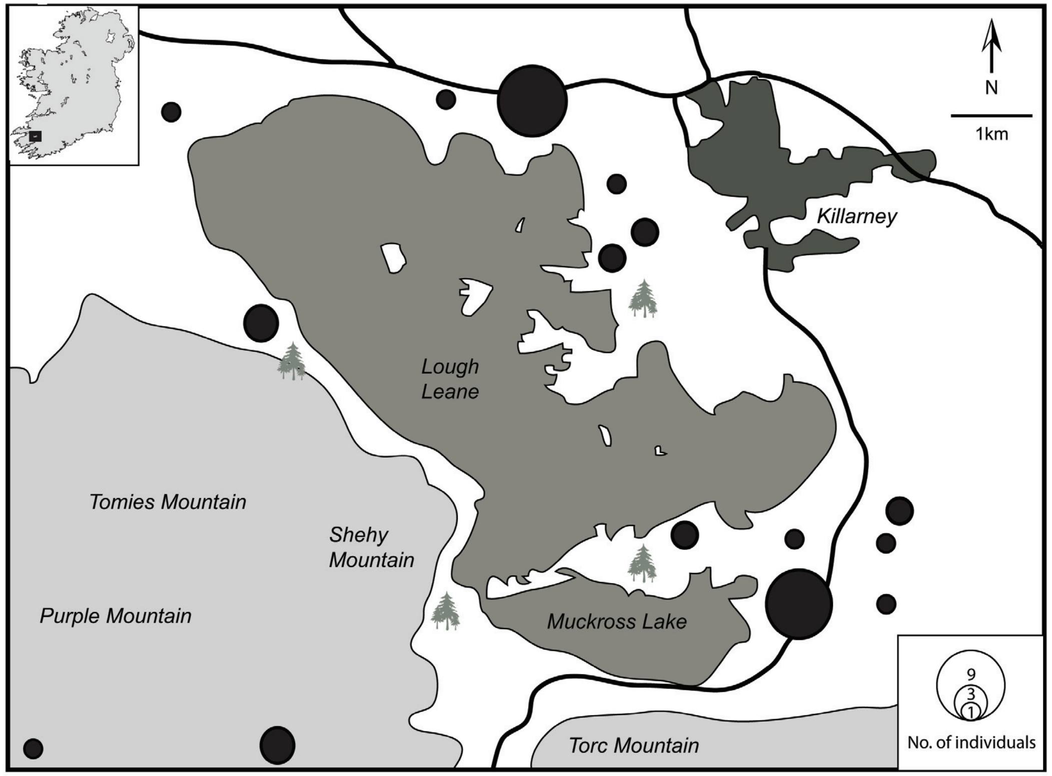
Genetic analysis

Nine polymorphic microsatellite loci, RT1, RT7, RT13 (Wilson *et al.* 1997), BL42, BM203, BM757, BM4513 (Bishop *et al.* 1994), IDVGA55 (Mezzalani *et al.* 1995) and OarFCB193 (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 KNP 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 point 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 period of 100 000 iterations.

We tested for isolation by distance (IBD) patterns by conducting spatial autocorrelation analysis (the analysis of genetic relatedness between pairs of individuals as a function of geographical distance) implemented in SPAGeDI v. 1.2 (Hardy and Vekemans 2002). As suggested by Vekemans and Hardy (2004), the kinship coefficient (F_{ij}; Loiselle *et al.* 1995) was chosen as a pairwise estimator of genetic relatedness as it is a relatively unbiased estimator with low sampling and has been used previously on a study of fine scale genetic structure in red deer (Frantz *et al.* 2008).

Figure 1. Study area within Killarney National Park. Killarney town is represented by dark grey shading, loughs/lakes are represented by intermediate grey shading (relative to the town and mountains) and mountainous regions are represented by light grey shading. Bold lines represent roads (major roads only), tree symbols represent forested areas and sampling locations (culling sites) are represented by black circles, each one proportional to the number of individuals sampled at each location.



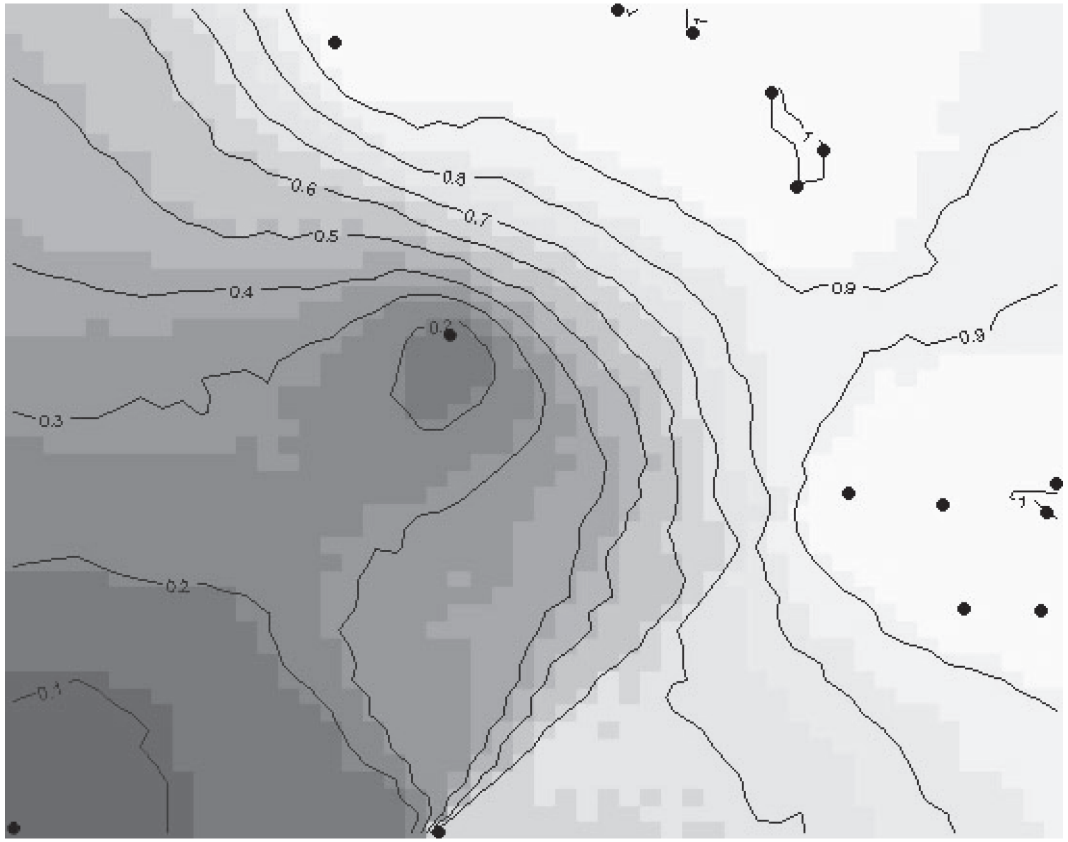
Conventional FST analysis (Weir and Cockerham 1984) was performed on clusters identified by GENELAND using the software FSTAT v. 2.9.3 (Goudet 1995) to test if identified clusters were significantly differentiated. Allelic richness (AR) and expected heterozygosity (HE) were also calculated for each cluster. 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.

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 KNP (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

Figure 2. Graphical output from GENELAND showing presence of two genetic clusters. Sampling locations are represented by black circles. Shading represents clines of probability of belonging to a particular cluster. In this case, lighter shading (white) represents cluster 2 east of Lough Leane with all individuals strongly assigned (probability >0.9). Cluster 1 is represented by darker shading (dark grey). See Figure 1 for corresponding sampling locations and associated landscape features.



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 KNP1 and KNP2 (McDevitt *et al.* 2009b). Haplotype KNP2 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; $P = 0.309$).

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 five 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 (Coulon *et al.* 2006, Pérez-Espona *et al.* 2008). Coulon *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-Espona *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 (10-50 km; Clutton-Brock *et al.* 1982; Catt and Staines 1987). Human development and infrastructure does not appear to impede gene flow on the eastern side of the Lough (Figs 1 and 2). In North America, *C. elaphus* are known to use areas of human activity as a means of refuge from wolves (Muhly *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-Espona *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 the way 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 bottleneck event 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 (Tomies, 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 (Petit *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 deducing strong 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 and management. 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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