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Genetic variation among endangered Irish red grouse (*Lagopus lagopus hibernicus*) populations: implications for conservation and management

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Abstract

Extant populations of Irish red grouse (*Lagopus lagopus hibernicus*) are both small and fragmented, and as such may have an increased risk of extinction through the effects of inbreeding depression and compromised adaptive potential. Here we used 19 microsatellite markers to assay genetic diversity across 89 georeferenced samples from putatively semi-isolated habitat fragments throughout the Republic of Ireland. In addition, we genotyped 28 red grouse from Scotland using the same markers. The genetic variation within Ireland was low in comparison to previously published data from Britain and the sample of Scottish red grouse, and comparable to threatened European grouse populations of related species. Irish and Scottish grouse were significantly genetically differentiated ($F_{ST}=0.07$, 95% CI=0.04–0.10). There was evidence of a weak population structure within Ireland with indications of four distinct genetic clusters. These correspond approximately to grouse populations inhabiting suitable habitat patches in the Northwest, Wicklow Mountains, Munster and Cork, respectively, although some admixture was detected. Pair-wise F_{ST} -values among these populations ranged between 0.019 and 0.040 and the overall mean allelic richness was 5.5. Effective population size in the Munster area was estimated to be 62 individuals 95% CI=33.6–248.8). Wicklow was the most variable population with an AR-value of 5.4 alleles / locus. Local (Munster) neighbourhood size was estimated to 31 individuals corresponding to a dispersal distance of 31km. In order to manage and preserve Irish grouse we recommend that further fragmentation and destruction of habitats need to be prevented in conjunction with population management to maximise population size.

Key words: red grouse; Ireland; fragmented; genetic diversity, differentiation

Introduction

Both genetic and demographic processes are important in determining extinction risk in small populations. Small populations may be at risk because of reduced fitness due to inbreeding and lost genetic variation. (Frankham et al. 2007). It has been argued that demographic or environmental stochasticity may entail a more immediate risk for small and isolated populations (e.g. Caughley 1994). Even if this would be the case, genetic data has important implications for conservation because if it can be shown that effective population size is small and gene flow among subpopulations is reduced, it is very likely that demographic and environmental stochasticity may become important.

Maintaining genetic diversity is also important for the long term survival of small and fragmented populations. Small population size may lead to lower average fitness of individuals through the effects of inbreeding depression (Keller and Waller 2002). Moreover, reduced genetic diversity can compromise the ability of species to adapt and evolve to changing environments (Frankham et al. 2007, Höglund 2009). Inbreeding is inevitable in small natural populations in species where suitable habitats have become fragmented, and especially where habitat destruction and exploitation are ongoing (Hartl and Clark 1997). As inbreeding (and genetic drift) increases, the level of genetic variability decreases. This, in turn, affects the viability of new individuals, which might give rise to even more inbred offspring which have a higher mortality and lower reproduction success. Escaping such an extinction vortex (Gilpin and Soulé 1986) is a primary focus of conservation genetics. Pragmatically, maximizing natural rates of gene flow, or moving individuals among populations, is the only strategy available to positively affect levels of genetic diversity in natural populations.

The Irish red grouse (*Lagopus lagopus hibernicus*) is an example of a species where small population size may have a negative effect on genetic diversity and population viability. It is one of four bird species that is considered to be endemic to Ireland and is the only grouse species that occurs (Dempsey and O'Clery 2002). With an estimated dwindling number of 4,200 birds scattered around the Irish bogs and moors, it is a red listed species (Lynas et al. 2008). As a game bird, and one of Ireland's native species, it has long held the status of iconic symbol of national biodiversity, and as such there is considerable emphasis placed on long term conservation. Although research has been carried out on the Irish red grouse since the early 1900's (Allen et al. 2004), focus has increasingly shifted towards ascertaining the genetic status of extant populations.

Irish grouse are morphologically and ecologically similar to red grouse in Britain (*L. l. scoticus*), both of which are divergent from the willow grouse (*L. lagopus spp.*) which occurs in mainland Europe, Asia and North America (Quintela et al. 2010). The red grouse on Ireland and Britain do not moult into a white winter plumage as with the Continental Willow grouse. It might be hypothesized that the Irish grouse have evolved differences relative to conspecific populations in Britain as an ancestral population may have survived the last glacial maximum in a refuge at or near Ireland (Rowe et al. 2006; Martinkova et al. 2007). A disputed, subtle difference between the British and the Irish red grouse is their possible association with different habitats. Red grouse habitats in Britain are often found in peatlands and upland moors dominated by *Calluna vulgaris*, while the Irish grouse occur in peatland areas with more grass (Hutchinson 1989). The darker colour of the British and lighter colour of Irish red grouse is thus thought to reflect adaptations to the background habitat in each of the island. This possible subtle difference in plumage colour is one reason to regard the Irish red grouse as a subspecies separate from the British (Potapov 1985). However, Freeland and

co-workers (2007) found no clear genetic differentiation between red grouse from Ireland and Britain and willow grouse from mainland Europe. However, their analyses were based on a relatively short region of mitochondrial DNA. Thus this may not present an accurate description of the overall genetic relationship between the two forms. Due to the fact that peatlands and upland moors in Britain often are managed in order to maximize the production of red grouse (for commercial hunting) (Tharme *et al.* 2001) while Irish peatlands are usually managed for forestry and livestock (which results in habitat destruction), there are huge differences in the densities of birds in the two islands. Where Ireland, according to a survey made during 2006 – 2008 (Cummins *et al.* 2010) has 1.1 birds km⁻², Scotland will have close to 60 birds km⁻² or more in some areas (Thirgood *et al.* 2002).

Here we characterise levels of microsatellite DNA diversity among grouse within Ireland to:

1) determine levels of genetic divergence among putative populations; 2) estimate the effective population size and neighbourhood size of Irish grouse; 3) compare levels of genetic diversity in Ireland to other populations of grouse in Britain and Continental Europe; 4) assess the extent of genetic differentiation between Scottish and Irish populations.

Materials and Methods

Sampling and Study Areas

DNA was extracted from 89 samples collected across Ireland between 2006 and 2009 (Fig. 1). An additional 28 samples from Scotland were obtained from birds collected in Aberdeenshire (Piertney *et al.* 1998). All samples had an associated sampling reference that varied in accuracy from known grid reference sampling location, to the scale of the nearest town (<10

kms). Sample sizes are roughly corresponding to the relative abundance of reed grouse in the different areas of Ireland, the Wicklow population being the largest and most abundant.

DNA extraction

DNA was extracted according to Freeland et al. (2007). In brief, a 2-5mm piece of the feather end was taken using a sterile blade, divided into two and placed in a sterile 2 ml Eppendorf tube along with two sterile steel balls. Samples were then frozen in liquid nitrogen and shaken for one minute at 2500rpm. Afterwards 180µl of QIAGEN DNeasy ATL buffer was added together with 20µl of proteinase K and 20µl 1M DTT. Extraction then followed the manufacturer's recommendations with final elution in 100 µl of buffer AE.

PCR and genotyping

In total 19 microsatellite loci were selected based on previous studies on grouse (e.g. Piertney and Dallas 1997, Sahlsten et al. 2008) and were amplified using PCR (Appendix). The markers were labelled with fluorescent dye (HEX, FAM and NED). Using QIAGEN Multiplex mix, PCR were performed on all samples with the following master mix: 5µl of multiplex mix; 2µl of ddH₂O; 1µl of Q-solution; 1µl of primer mix and 1µl of DNA. The primer mix consisted of 10µl of each 20µM primer stock (forward and reverse) with ddH₂O to a total volume of 100µl. PCR conditions for each multiplex were: denaturation at 95°C for 15 min; 40 cycles of; 94°C for 30s: annealing for 90s and 72°C for 60s followed by a final extension for 30 minutes at 60°C. Annealing temperatures for each primer pair are found in appendix 1. All samples were genotyped at least twice and most often three times to ensure the reliability of the genotypes. To rule out contamination of samples with exogenous DNA or

PCR products, tubes with water instead of sample/template were included in the DNA extraction and PCR amplification procedure as negative control.

PCR products were analyzed on a MegaBACE 1000. Each sample was prepared for genotyping by mixing 2µl of diluted (10 times) PCR product, 7.8µl ddH₂O and 0.2µl size standard. The output was analyzed with the software Fragment Profiler (Fragment Profiler 1.2, Amersham Biosciences, 2003). The scoring of microsatellite alleles was initially done automatically using a peak filter but each score was also checked manually. The majority of samples came from known individuals (i.e. shot birds) and therefore the identity of feather sample was known and extractions from multiple feathers could be pooled. In ambiguous cases (i.e. pick-up feathers), the multi-locus genotypes for each feather was checked with the genotype matching function in Microsatellite Toolkit plug-in for Excel (Park 2001) to search for feathers with the same genotype. Individuals were only included once.

Data analysis

Population genetics

To check for null alleles, stuttering and large allelic dropout, the dataset was run through MicroChecker 2.2.3 (Van Oosterhout et al. 2004). The only locus that consistently was found to contain null alleles was TUT4 (* in appendix 1) and this was therefore removed from all further analyses (yielding a total of 18 loci).

The calculation of Weir and Cockerham's (1984) pair-wise F_{ST} was done by considering all possible pairs of populations and assuming that they constitute the entire population in each calculation (Höglund 2009) and was performed using GENETIX 4.05.2 (Belkir et al. 2000). Significance was determined by bootstrapping the data 1000 among locations using the same

software. Allelic richness (AR), the rarefied number of alleles in a population (El Mousadik and Petit 1996) normalized to the smallest complete sample number (here 12 for comparisons within Ireland and 22 for comparing Ireland and Scotland), across loci was obtained using FSTAT 2.9.3.2 (Goudet 2001). Expected and observed frequencies of heterozygotes (H_e and H_o , respectively), for all loci, were obtained using GENETIX. Expected heterozygosity was calculated as according to Nei (1978). GENETIX was also used to create factorial correspondence analysis plots (AFC) to illustrate the multidimensional relationships between each individual genotype in a two dimensional plot. Effective population size (N_e) was calculated with the software LDNe (Waples 2006). This program uses a method to infer N_e , or rather the effective numbers of breeders N_b since we are dealing with a species with overlapping generations, from the observed levels of linkage disequilibrium.

Spatial distribution

We used the model based approach in the software STRUCTURE to assign individuals to genetic clusters (Pritchard et al. 2000). We used the admixture model to introduce population structure (clusters, K) to find clusters in Hardy-Weinberg equilibria (HWE) and linkage disequilibrium (LD) (Pritchard et al. 2000). We followed the approach suggested by Evanno et al. (2005) to infer the most likely number of K adjusting for an increase in variance as K increases and looking at the modal value of ΔK with the aid of the software STRUCTURE HARVESTER (http://taylor0.biology.ucla.edu/struct_harvest/).

We used runs both with and without a location prior each for 50 replicates at $K = 2 - 5$ with a burn-in of 50 000 and 100 000 iterations. To account for “label switching” and to take an average over all runs (50), the output files were aligned in CLUMPP (Jakobsson and Rosenberg 2007). The averaged Structure outputs were then visualized using the software DISTRUCT (Rosenberg 2004).

To describe the relationship between individual relatedness and distance, the software SPAGeDi 1.3a (Hardy and Vekemans 2002) was used. We used ten classes which were defined in such a way that there were approximately the same numbers of pair-wise comparisons within each class (Hardy and Vekemans 2002). The relatedness coefficient (r) (Queller and Goodnight 1989) was used to indicate relationships within each distance class.

Neighbourhood size, NS , can be estimated via the slope (b) and intercept of a regression line made over all distance classes with log-transformed geographic distance (Hardy and Vekemans 2002). We obtained b and intercept from the slope of the regression of r on \ln -transformed distance within each cluster determined by STRUCTURE (see above). Following Sahlsten et al. (2008) we calculated effective population density ($D = N_e / \text{Area}$). The mean axial dispersal distance (σ) was inferred through the relationship of NS and D . The area of suitable habitat for each region where the sampling took place was estimated from digitised maps (Urban Institute of Ireland, University College Dublin, Ireland) using ARCMAP 9.2 by calculating the area of a polygon covering mountainous land (excluding farmland and populated areas).

Results

Variation within Ireland

Structure runs and ΔK analyses for $K=1 - 5$ gave the highest support for four genetic clusters within Ireland. Individuals from Cork (C) were mostly assigned to the red cluster (11 + 3 admixed individuals of a total of 18), Munster (M) were assigned to the blue (14 of 19), individuals from the Northwest (NW) and West Ireland (WI) tended to belong to the green cluster (9 of 15) and individuals from Wicklow (W) belonged to the yellow cluster (17 of 37).

These numbers are based on the assumption that an admixture proportion (Q) higher than 0.70 indicates assignment to a specific cluster. However, Wicklow birds showed considerable evidence of admixture (Figure 2). The geographic structure and separation among the geographic areas can also be seen in the AFC-plot (Fig. 2) which explained 7.07% of the variation. Pair-wise F_{ST} (Weir and Cockerham 1984) among the four putative populations within Ireland are shown in Table 1. The global F_{ST} -value for Ireland was 0.028 (95% CI = 0.016 – 0.039).

Allelic richness within each putative population ranged from 1 – 10.5 alleles per locus, with mean values ranging from 4.8 to 5.4 alleles per locus (Table 2). The overall AR within Ireland was 5.5 alleles per locus. Unbiased H_e ranged from 0.63 – 0.67 with the Wicklow cluster as the most diverse. Wicklow also had the highest value of H_o (0.68) which ranged from 0.64 – 0.68. We were able to calculate two reliable values for N_e , for Munster and Wicklow, which gave the estimates 62 and 151, respectively. The calculations regarding Cork provided a negative value and Northwest-West displayed an upper 95% CI of infinity. An overall estimate of N_e was calculated by taking the harmonic mean of the four region estimates and multiplying it with four, giving 456 (95% CI = 47 – ∞) (Table 2).

Relatedness decreased with distance (Fig. 4). Local (Munster) neighbourhood size was estimated to 32 individuals (using the values: $F = 0.198$ and $b_{log} = -0.025$ intercept and slope, respectively) from a regression analysis made on relatedness data from Munster. Dispersal distance within the Munster sampling region was thus estimated to 5.6 km using these values: $NS = 32$, $N_e = 62$ and $Area = 756 \text{ km}^2$.

Differentiation between Ireland and Scotland

In the factorial component analysis ordination plot Irish and Scottish multilocus genotypes did not overlap (Fig. 5). The two major factorial components explained 7.22% of the variation observed in this dataset. The Irish samples were more tightly clustered than the Scottish samples which indicate a higher variability in the Scottish red grouse. Irish grouse thus appeared less genetically variable as compared to the limited sample of Scottish red grouse genotyped in this study and also less variable than published data on larger and more extensively sampled populations of red grouse in Scotland (Table 3). The level of heterozygosity in Irish red grouse was comparable to published records on threatened and isolated populations of other grouse species (Table 3). The pair-wise F_{ST} -value between Ireland and Scotland was 0.068 (95% CI = 0.043 – 0.098). Allelic richness among the Scottish samples was 6.86 ± 3.06 (1 SD) and 6.66 ± 2.47 in Ireland.

Discussion

The results of this study show that the Irish red grouse, as predicted from low population size and fragmented habitat structure, have indeed a low genetic variability. A recent survey (Cummins et al. 2010) estimated the red grouse population on Ireland to be in the range of 4,200 birds (95% CI = 3,800 – 4,700). The same study also estimated that the population on Ireland has experienced a 50% decrease in numbers over the last 40 years. Values of allelic richness, H_o and H_e estimated for threatened populations of black grouse and capercaillie in Europe and Irish grouse display similar levels of genetic variation comparable with other isolated and threatened grouse populations in Europe that have been assayed using microsatellites.

The Wicklow grouse does not appear a homogenous group. Several of the individuals show genotypes perhaps better fitted in either Munster or in the Northwest groups. Wicklow furthermore have lower pair-wise F_{ST} values than any population combinations excluding Wicklow. This suggests that red grouse from Wicklow are in general more diverse than birds from other areas of Ireland. The population in Wicklow is very likely the most viable population on Ireland, with the highest estimates of genetic variation of all putative Irish populations sampled. In view of this and regarding the historical distribution maps (Cummins et al. 2010) a possible conclusion is that the population in Wicklow may be used as a source population for a possible relocation programme within Ireland.

Even though microsatellites have important limitations for comparing populations that have been isolated for long periods due to chance of detecting low differentiation levels due to homoplasy (same alleles appearing in each population independently) we propose that this is unlikely to be a major problem in this study. The fragmentation red grouse habitats on Ireland is fairly recent (< a few hundred years) since loss of habitat is mainly determined by recent afforestation, peat mining and change in agriculture (Cummins et al. 2010). Furthermore, the Structure-based analyses and AFC-plots also suggest population structure. Our results suggest that Wicklow, Cork, and Munster are different groups while the additional grouping may occur among Northwest and West Ireland groups. For these analyses we used the option without using prior information on the location of sampled individuals. Adding this information may improve analyses of geographic structuring (Hubisz et al. 2009). However, when we included this information, the outcome was not altered. To more clearly identify all possible management units (MU) among the red grouse on Ireland it would be useful to include more samples especially from small populations, mainly in the west and northwest areas of Ireland.

There are some limitations in our estimates of the mean axial dispersal distance (σ), mainly the size of suitable grouse habitat which here was estimated from rough estimates of mountain region. Mountainous areas were identified and size estimated in ARCMAP 9.2 using a simple polygon area calculation. A wrongly estimated area would thus give a wrong estimate of mean dispersal distance and since the area can be many times higher than any other entry, this error can be large. It is also important to bear in mind that since there is structure among the sampled individuals, a separate N_e has to be obtained for each putative population. N_e is one of the most important values in conservation genetics and a low N_e -value is associated with accelerated depletion of genetic variability (Pertoldi et al. 2007). In two putative populations (Cork and Northwest) estimates of N_e provided infinity as the upper 95% CI limit, which is indicative of a too small sample size. N_e/N -ratio in wild populations is approximately estimated to be in the range of 0.10 (Frankham 1995). This estimate would for Irish red grouse around Munster give a population size of 620 individuals which is not an unrealistic number given that there is an estimated total population size of 4200 birds in all of Ireland. In the Wicklow area, the same estimations would give a total population size of around 1500 birds. The overall estimate of N_e times 10 will give a value of 4560 birds which falls within the 95% CI of the 2006 – 2008 survey (Cummins et al. 2010). Although the effective population size for a meta-population (harmonic mean of the sub-populations) can be calculated (see eg. Vucetich et al. 1997) it is not clear what this means as it is highly dependent on the size and direction of gene flow and if the sub-populations are of the same size (Wang and Caballero 1999).

In the areas where we obtained estimates of N_e (Munster and Wicklow) regions, only Munster provided an estimate of neighbourhood size with reasonable confidence limits. Hence we only

estimated dispersal distance for birds from Munster. Grouse species are typically not long distance dispersers. The mean estimated dispersal distance for Swedish hazel grouse (*Bonasa bonasia*) was 1514m (Sahlsten et al. 2008) and for English black grouse the estimated mean dispersal distance during early spring was 5.8 km and during fall 10.5km (Warren and Baines 2002). In comparison the estimated dispersal distance of the Irish red grouse of 5.6 km is not unreasonable and together with evidence of dispersal limitations (Piertney et al. 1998), an indication that natural dispersal between the putative remaining population fragments is unlikely.

There is much debate whether or not the Irish birds should constitute a separate subspecies of *L. lagopus* (Allen et al. 2004). When mtDNA was used to investigate the relationship between Irish and Scottish red grouse, no large differentiation in any subspecies of *Lagopus lagopus* studied was found (Freeland et al. 2007). However, this study was based on a short section (ca 300 bp) of the control region of maternally inherited, mtDNA. It is thus possible that more haplotypes would have been discovered if more sequence data would have been added and the study does not provide sufficient evidence to rule out the possibility that the Irish red grouse is a separate subspecies of *L. lagopus*. Although there has been many recorded and suspected introductions since the early 1900's of British red grouse into Ireland (Allen et al. 2004), our analyses do not show any overlap between the Irish and Scottish multi locus genotypes. However, our data do not provide a definitive solution to the "*L. lagopus hibernicus*-question", although they indicate a genetic differentiation with Scottish birds. This may suggest a reason for why birds transplanted from the UK to Ireland seem unsuccessful in surviving and reproducing within the Irish populations (Allen et al. 2004). The management unit of the Irish red grouse according to microsatellite differences appears to be the island of Ireland. A recent similar study of the Golden Eagle (*Aquila chrysaetos*) suggested the

management unit was to be Britain and Ireland for this species (Bourke et al. 2010). Given the differences in dispersal ability among the species, with grouse showing much more localized dispersal, these different conclusions and recommendations are not surprising.

The most common threats to grouse populations in Europe are habitat destruction and habitat fragmentation (Storch 2000). Many peatlands in Ireland use the heather moors for forestry and therefore destroy large amounts of suitable red grouse habitats. It is critical to understand that the habitat of the red grouse cannot be rapidly recreated as it may take thousands of years for peatlands to establish. Therefore the focus should be to protect the habitat that is present and enhance the Irish red grouse population where possible. Firstly, efforts should be taken to allow local population sizes should become larger. Secondly, facilitation of gene flow between the remaining population fragments should be facilitated. Thus small isolated populations could be augmented with transplanted birds from a larger population (e.g. Wicklow). However, as our data showed no overlap between Irish and Scottish samples, we do not recommend to introduce British red grouse to Ireland and the genetic integrity of the Irish population should be protected. The red grouse is a bird that depends on peatland covered by a fair amount of heather. Approximately 0.5% and 3% of the breeding birds observed on raised bogs and montane blanket bogs, respectively, are red grouse (Bracken et al. 2008). Red grouse is also closely associated to the presence and abundance of ling heather (Finnerty et al. 2007), *Calluna vulgaris*, for food and protection and it is the only bird species found in Ireland that is exclusively associated with peatlands (Feehan et al. 2008). Studies have shown that the diet can be made up of around 90% ling heather (Lance and Mahon 1974). Since Irish peatlands may be quite different from British moors and uplands it is thus possible that Irish birds are locally adapted to a different habitat compared to what is most common in Britain. With an estimated population size around 4000 birds and genetic

variation of the Irish red grouse is weakly comparable to some of the threatened grouse populations in Europe, it is vital that remaining populations and habitats are protected, managed and conserved in order to maintain viability of the species.

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Table 1. Pair-wise F_{ST} (Weir and Cockerham 1984) for the four putative populations discerned from Structure analyses. All values were significant at the Bonferroni corrected P-value ($\beta=0.05/6=0.0083$) after 1000 bootstrap replicates.

	Munster	Northwest	Wicklow
Cork	0.038	0.037	0.027
Munster		0.040	0.019
Northwest			0.022

Table 2. Summary of sample size n (the Northwest include 3 birds from the West see Fig. 1, all calculations are based on $n=12$ from the North West), unbiased H_e (Nei 1978), H_o and average values of allelic richness (AR) for the four putative populations and overall values. AR-values are rarefied to a sample size of $n=12$. N_e -values are presented with Jack-knifed 95% CI. The overall N_e -value is calculated as four times the harmonic mean over all areas.

Area	n	H_e	H_o	AR	N_e (95% CI)
Cork	18	0.63	0.64	4.8	-785 (93.6 – ∞)
Munster	19	0.65	0.64	4.8	62 (33.6 – 248.8)
Northwest	12+3	0.64	0.66	5.1	74 (32.1 – ∞)
Wicklow	37	0.67	0.68	5.4	151 (87 – 465.7)
Overall		0.67	0.62	5.5	456 (47 – ∞)

Table 3. Comparisons of F_{is} (Weir and Cockerham (1984)) and observed heterozygosity (H_o) found in threatened and non threatened European red grouse, black grouse (*Tetrao tetrix*) and capercaillie (*Tetrao urogallus*) populations. † denotes threatened populations.

Species	Location	F_{is}	H_o
Red grouse*	Ireland [†]	0.028	0.62
	Scotland	-0.019	0.81
	Scotland	0.119	0.78
Black grouse**	Netherlands [†]	0.025	0.53
	Norway	-0.023	0.72
	Austria	-0.056	0.74
	England [†]	0.108	0.52
Capercaillie***	Spain [†]	-0.022	0.36
	Pyrenees [†]	0.091	0.48
	Archangelsk (Russia)	-0.004	0.72
	Jaroslavl (Russia)	0.071	0.68

*For Ireland see this study, for Scotland see Piertney *et al.* 1997 and 1998

** Data from Larsson *et al.* 2008

*** Data from Rodríguez-Muñoz *et al.* (2007)

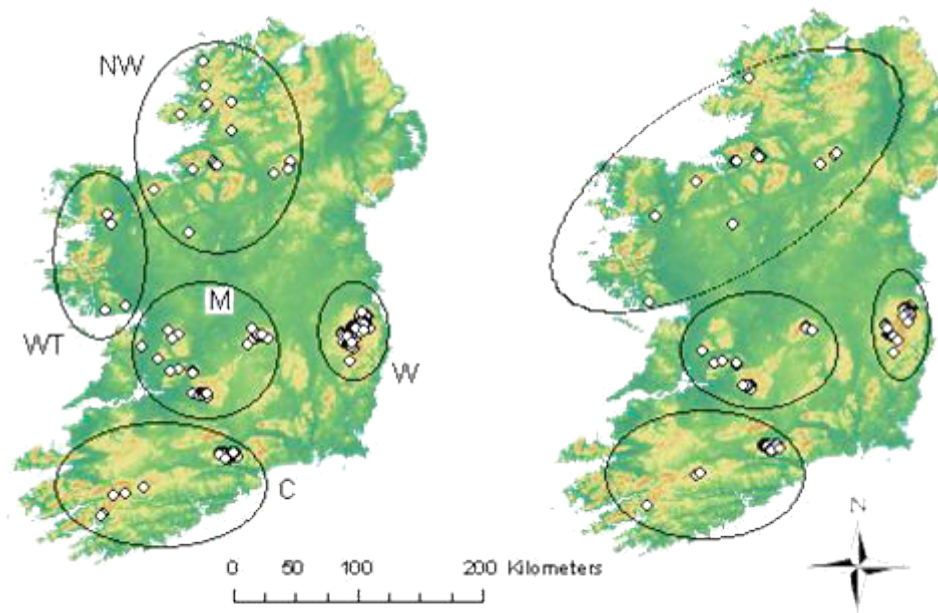


Fig. 1. Map of Ireland showing all the sampling locations of all feathers (left) and those successfully genotyped (right). In the left map, the five sampling areas are encircled. In the right map the approximate geographic location of the four clusters found by Structure are shown. From north to south in the left map is: Northwest (NW), West (WT), Wicklow (W), Munster (M) and Cork (C). From north to south in the right map is: Northwest, Wicklow, Munster and Cork. The map is used with permission from Urban Institute of Ireland, University College Dublin, Ireland.

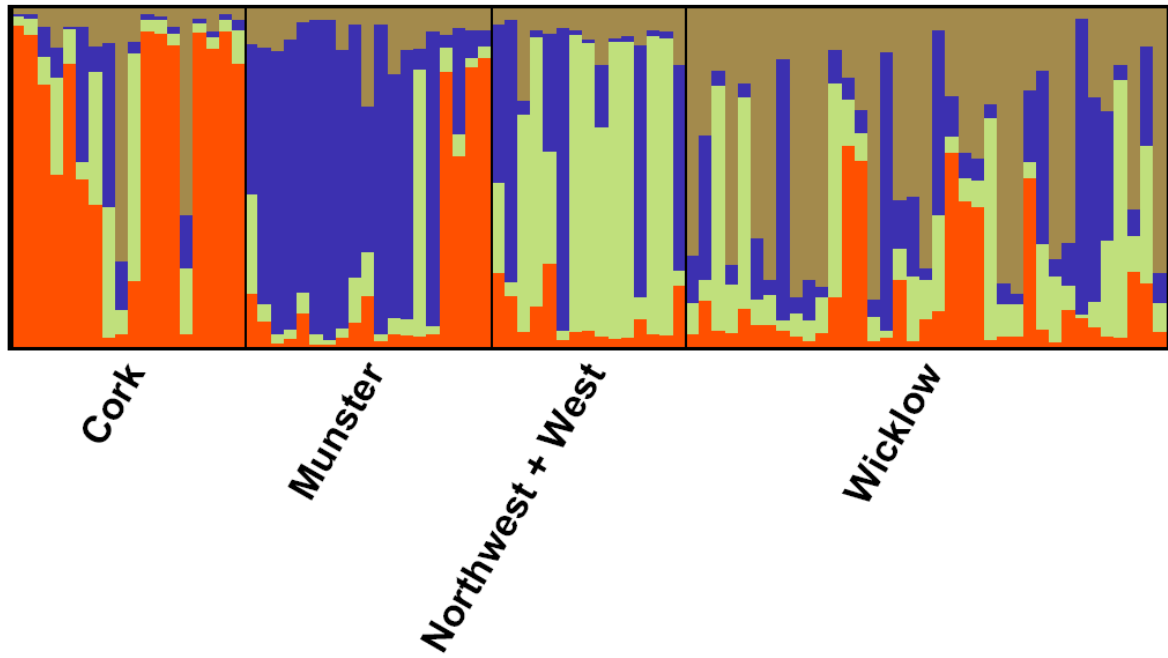


Fig. 2. The averaged output from 50 Structure runs using the admixture model without loc prior, $K=4$.

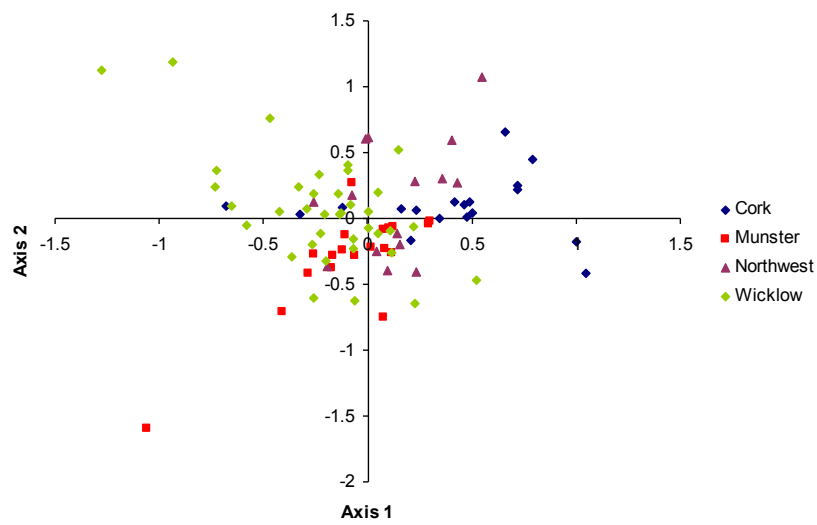


Fig. 3. AFC 2D plot showing the 89 Irish samples coloured according to their belonging to four putative populations. Axis 1 and 2 constitutes the two major components and explain 7.07% of the variation observed in the data set. Northwest and West Ireland are grouped under “Northwest”.

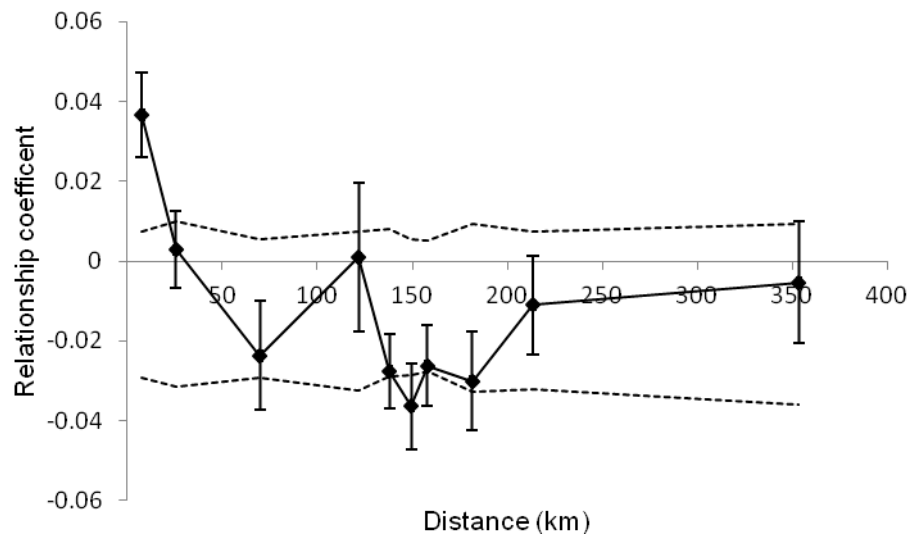


Fig. 4. Mean relatedness in distance classes of Irish grouse in relation to geographical distance 95% confidence limits are indicated.

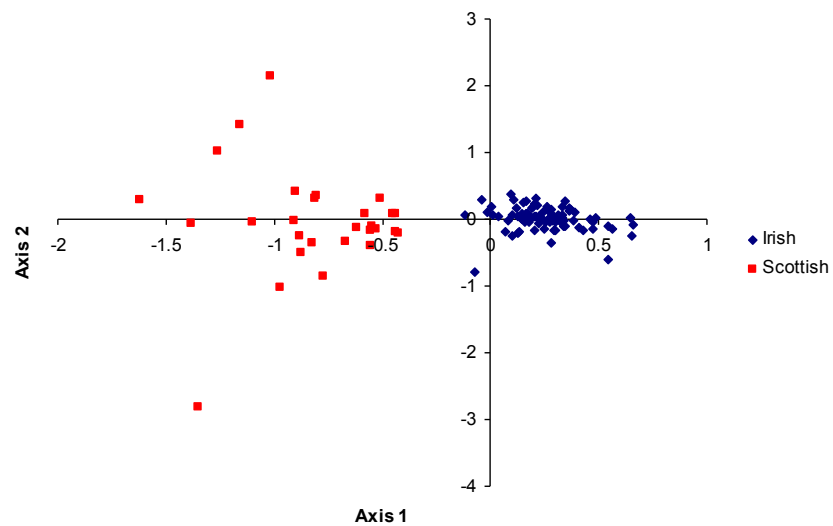


Fig. 5. AFC 2D plot of the 89 Irish (blue) and 27 Scottish (red) samples. Axis 1 and 2 constitutes the two major factorial components and they explain 7.22% of the variation in the data.

Appendix.

Compilation of the microsatellite markers used arranged in multiplexes. Size is given in base pairs (bp); spacing is the repeat number of the microsatellite locus, also in bp. Observed heterozygosity (Obs H) and allele numbers (NA) are given for the Wicklow population.

Marker	Dye	Size	Spacing	Annealing T(°C)	Obs H	NA
Multiplex 1						
ADL 230	FAM	90 - 115	2	48	0.49	4
ADL 142	HEX	207 - 255	2	48	0.72	10
Multiplex 2						
ADL 184	NED	110 - 160	2	54	0.51	7
BG 15	HEX	130 - 173	4	54	0.72	10
BG 16	NED	130 - 170	4	54	0.24	8
BG 18	FAM	110 - 180	4	54	0.68	11
Multiplex 3a						
LEI098	NED	135 - 165	2	60	0.51	5
TUT2	HEX	135 - 175	4	60	0.72	9
TUT4*	FAM	157 - 215	4	60	0.68	9
Multiplex 3b						
TUT1	NED	178 - 220	4	60	0.30	9
TUT3	FAM	150 - 180	4	60	0.62	8
Multiplex 4						
LLSD4	HEX	185 - 220	2	58	0.65	11
LLSD6	FAM	88 - 126	2	58	0.11	4
LLSD7	HEX	140 - 176	2	58	0.70	15
LLSD8	FAM	138 - 170	2	58	0.86	9
Multiplex 5						
LLST1	NED	120 - 170	3	54	0.72	8
LLSD2	HEX	100 - 117	2	54	0.57	3
LLSD3	HEX	123 - 145	2	54	0.46	5
LLSD5	FAM	120 - 140	2	54	0.59	5

The following loci showed signs of possible null alleles: BG16, BG18, TUT1, TUT3, LLSD4, LLSD6, LLSD10