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Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers

STUART B. PIERTNEY,* ANDREW D. C. MacCOLL,*† PHILIP J. BACON* and JOHN F. DALLAS*

**NERC Molecular Genetics in Ecology Initiative, Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK, †Institute of Terrestrial Ecology, Banchory Research Station, Banchory, Kincardineshire AB31 4BY, UK*

Abstract

Allelic variation at seven hypervariable tri- and tetranucleotide microsatellite loci was used to determine levels of population differentiation between 14 populations of red grouse (*Lagopus lagopus scoticus*) in northeast Scotland, UK. Despite the potential for long-distance dispersal in grouse, and a semicontinuous habitat, significant population divergence was observed (mean $R_{ST} = 0.153$; $P < 0.01$) and an isolation-by-distance effect detected (Mantel test: $P < 0.001$). Examination of the spatial trend in principal component scores derived from allele frequencies among populations highlighted a barrier to gene flow that was confounding a simple isolation-by-distance effect. This barrier corresponded to an area of unsuitable habitat for grouse associated with a river system that bisected the study area. Mean genetic relatedness was higher for males than for females in all but one of the study populations, suggesting that the territorial behaviour and natal philopatry displayed by cocks have a manifold effect in generating the observed spatial genetic structure. Lower female relatedness values suggest a higher level of female-mediated gene flow, which is sufficient to prevent the loss of genetic variation from within populations and the onset of inbreeding effects. The potential consequences of local subdivision for red grouse populations are discussed.

Keywords: gene flow, geostatistics, grouse, *Lagopus*, microsatellite, population structure

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Introduction

Inconsistencies in the availability or suitability of habitat tend to result in a heterogeneous distribution of individuals and hence spatially segregated demes. The dispersal capacity of a species is therefore of primary importance in determining whether gene flow can prevent population differentiation between demes by the combined effect of stochastic and deterministic microevolutionary forces. It is often assumed that a species with a high potential for dispersal should remain close to panmixia despite the fragmentation of populations. Avian species have long been considered a paradigm for such arguments (Barrowclough 1983). Through flight, and a propensity for migration, birds are expected to have considerable levels of dispersal, and concomitantly high gene flow. The broad geographical ranges of some bird species, plus small genetic distance between conspecific populations and congeners, relative to nonavian vertebrates (see Avise & Aquadro 1982; Barrowclough 1983; and Ward *et al*. 1992 for reviews), would tend to confirm this assumption. However, it is becoming increasingly apparent that avian species display a wide variety of population genetic structures (Avise & Ball 1991; Avise 1996), partly because the maximum potential for dispersal in a species is not often realised, and also because social structure and individual behaviour tends to prevent the complete admixture of genes over areas (Sugg *et al*. 1996; Dobson *et al*. 1997). Ultimately, these factors lead to the formation of isolated, finite-sized populations that are prone to the effects of genetic drift and disruptive selection.

This scenario is well illustrated in the red grouse (*Lagopus lagopus scoticus*). Although distributed throughout northern and western Britain, red grouse populations are limited to moorland areas dominated by heather (*Calluna vulgaris*), which provides its primary food source

Correspondence: S. B. Piertney. Fax: +44 (0)1224 272396; E-mail: s.piertney@abdn.ac.uk

and cover (Hudson 1986). Despite the potential for long-distance flight in red grouse, only very localized dispersal has been documented, and this data is geographically somewhat patchy. At Kerloch moor in northeast Scotland, UK 94–98% (n = 218) of cocks and 79–83% (n = 238) of hens moved distances of less than 1.5 km from where they were tagged (Jenkins *et al*. 1963). A second, more comprehensive survey recovered 84% of birds (n = 669) within 1.5 km and 94% within 5 km from the point at which they were marked (Jenkins *et al*. 1964). In general, female grouse moved greater distances than males. Similar levels of dispersal have also been recorded in the north of England, UK (Hudson 1986).

Such restricted vagility in cocks is brought about, at least in part, by a combination of extreme natal philopatry and territorial behaviour. Red grouse cocks obtain territories in autumn, remain on these over winter, and then father a clutch of chicks in the spring. Young males from these broods compete among themselves and with established cocks to obtain a territory in close proximity to their natal breeding site the following autumn. Such territory acquisition is essential for recruiting to the population and is the major factor controlling yearly changes in breeding density (Moss *et al*. 1996). The fate of those birds that do not obtain territories is uncertain. Some will die, whilst others become 'floaters' that inhabit marginal habitat on the periphery of territories, or move away from the natal area in an attempt to recruit elsewhere (Jenkins *et al*. 1963).

It is unknown to what extent male philopatry, female dispersal and the occasional movement of males unsuccessful in philopatric recruitment promotes population genetic structuring. Inference of gene flow by direct methods can be misleading because of an underestimation of rare and long-range dispersal events that can have a strong effect on homogenizing population structure. Three potential scenarios for gene exchange between local grouse populations can be envisaged: (i) all individuals can disperse at a local level resulting in overall panmixia through stepping-stone gene flow; (ii) natal philopatry precludes male-mediated gene flow, leading to the establishment of male lineages within populations, but female dispersal and occasional movement by floaters moderates the extent of overall structuring; and (iii) both male and female gene flow is limited, resulting in significant population differentiation.

In the present study we estimate the degree of genetic divergence between local populations of red grouse by examining the spatial distribution of microsatellite variation in 14 populations from NE Scotland. Within-population relatedness estimates are also determined to assess the relative contributions of male- and female-mediated gene flow.

Given limited individual dispersal, it is expected that gene flow would act predominantly in the local vicinity, leading to isolation by distance (Wright 1943, 1946; Kimura & Weiss 1964). However, this ignores the possible effects of habitat in moderating individual movement and thus affecting the distribution of allelic variation. With the high levels of variability that are resolvable at microsatellite loci and the detailed picture of population differentiation this affords, it is possible not only to detect localized structure, but also to map trends in spatial allele frequency data. Under isolation-by-distance models, one would expect the frequency of alleles to change gradually across two-dimensional space. Any areas where there are rapid changes in allele frequency can be viewed as barriers to effective dispersal. In recent years a panoply of statistical methods have been developed to detect such ecotones from maps of biological variables (e.g. Barbujani *et al*. 1989; Legrande & Fortin 1989, Fortin 1994; Fortin & Drapeau 1995). Here, we utilize one such approach to identify those areas that may be acting to prevent gene flow and confounding a simple isolation-by-distance effect, then attempt to determine the physical features responsible, by reference to geographical databases.

Materials and methods

Sampling of populations

Feather samples were taken from all the shot birds following grouse shoots at the locations shown in Fig. 1. Grid references and sample sizes are given in Table 1. The mean number of individuals per sample was 30. The maximum distance between populations was ≈ 52.5 km, the minimum distance < 1 km. Only adult individuals were genotyped to prevent the sampling of families that would potentially bias population allele frequencies by overrepresentation of individual lineages.

DNA extraction

DNA was extracted from the feather samples using an approach analogous to that of Ellegren (1991). Each feather tip was excised and incubated for 12–16 h at 55 °C in 200 μL of 10 mM Tris-HCl (pH 8.0) containing 5% Chelex (Bio-Rad Ltd) and 25 mg/mL proteinase K. The solution was then vortexed at high speed for 30 s, heated to 100 °C for 8 min and centrifuged for 2 min at 15 000 ***g***. A volume of 5 μL of the supernatant was used in subsequent PCR reactions.

Microsatellite development

Hypervariable tetranucleotide microsatellites were isolated using an enrichment protocol similar to that of Armour *et al*. (1994) and modified from Piertney *et al*. (1998). A total of 10 μg of grouse genomic DNA was

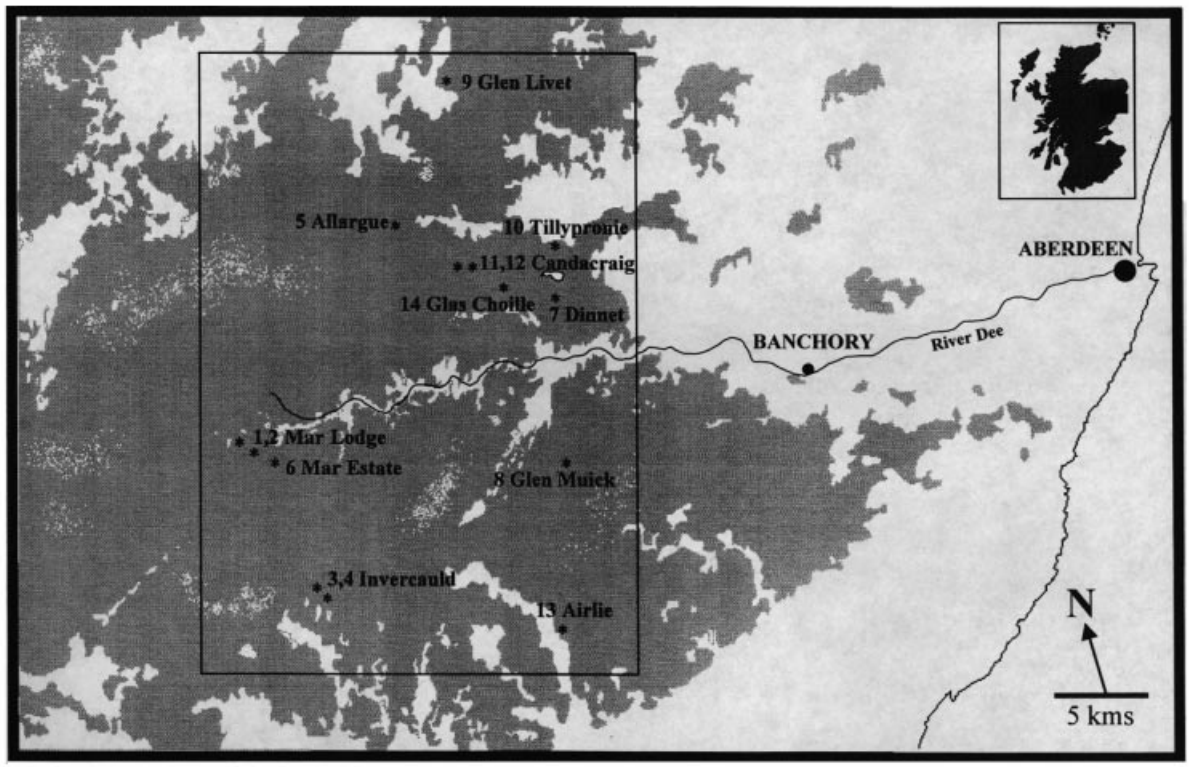

Fig. 1 Map of NE Scotland showing the locations of the 14 grouse populations sampled in the present study. The square delimiting the sampled populations represents the area over which kriging interpolation of first-axis principle component (PC1) scores was undertaken. The areas in grey represent suitable grouse habitat as defined by the DeeCAMP GIS database. White regions are deemed unsuitable grouse habitat.

digested to completion with *Sau*3AI restriction endonuclease, and a 300–800 bp fraction isolated. This DNA was ligated to a SAU linker molecule, which was formed by the ligation of equimolar amounts of the SAULA oligonucleotide (5'-GCGGTACCCGGGAAGCTTGG-3') and the phosphorylated SAULB oligonucleotide (5'-GATCCCA-AGCTTCCCGGGTACCGC-3'). Resultant fragments were denatured and hybridized to a 1-cm^2 piece of Hybond N^+ membrane (Amersham International Ltd) saturated with a synthetic microsatellite polymer $(CAAA)_n$ or $(GAAA)_n$. Hybridization took place at 55 °C for 12 h in 200 μL of 1× SSC, 0.1% SDS after which the membranes were washed at low stringency (three washes of 2× SSC, 0.1% SDS) to remove DNA devoid of microsatellite regions, then heated to 95 °C to remove the enriched microsatellite fraction. DNA was precipitated from the high-stringency wash using normal procedures, and complementary strands were reformed in a PCR reaction (20 cycles at 94° denaturation and 55 °C annealing) using the SAULA oligonucleotide as a primer. The SAU linker molecules were removed from the enriched genomic fraction by overnight digestion with *Sau*3AI endonuclease and the genomic DNA was ligated into a phosphorylated, *Bam*H1-cut pUC18 vector (Pharmacia Ltd). Vector molecules were transformed into highly competent INVαF' One Shot *Escherischia coli* (Invitrogen) which were then grown overnight on Luria–Bertani (LB) plus ampicillin medium. Direct lifts were made from the plates onto Hybond N membrane and screened, using standard techniques, for

Table 1 Location and sampling information for the grouse populations examined

	Estate	O/S Ref.	No.
1	Mar Lodge	NO030840–030860	31
2	Mar Lodge	NN980840–990840	36
3	Invercauld	NO143753–147774	24
4	Invercauld	NO147753–152772	29
5	Allargue	NJ190080	28
6	Mar Estate	NO070850–070840	42
7	Dinnet	NJ370030–380030	22
8	Glen Muick	NO360880	16
9	Glenlivet	NJ260220–260210	23
10	Tillypronie	NJ370050–360040	20
11	Candacraig	NJ270060	23
12	Candacraig	NJ280060	20
13	Airlie	NO380700	50
14	Glas Choille	NJ300030–310030	50

the presence of microsatellites with a random primed synthetic microsatellite array (Sambrook *et al*. 1989). Those clones containing arrays were sequenced using an ABI 377 automated sequencer (dideoxy end-labelling procedure according to the manufacturer's instructions), and PCR primers designed using the criteria described in Piertney & Dallas (1997).

PCR amplification

All individuals were genotyped at seven microsatellite loci. PCR amplifications were performed in a total volume of 10 μL using a MJ Research PTC-100 thermal cycler. Each reaction mix contained 5 μL of Chelex DNA extraction (equates to ≈ 10 ng of template DNA), 2.5 mM $MgCl_2$, 75 mM Tris-HCl (pH 9.0), 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween-20, 0.2 mM of each nucleotide, 5 pmoles of each primer (forward primer end-labelled with [γ^{32}P]-dATP) and 0.5 units of *Taq* polymerase. PCR profiles followed a 'touchdown' (Don *et al*. 1991) procedure, whereby after an initial denaturation step of 3 min at 90 °C, 20 cycles of PCR were performed, each cycle consisting of 30 s denaturation at 91 °C, and 30 s of annealing starting at 60 °C and dropping by 0.5 °C per cycle. A further 15 cycles were then performed with 30 s denaturation at 90 °C and 30 s annealing at 50 °C. No extension steps were included in the program, apart from a 2-min period at 72 °C following the final annealing step. PCR fragments were resolved by electrophoresis on 6% denaturing polyacrylamide gels (Sambrook *et al*. 1989), and allele sizes were determined by reference to a M13 mp8 DNA sequencing standard run simultaneously.

Statistical analysis

Allele frequencies, Nei's (1978) unbiased estimates for expected heterozygosity (H_E), observed heterozygosity (H_O), and mean number of alleles were calculated for each locus using BIOSYS-1 (Swofford & Selander 1981). Deviation from Hardy–Weinberg expectations for panmixia was tested using GENEPOP version 3 (Raymond & Rousset 1995), which employs the Markov chain method of Guo & Thompson (1992) at each combination of locus and population. A global probability value over all loci was obtained using Fisher's method of combining independent *P*-values (Sokal & Rohlf 1981). Multilocus F_{IS} was also calculated for each population, then tested by permutation, using Goudet's (1995) FSTAT version 1.2.

Tests for linkage disequilibrium between loci for each population were performed by generating exact probabilities of type-I error for the null hypothesis that a pair of loci are unlinked. The test was performed for all locus pairs within each population and over all populations using GENEPOP version 3 (Raymond & Rousset 1995).

Population divergence estimates were obtained from unbiased, multilocus values of Slatkin's (1995) R_{ST} using the R_{ST}CALC program of Goodman (1997). The significance of all values was ascertained by permutation. R_{ST} estimates were then used to test for isolation by distance using the Mantel procedure (Manly 1986), following the recommendations of Rousset (1997), whereby correlation is examined between $R_{ST}/(1 - R_{ST})$ and the logarithm of euclidean geographical distance between populations in kilometres. Relatedness estimates were calculated separately for males and females within each population using the program of Queller & Goodnight (1989).

Wherever significance testing occurred, the sequential Bonferroni technique was employed to eliminate the false assignment of significance by chance (Rice 1989).

Populations were spatially clustered according to genetic similarity using principle component (PC) analysis, which utilized the allele frequencies of populations at all loci as the variables. The first-axis principle component scores (PC1) were also used to delineate any barriers to gene flow among the study populations (following Dickson *et al*. 1990 and Brierley *et al*. 1995). The kriging interpolation procedure (Journel & Huijbregts 1978) was employed to interpolate hypothetical PC1 scores from the actual sampling points at the 'nodes' of a regular grid that encompasses the whole sampling area (this area is delimited as a solid box in Fig. 1). Kriging interpolates based on the spatial autocorrelation of the variable at sampled points, and in this case was performed using Surface III software (Kansas Geological Survey). Barriers were identified as the zones of maximum slope following the contours of the PC1 scores. The inferred boundary zones were overlaid onto a variety of land-use maps within the DeeCAMP Geographic Information System (GIS) database (Bacon & Mackay 1996; Webb & Bacon 1998) to highlight environmental features that might be responsible for the discontinuities. In particular, the 25 landcover types within the Institute of Terrestrial Ecology's Land Cover Map of Great Britain (LCMGB; Barr *et al*. 1993) were reclassified with respect to grouse habitat (see Fig. 1): various classes of heather moor (including small patches of upland grass) were reclassified as 'good' grouse habitat; all other habitats were classed as 'bad'.

Results

Microsatellite development

The enrichment technique described here generated ≈ 15 microsatellite-containing clones (1% of total recombinants). This represents a 15-fold increase in the number of tetranucleotide-containing clones obtained over the nonenrichment method described in Piertney & Dallas

(1997), that had been previously used to isolate equivalent tetranucleotide markers with minimal success. The number of positive clones detected could have been increased further by undertaking multiple rounds of enrichment, as has been described in other enrichment protocols (Waldbeiser 1995). Of the 15 clones obtained, 10 primer pairs were designed and these resolved seven loci. Primer sequences and array types are given in Table 2. All loci were shown to be highly variable with a mean observed heterozygosity of 0.81 and a mean number of alleles per locus of 18.86.

No evidence could be found for a high incidence of null alleles (Pemberton *et al*. 1995), nor genotypic linkage disequilibrium between loci within population samples. Of the 294 Fisher exact probability tests undertaken, only 14 were significant ($P < 0.05$; data not shown) after Bonferroni correction, and these were scattered randomly across locus pairs. As such it is valid to utilize the data generated using these markers to assess levels of genetic structure between grouse populations.

Population structure

Considerable levels of population differentiation were apparent. Multilocus, unbiased estimates of Slatkin's R_{ST} are given in Table 3. Values ranged from –0.045 to 0.641, with a large proportion of these values being deemed significant by permutation testing (52 of 91; 57%). The overall R_{ST} value among all populations was 0.157. The allelic size distribution was practically continuous, which would suggest that mutation is underpinned by a stepwise process. As such it is valid to use R_{ST} as a fixation metric.

No significant deviation from Hardy–Weinberg expectations was observed within populations. Both exact probability testing and permutation testing of F_{IS} values (Table 4) failed to detect a significant deviation from Hardy–Weinberg expectations in any population. The majority of populations showed a slight, nonsignificant, heterozygote excess, manifested as a small, negative F_{IS} value.

Male and female relatedness estimates are also given in Table 4. In all populations the average male relatedness was comparable to, or higher than (significantly so in 8/14 cases; $P < 0.05$), the average female relatedness.

When $R_{ST}/(1 - R_{ST})$ is plotted against the logarithm of euclidean geographical distance between populations in kilometres, a significant, positive correlation (Mantel test; $P < 0.001$) is observed. The regression line accounts for ≈ 10% of the variation observed. Regression analyses were also performed among the populations to the north and south of the River Dee, which bisects the study area. As highlighted by Slatkin (1993), any form of noncontinuous sampling can potentially generate spurious conclusions regarding the extent of isolation by distance. In this case, however, the isolation-by-distance effect was still apparent when the samples were split (Mantel test; $P < 0.05$ both north and south of the river), although the slope of the associated regression line was less (regression equations were $y = 0.0172\ln(x) + 0.0516$ and $y = 0.0172\ln(x) + 0.0654$ for those populations north and south of the river, respectively).

The populations are ordinated using principle component analysis in Fig. 2. Populations to the north and south of the river form two separate groups of points, with the populations to the north of the river having negative PC1

Table 2 Description of seven microsatellite loci for *Lagopus lagopus scoticus*. Primer sequences are given in 5′–3′ orientation together with array length/type, number of alleles resolved, observed heterozygosity (direct count) and expected heterozygosity. The clone sequences from which the primers were designed have EMBL Accession nos X99051, and Y16826 to Y16831

Name	Sequence (5'–3')	Repeat	Size	No. of alleles	H_O	H_E
LLST 1	AAATTCTTTTCTTGTTGATGAG TGAGGGTTATGACATTATTAGG	$(AAT)_{21}$		15	0.96	0.93
LLST 2	TCTTGCTAGAAACTCTTTATGC AGAACTTGTGTATTGTGTATAAGG	$(CAAA)_{20}$	210	21	0.82	0.95
LLST 3	CCAATTATTTTGTCTTTCATTTG CCAAGTTTCAAGTAACAGAAAAC	$(CAAA)_{18}$	260	15	0.84	0.93
LLST 4	CTATTCCTTCACGTATCCTGAC GTCATATTCAGGTCCTCATTTG	$(GAAA)_{44}$	339	25	0.9	0.96
LLST 5	AAGACAACAGATGGAGTGACAG GTTGTCCTTTGGTGAATGTATC	$(GAAA)_{47}$	355	23	0.74	0.96
LLST 6	GCTGCTGTTATTATGTGGTTAC ATCCTGGTCACTAGATAGAAGC	$(GAAA)_{25}$	286	19	0.64	0.95
LLST 7	TCAGTGAAAGAGTGGTGTGG GGGCATCCATAACTTTTCTG	$(GAAA)_{18}$	346	14	0.75	0.93
Mean				18.86	0.81	0.94

Table 3 Unbiased, multilocus estimates of Slatkin's (1995) R_{ST} between all population pairs. Significant values are underlined

	Mar Lodge 1	Mar Lodge 2	Invercauld 1	Invercauld 2	Allargue	Mar Estate	Dinnet	Glen Muick	Glen Livet	Tillypronie	Candacraig 1	Candacraig 2	Airlie
Mar Lodge 1													
Mar Lodge 2	0.039												
Invercauld 1	0.227	0.060											
Invercauld 2	−0.020	−0.011	0.117										
Allargue	0.048	0.174	0.380	0.075									
Mar Estate	0.152	0.285	0.477	0.177	0.009								
Dinnet	0.017	0.137	0.352	0.040	−0.016	0.049							
Glen Muick	−0.032	0.009	0.153	−0.033	0.024	0.110	−0.002						
Glen Livet	0.181	0.309	0.491	0.204	0.028	−0.012	0.074	0.137					
Tillypronie	0.328	0.448	0.619	0.334	0.117	0.026	0.195	0.252	−0.004				
Candacraig 1	0.332	0.458	0.641	0.333	0.100	0.009	0.185	0.243	−0.017	−0.039			
Candacraig 2	0.236	0.374	0.565	0.254	0.040	−0.019	0.103	0.173	−0.030	−0.025	−0.045		
Airlie	0.249	0.384	0.574	0.263	0.051	−0.008	0.166	0.182	−0.020	−0.012	−0.028	−0.039	
Glas Choille	−0.001	0.040	0.208	−0.013	0.044	0.136	0.017	−0.025	0.163	0.239	0.291	0.209	0.220

scores and the populations to the south of the river having positive PC1 scores. The populations to the north also form a more diffuse cluster of points. A contour plot of the first-axis principle component scores derived from the actual sampling points and interpolated over an area delimiting the sample sites (seen as the solid line in Fig. 1) is given in two dimensions in Fig. 3a and in three dimensions in Fig. 3b. The surface can be viewed as being on three levels. The area north of the river is on one level, and south of the river is split into two levels; one to the east encompassing the populations at Glen Muick (8) and Airlie (13), and one to the west encompassing the populations from Mar Lodge (1 and 2), Mar Estate (6) and Invercauld (3 and 4). The change in PC1 scores as one moves from one level to another is considerably greater than would be expected if the difference between populations was due to an isolation-by-distance effect alone, and suggests that these areas represent barriers to gene dispersal. These high-gradient regions show considerable correspondence with the major areas of poor-quality grouse habitat defined by the DeeCAMP GIS database (shown in Fig. 1 in white) that are associated with the low-level arable land adjacent to the River Dee.

Discussion

It is immediately apparent from an examination of the spatial distribution of allele frequency among the 14 grouse populations studied here that there are considerable levels of population structuring. A large proportion of between-population R_{ST} values were deemed significant, indicating that the populations examined do not form a single panmictic breeding unit. Such localized population subdivision is relatively uncommon among avian taxa, even among other related species with similar life histories (e.g. Gyllensten *et al*. 1985; Ellsworth *et al*. 1994; Schreiber *et al*. 1998).

Two factors appear to be acting to promote such localized divergence in red grouse. First, the significant Mantel correlation between genetic and geographical distance among populations, even in the two small-scale transects, indicates that a major factor generating population structuring is an isolation-by-distance effect. As such, movement of individuals is limited to the local vicinity. This is perhaps initially surprising given the potential for long-distance dispersal in red grouse, but has been suggested from previous direct estimates of dispersal capacity derived from mark and recapture (Jenkins *et al*. 1963, 1964) and radiotelemetry (MacColl & Elston, unpublished) based studies.

The behavioural factors that are preventing the complete admixture of individuals over large areas are territoriality and extreme natal philopatry. The precise effects of behavioural mechanisms such as philopatry

Table 4 The genetic relatedness and inbreeding coefficient (F_{IS}) for each of the populations. Relatedness estimates are provided for males and females separately, together with standard error. The exact test probability associated with the Hardy–Weinberg test are also provided. The populations with a significantly higher ($P < 0.05$) male relatedness than female relatedness are asterixed

	Male relatedness	Standard error	Female relatedness	Standard error	F_{IS}	HW Exact Probability
Mar Lodge 1*	0.064	0.03	0.004	0.02	−0.088	0.076
Mar Lodge 2*	0.058	0.03	0.011	0.02	−0.026	0.099
Invercauld 1*	0.114	0.04	0.085	0.01	−0.028	0.777
Invercauld 2*	0.230	0.04	0.009	0.03	−0.067	0.620
Allargue	0.008	0.07	−0.116	0.06	0.061	0.441
Mar Estate	0.003	0.06	−0.006	0.03	−0.122	0.056
Dinnet	0.089	0.03	0.065	0.02	0.033	0.398
Glen Muick*	0.001	0.03	−0.179	0.06	0.105	0.153
Glen Livet	0.045	0.05	0.070	0.03	0.068	0.053
Tillypronie*	0.022	0.04	0.001	0.02	−0.073	0.731
Candacraig 1*	0.207	0.04	0.105	0.03	−0.066	0.455
Candacraig 2	−0.001	0.02	−0.106	0.06	−0.039	0.841
Airlie*	0.208	0.06	−0.144	0.02	0.070	0.058
Glas Choille	0.023	0.06	0.013	0.02	−0.096	0.557

and assortative mating on population genetic structure have been the focus of debate for a considerable period of time. Theoretically, philopatry should promote genetic heterogeneity among populations by limiting gene flow, and also reducing genetic variation within populations by organizing individuals into breeding lineages (Sugg *et al*. 1996; Dobson *et al*. 1997) and therefore providing opportunities for inbreeding. This was originally seen as potentially adaptive, as it will generate complexes of homozygous alleles that are locally advantageous, improving the overall fitness of subsequent generations (e.g. Shields 1982). However, an increasing number of studies are documenting high heterozygosities within social lineages and describing behavioural processes that prevent breeding between closely related individuals (e.g. Greenwood 1980; Pusey 1987). Such behaviour has probably evolved as a mechanism to prevent the onset of deleterious inbreeding effects promoted by philopatry. In red grouse, the philopatric tendencies of males will tend to promote inbreeding effects unless the dispersal capacity of the females is sufficient to augment the allelic variation present in male lineages. This appears to be the case: the mean relatedness of females is comparable to or lower than that of males in every population, suggesting greater mixing of females between populations. Consequently, no loss in variation, which would be manifested as a significant deviation from Hardy–Weinberg expectations, is observed within populations. Indeed, the slight heterozygote excesses (i.e. negative F_{IS} values) observed in the majority of populations are indicative of a species with considerable sex-biased dispersal. Prout (1981) demonstrated that under sex-dependent migration, local differences in gene frequency between the two sexes could result, which upon random mating will generate heterozygote excess in a way analogous to the Wahlund (1928) effect.

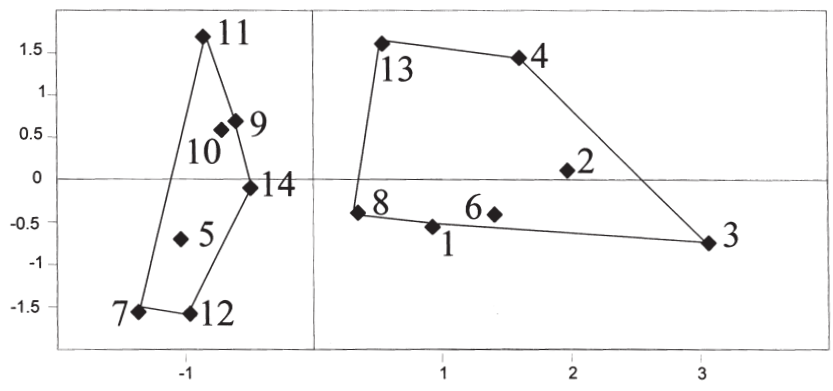

Fig. 2 Principal component analysis plot showing the similarity between the 14 populations of red grouse based on similarities in allele frequencies. The two principal components used in this plot account for 64% of the total variance. Populations have been clustered into two groups according to whether they are located north or south of the River Dee. Sample numbers are as given in Table 1.

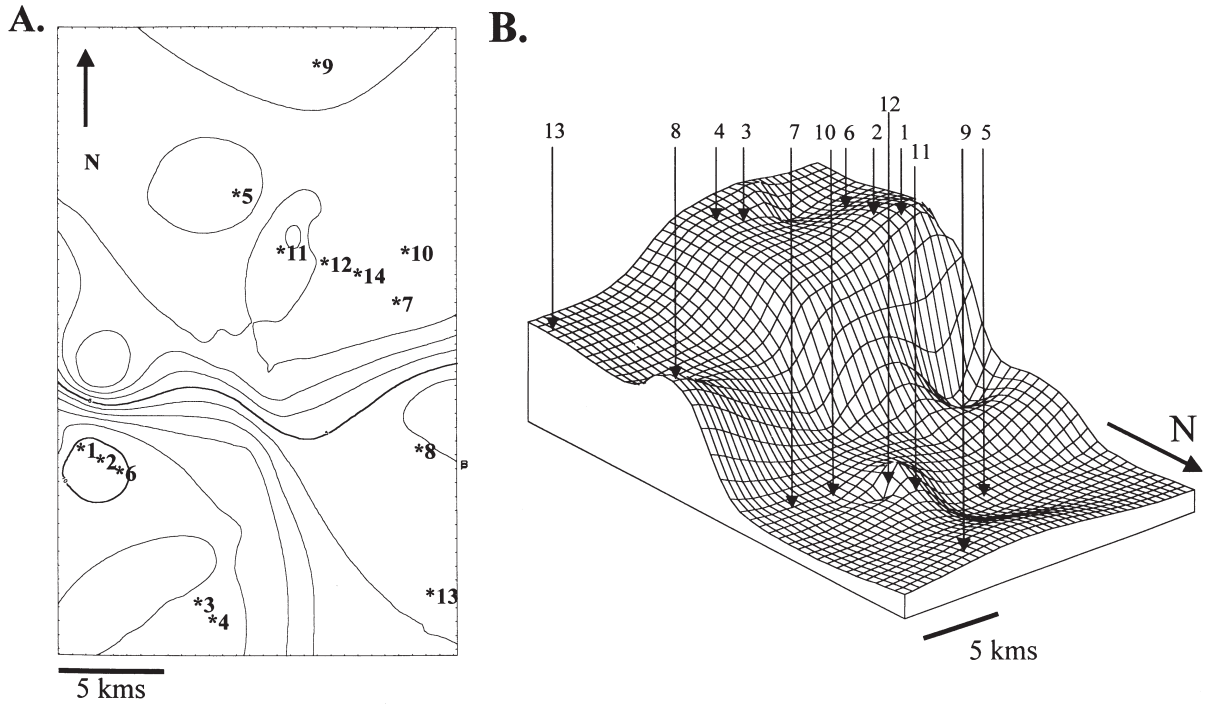

Fig. 3 Contour plot showing the hypothetical first-axis principal-component (PC1) scores derived from the kriging procedure in (A) two dimensions, and (B) three dimensions for the area delimiting the sampled populations given in Fig. 1. The lines connect points of equal PC1 score, and the actual sampling points are highlighted (sample numbers are as given in Table 1). To aid viewing, the closest point in the three-dimensional plot represents the NE corner of the box shown in Fig. 1.

The second factor probably promoting local population structure is the effect of physical barriers to gene flow. Despite the significant correlation of genetic and geographical distance, the regression coefficient associated with the correlation was relatively low, with the line accounting for only 10% of the variation observed in the points. Slatkin (1993) and Rousset (1997) have highlighted that the number of loci examined, the equilibrium/nonequilibrium status of the populations, the mutation rate of the markers and the distribution of pairwise distances between populations can all affect the patterns of isolation by distance. Here, we suspect that barriers to gene flow may also be confounding a simple isolation-by-distance relationship by inflating the extent of differentiation per unit distance for those populations that straddle a physical boundary to effective dispersal. This is manifested by a shallower slope and higher regression coefficient when genetic vs. geographical distance is examined within the populations forming the two main clusters identified by the principle component analysis, relative to the analysis involving all populations simultaneously.

When the first axis principle component scores are interpolated onto a regular grid then contoured to provide a visual representation of allele distribution among the populations, two distinct discontinuities can be resolved. Under isolation-by-distance models, the frequency of alleles, and hence PC1 scores, is expected to change gradually across two-dimensional space. Any discontinuities can be viewed as barriers to gene flow. In this case, the discontinuity showed considerable correspondence with areas of 'unsuitable' grouse habitat flanking the River Dee system that bisects the sampled areas. Associated with the area are large swathes of agricultural and forestry land, plus several villages and water bodies, which would presumably disrupt the connectivity of areas of habitable heather moorland. Given that grouse could potentially cross such barriers in a single flight, the size of the barriers is appearing to have a disproportionate effect on limiting allelic movement.

High levels of genetic structuring and within-population male relatedness have significant implications for the persistence of grouse in natural populations. These parameters imply that when a male fails to obtain a territory and recruit to the adult population at his natal site he is not generally successful in recruiting elsewhere. This may reflect the fact that either the male does not actually emigrate and attempt to recruit in another population, but remains as a 'floater' on the periphery of the natal moor, or the male cannot successfully recruit if he does disperse. This has been implied previously from observation of territory-holding cocks that were killed during the winter, being replaced by local individuals from the same

cohort not known to have previously held a territory (A. D. C. MacColl, personal observation). This might ultimately mean that the breeding densities of grouse populations are controlled largely by the success of recruitment from locally reared cohorts. Overdepletion of territory-holding or resident cocks by shooting could potentially limit overall population size. This situation is exacerbated further in grouse because they undergo regular (5–10 years, depending on latitude) cycles in abundance (Potts *et al*. 1984). Catastrophic loss of resident males during a population trough or during the early incline phase of a population cycle could ultimately compromise the viability of populations.

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