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## Spatial distribution of genetic relatedness in a moorland population of red grouse (*Lagopus lagopus scoticus*)

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Several aspects of the ecology and biology of red grouse (*Lagopus lagopus scoticus*) could prevent the complete admixture of genes within and between populations. Male red grouse display a high degree of natal philopatry, are territorial, and show less aggression to kin than to non-kin. Such factors acting in combination predict limited male-mediated gene flow, which will promote social structure within a population by the formation of stable kin clusters, and facilitate a rapid rise in allelic coancestry and/or inbreeding. In this study we utilize hypervariable microsatellite polymorphisms to examine the extent of social affiliation between relatives in a moorland population of grouse from NE Scotland. Levels of genetic relatedness between individual male red grouse occupying territories at Glas Choille in the spring and autumn of 1995 were examined, and kin clusters delimited. Nine kin groups (mean size = 2.4 individuals) were identified prior to breeding in the spring, which increased to 11 kin groups (mean size = 4.0 individuals) when territories were reformed in the autumn. The majority of those individuals that were recruited into the adult population during the autumn already had a first-order male relative established, supporting the hypothesis that recruitment is facilitated by behavioural interactions among relatives. The demographic and population genetic consequences of philopatric recruitment and kin clustering are examined and discussed.

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ADDITIONAL KEY WORDS:—dispersal – DNA – gene flow – kin – microsatellite – philopatry – recruitment – social structure – territory – altruism.

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## INTRODUCTION

Population geneticists have traditionally viewed a species distribution as a set of semi-discrete subpopulations, each comprised of males and females mating at random. Such a view facilitated the development of models that describe how genetic diversity can be partitioned into components that reflect population genetic structure in terms of inbreeding within subpopulations and differentiation between them (Wright, 1969, 1978). Behavioural ecologists and population biologists, however, have long appreciated that social structures exist in many species. These can range from individuals interacting more or less equally with all other individuals in a population, to specific groups of individuals forming cohesive reproductive units. Such structure can limit the number of breeding individuals, and control the extent of dispersal of the sexes and specific individuals (e.g. Greenwood, 1980; Pusey, 1987). This ultimately prevents random mating within subpopulations, one of the central assumptions of classical population genetics theory. Considerable emphasis, therefore, is now being placed on determining the extent of social affiliation within populations (e.g. Pope, 1992; vanStaaen, 1995; Girman *et al.*, 1997; Dobson, 1998; Dobson *et al.*, 1997, 1998; Ishibashi *et al.*, 1998), and on developing a theoretical framework to model gene dynamics in socially structured populations (Chesser, 1991a, b; Sugg & Chesser, 1994; Sugg *et al.*, 1996; Berg *et al.*, 1998).

The application of hypervariable molecular markers, such as microsatellites, to studies of natural populations has provided a wealth of new information about a range of behavioural and ecological parameters such as social structure, reproductive success and dispersal (Avisé, 1994, and references therein). In particular, the ability to utilize the high allelic variability and heterozygosity resolved at microsatellite loci to ascertain the genetic relatedness of individuals, and hence determine family relationships and reconstruct pedigrees, has clarified the pattern and context of conspecific individual interaction (e.g. Morin *et al.*, 1994; Richard *et al.*, 1996; Smith *et al.*, 1997; Taylor *et al.*, 1997; Martinez *et al.*, 1998; Prodohl *et al.*, 1998).

In this paper we use microsatellite DNA polymorphisms to examine relatedness and kin structure within a population of red grouse (*Lagopus lagopus scoticus*). We test several specific hypotheses and predictions on the degree of kin structure and social affiliation that follow from observations on grouse behaviour, then examine the extent to which kin structure affects population genetic patterns and demographic processes.

Red grouse are locally common throughout northern and western Britain, in moorland areas dominated by heather (*Calluna vulgaris*), which provides its primary food source and cover (Hudson, 1986). A number of aspects of the biology and ecology of grouse suggest that individual dispersal is somewhat limited, and will preclude the complete admixture of genes within and between areas. First, in common with the vast majority of avian species, males display a high degree of natal philopatry. Mark and recapture studies have highlighted extremely limited

male dispersal. At Kerloch moor in Scotland, 94–98% ( $n=218$ ) of marked cocks were recovered within 1.5 kms of their natal site (Jenkins *et al.*, 1963). Female dispersal is considerably greater than that of males, but the actual estimation of distance has been difficult to ascertain unambiguously (Hudson, 1986). Second, male grouse are territorial. Cocks obtain territories in autumn, remain on these over winter, and then father a brood of chicks in the spring. Young males from these broods compete among themselves and with established cocks to obtain a territory the following autumn. The recruitment of males to the local adult population is contingent on an individual successfully gaining a territory. Third, male grouse show greater levels of aggression to non-kin than to kin. Watson *et al.* (1994) showed that neighbouring territory holding individuals had fewer boundary disputes if those individuals were closely related than if they were unrelated. In the context of young birds attempting to establish in the adult population, this would suggest that it is less costly for an individual to recruit within the immediate vicinity of a first order relative (MacColl, 1998).

Large-scale population genetic surveys in NE Scotland (Piertney *et al.*, 1998) have highlighted that male philopatry and territoriality lead to a heterogeneous distribution of microsatellite genotypes over short distances (<10 km), indicating significant local population differentiation. Within-population relatedness estimates ( $R$ ; Queller & Goodnight, 1989) are higher for males than for females, suggesting that limited male dispersal has a major effect on how genetic variation is apportioned within and between populations. A subsequent survey (Piertney *et al.*, in press) examining levels of population divergence using mitochondrial DNA haplotypes could resolve no differences between areas, confirming that female-mediated gene flow is high and that the major factor promoting genetic structure is limited male dispersal.

However, at a more local scale, it is unknown whether limited male dispersal promotes social organization within populations, by maintaining related individuals in close proximity for extended periods. Nor are the demographic and genetic consequences of such structuring understood. Certainly, it is unclear whether such clustering of relatives will increase levels of allelic coancestry (the correlation of genes between individuals within the same population), and whether this can be maintained in the absence of local inbreeding. It has been proposed that kin structure may play a fundamental role in the temporal demographics of red grouse populations by providing a mechanism that can drive the unstable population dynamics characteristic of many populations (Mountford *et al.*, 1990; Watson *et al.*, 1994). The aims of this study are therefore to use molecular techniques to examine levels of genetic relatedness between individuals within a natural population, to infer from this the extent of social affiliation between relatives, and finally to assess the impact kin structure may have on population genetic and demographic parameters.

## MATERIAL AND METHODS

### *Study population*

Since 1993, we have studied intensively a population of red grouse at Glas Choille (57°07'N, 3°19'W) on Invercauld Estate, approximately 60 km west of Aberdeen. Glas Choille is a small valley about 2.5 km long and 1 km wide, which is managed

for red grouse by rotational burning of heather. A stream, a road and the watershed at the top of the valley form the boundaries of the 65 ha study area (MacColl, 1998). In the present study we focus on the demographic patterns observed during the 1994 breeding season, specifically the patterns of territory establishment.

Grouse were located and captured at night during systematic searches of the study area using the spotlighting technique described in Hudson (1986). Individuals were ringed, and marked with a characteristic pattern of conspicuous patagial streamers (Boag *et al.*, 1975). Blood samples were taken from the brachial vein of each individual and stored in sterile isotonic SSC buffer (Bruford *et al.*, 1992).

Male territories were mapped twice a year (March through to May, then August through to November) using the procedure described in Jenkins *et al.* (1963), whereby incidences of territorial behaviour (standing alert, ground calling, becking, fighting and boundary disputing; Watson & Jenkins, 1964) for identifiable individuals were collated on 1:2500 scale vegetation maps, then minimum convex polygons were drawn around the outer points to delimit territory boundaries (MacColl, 1998). The two territory maps represent the male population in 'spring', when the males present form the breeding cohort for that year, and 'autumn', which is the breeding population after recruitment of that year's young males.

#### *Microsatellite genotyping*

DNA was extracted from blood samples using the standard procedures described in Bruford *et al.* (1992). All individuals were genotyped at between 12 and 17 di-, tri- and tetranucleotide microsatellite loci (Piertney & Dallas, 1997; Piertney *et al.*, 1998). PCR amplifications were performed in a total volume of 10  $\mu$ l using an MJ Research PTC-100 thermal cycler. Each reaction mix contained approximately 10 ng of template DNA, 2.5 mM MgCl<sub>2</sub>, 75 mM Tris-HCl (pH 9.0), 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.01% (v/v) Tween-20, 0.2 mM of each nucleotide, 5 pmoles of each primer (forward primer end-labelled with [ $\gamma$  <sup>32</sup>P]-dATP) and 0.5 units *Taq* polymerase. PCR profiles followed a 'touchdown' (Don *et al.*, 1991) procedure, whereby after an initial denaturation step of 3 minutes at 90°C, 20 cycles of PCR were performed, each cycle consisting of 30 seconds denaturation at 91°C, and 30 seconds of annealing starting at 60°C and dropping by 0.5°C per cycle. A further 15 cycles were then performed with 30 seconds denaturation at 90°C and 30 seconds annealing at 50°C. No extension steps were included in the program, except for a 2 minute 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*

There is considerable debate about the number of loci that are required to accurately assign the relatedness between two individuals of unknown pedigree, and to differentiate first-order relatives (father-son and full siblings in this case) from unrelated individuals (Capy & Brookfield, 1991; Brookfield & Parkin, 1993; Blouin *et al.*, 1996). Here we used two approaches to highlight that the markers used in

the present study provide accurate estimates of relatedness from which individual relationships can be inferred. Firstly, we estimated the number of loci required to provide robust estimates of relatedness using the rarefaction algorithm described in Girman *et al.* (1997). For this we selected a locus at random and calculated the relatedness for a single pair of individuals ('dyad'). A second locus was chosen (without replacing the first) and relatedness was recalculated using both loci. This procedure continued so that relatedness was calculated with an increasing number of loci until all loci were selected. The change in relatedness with increasing number of loci was then expressed as a function of the number of loci drawn. The minimum number of loci to employ is that which provides little or no change in the relatedness estimate. Secondly, the potential of the markers to distinguish between first-order relatives and unrelated individuals was assessed by simulation using Kinship 1.2 (Queller & Goodnight, 1989). From population allele frequencies at Glas Choille in 1994, we constructed hypothetical dyads, each related according to a specific hypothesis (with a probability of zero that alleles are identical by descent for unrelated simulations, with a probability of 1 that one allele is identical by descent in a parent-offspring simulation, or with a probability of 0.5 that an allele is identical by descent from both parental lines in a full-sibling simulation). Relatedness values were calculated for all three sets of hypothetical dyads, and the distribution of  $R$  values for the specified relationships was ascertained. Non-overlapping distributions of  $R$  values associated with non-relatives and first-order relatives would indicate that sufficient loci have been scored to infer correctly a relationship from a relatedness estimate with minimal type I or type II error.

First-order relatives holding territories at Glas Choille were identified using the following approach. First, pairwise relatedness estimates were calculated between every territory-holding cock according to Queller & Goodnight (1989) using the Kinship 1.2 package. Confidence limits around these estimates were obtained by jack-knife permutation over loci. Those dyads with relatedness estimates greater than 0.4 were deemed to be first-order relatives. In the majority of cases, parent-offspring relatives could be distinguished from full-siblings in this set from the age of the individuals concerned (MacColl, 1998). Parent-offspring relationships identified from the Queller & Goodnight (1989) relatedness values were re-examined using likelihood approaches within CERVUS (Marshall *et al.*, 1998). This program provides an estimate of the relative likelihood of parentage, plus a level of confidence for parental assignment given a specified level of genotyping error and the possibility that the true relative may not have been sampled and subsequently genotyped. In this case, paternity was assigned with 95% confidence and a 1% probability of genotyping error from a pool 100 candidate males of which only 60% were present in the study area and hence sampled. Kin clusters on the spring and autumn 1994 territory maps were identified by linking related individuals in 'neighbouring' territories. We define neighbouring territories as having no intervening territory (and thus a common territorial boundary where birds can interact), plus having centres within 3 mean territory radii (i.e. three times the mean distance from the centre to the periphery of the territory for the population; Watson *et al.*, 1994).

The significance of spatial clustering of kin at Glas Choille was assessed by Monte-Carlo simulation (Kalos & Whitlock, 1986). Two simulations were performed. In the first, each territory-holding individual was moved to a random territory, and the frequency of kin clusters and the number of individuals present in a kin cluster were recalculated. This procedure was repeated 1000 times, and the results of all

simulations were then ranked according to the number of kin groups or individuals involved. Under the null hypothesis of random distribution of individuals, all of the kin cluster sizes and frequencies are equally probable, and so the significance of the actual frequency equals the proportion of permuted frequencies greater than or equal to the observed value. In the second simulation, the same procedure was employed except only the birds that recruited in the winter were reassigned territories; previously established birds remained fixed. The latter is probably the more biologically meaningful scenario, given birds that hold territories in the spring will try and re-establish in the same territory in the autumn rather than relinquish space.

The extent to which natal philopatry and social affiliation between related individuals might affect population genetic structure was examined using computer simulation. Chesser & Baker (1996) developed an algorithm to model the spatial dynamics of diparentally and uniparentally inherited genes and generate  $F$ -statistic analogues in subdivided populations given variation in breeding group sizes, the number of female mates per male, dispersal rates and number of offspring per reproductive individual. In the context of examining how kin clustering can affect gene dynamics within and between populations of red grouse, Chesser & Baker's (1996) model can be utilised to predict  $F_{IS}$  and  $F_{ST}$  statistics given an increasing number of extant patriline within a population and various levels of male and female immigration. The other variables within the model are parameterized with empirical data obtained from Glas Choille (MacColl, 1998; Piertney *et al.*, 1998).

## RESULTS

From observational analysis, 61 territories were identified within the study area in spring and 82 territories the following autumn (Fig. 1A, B). This represents an increase in population size of approximately 34% following recruitment of the new cohort of young males. Of all the individuals holding territories, 96% were captured to obtain blood for DNA analysis. A number (43) of the individuals present on the spring map were also present in the autumn map (highlighted by the grey shading in figure 1b). All except five of these individuals had territories in autumn that were in approximately the same position as they had been the previous spring (i.e. spring and autumn territory areas overlapped by at least 20%), though the shape and size may have altered somewhat.

Well-resolved and easily scorable genotypes were obtained at up to 17 microsatellite loci for all of the territory holding birds sampled. Mean observed heterozygosity for these loci for the combined spring and autumn data was 0.78 ( $\pm 0.10$ ), and the mean number of alleles resolved per locus was 8 ( $\pm 5$ ).

The extent to which the Queller & Goodnight (1989) relatedness parameter  $R$  changes for an individual dyad with an increasing number of loci is shown in Figure 2. The value changed by less than 1% after calculation was derived from eight loci. The use of between 12 and 17 loci for all relatedness estimates in the present study would suggest that the values obtained were precise and robust.

The distribution of relatedness estimates derived from simulated unrelated and first-order dyads is shown in Figure 3. There is some variation associated with the estimates from both of these relationships, though the overlap is minimal. This suggests that only 0.004% of first-order relatives will potentially be incorrectly

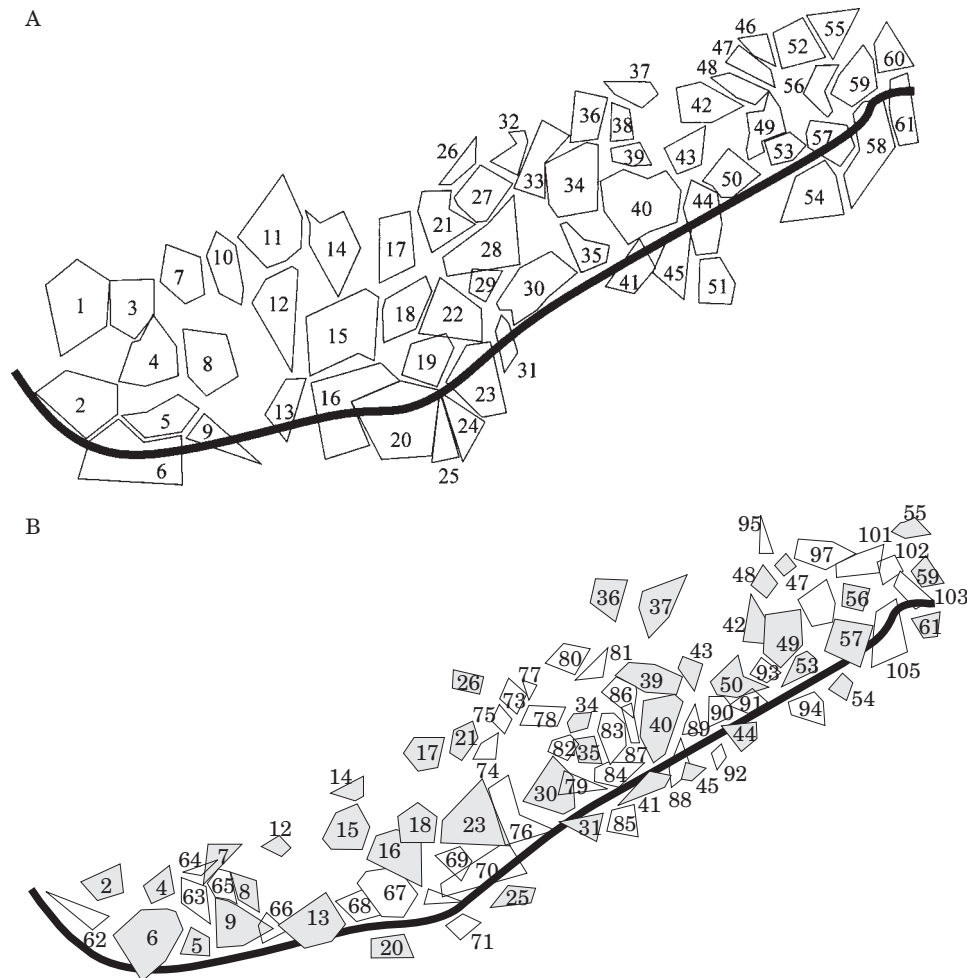


Figure 1. Map of Glas Choille showing the distribution of red grouse territories in (A) spring 1995, and (B) autumn 1995. Those polygons highlighted in grey belong to individuals who have retained a territory between spring and autumn. Territory identifiers correspond to the identifiers used in Table 1.

classified as unrelated individuals, or vice versa. There is a possibility that second-order relatives (e.g. half-siblings) will be mis-classified as full siblings. The overlap between relatedness distributions for full and half-sibling relationships (not shown) indicates that potentially 11% of dyads could be mis-classified in this way. However, given that the frequency of polygamy in grouse populations is low, half-siblings must be derived from different yearly cohorts. As such, the age of birds can be used to separate half-sibling from first-order relationships, and hence the actual frequency of mis-classification is considerably less than that predicted from relatedness estimates alone.

The mean relatedness of neighbours was significantly higher than that of non-neighbours in autumn (Mantel test;  $P < 0.05$ ), and when spring and autumn were combined ( $P < 0.05$ ), though this was not the case in the spring ( $P > 0.05$ ). Kin groups

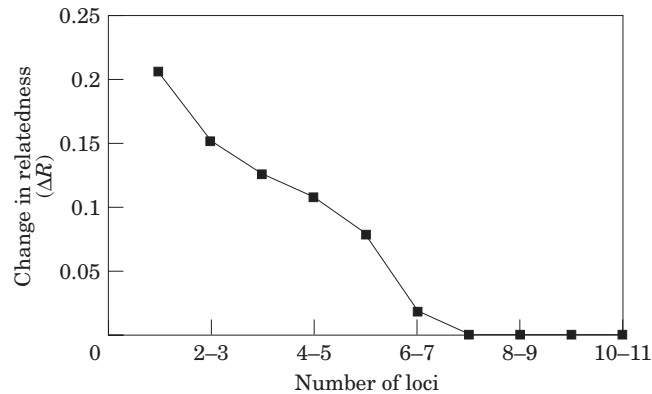


Figure 2. Rarefaction analysis plot highlighting how Queller & Goodnight's (1989) index of relatedness changes with an increasing number of microsatellite loci.

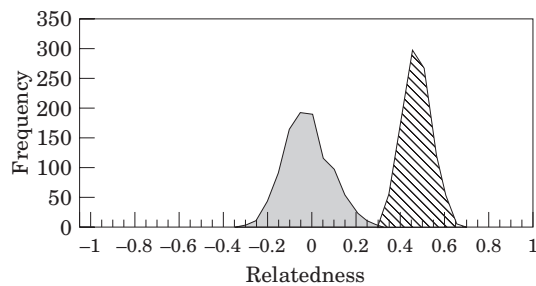


Figure 3. Simulated distributions of genetic relatedness estimates from microsatellite data for hypothetical unrelated (■) and first-order (▨) relationships (parent-offspring and full-sibling combined). Simulation derived from the allele frequency data of all the territory holding males in 1995.

are highlighted for both spring and autumn in Figure 4A and B respectively. Nine kin groups were defined in spring and 11 in autumn. The mean size of the kin groups for spring and autumn was  $2.4 (\pm 0.95)$  and  $4.0 (\pm 2.0)$  individuals respectively, which equates to 36% of all individuals being adjacent to a first-order relative in the spring, and 49% in the autumn. Several kin groups were present in both the spring and autumn maps (highlighted with consistent shading in Figure 4A, B), and in a number of cases the size of the kin cluster increased from spring to autumn. Of those individuals that recruited into the adult population in autumn by obtaining a territory, 19 (61%) established immediately adjacent to a first-order relative.

Those dyads ( $n = 16$ ) defined as parent-offspring using age and pairwise relatedness information were confirmed in every case using likelihood analyses. The most likely father was without exception the individual assigned using relatedness. In a small number of cases ( $n = 3$ ) the difference in the likelihood scores between the most likely and the second most likely father was insufficient for the CERVUS algorithm to assign paternity with sufficient probability to preclude error. Marshall *et al.* (1998) suggest that this may mean the actual father was not sampled. However, on at least one occasion we have evidence that the two most likely putative fathers were in fact



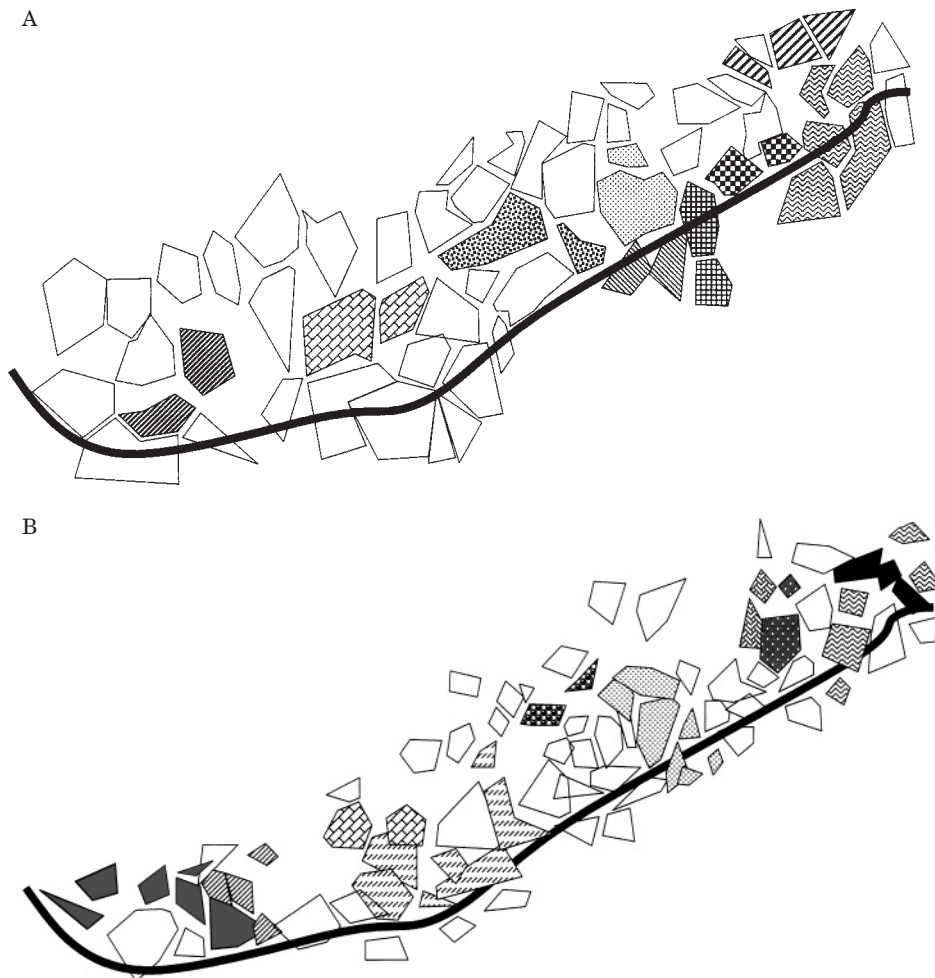


Figure 4. Kin groups present at Glas Choille in (A) spring 1995 and (B) autumn 1995. Kin groups are defined in the text. Identical shading schemes between the spring and autumn maps indicates that kin clusters have been maintained.

brothers, and that a small difference in likelihood score was due to allelic similarity between these individuals.

The extent of kin clustering in the autumn was considerably greater than would be expected by chance. The frequency distributions of the number of kin clusters and the number of individuals involved in kin groups, derived from the reassortment of all birds into random territories, are given in Figure 5A and B respectively. The observed number of kin groups (11) was higher than that generated in all 1000 Monte-Carlo simulations, and the number of individuals encompassed in kin groups (40) was higher than that generated in 998/1000 permutations. Similarly, the observed number of kin groups and the number of individuals was higher than those generated in 985/1000 and 960/1000 simulations (respectively) when just the

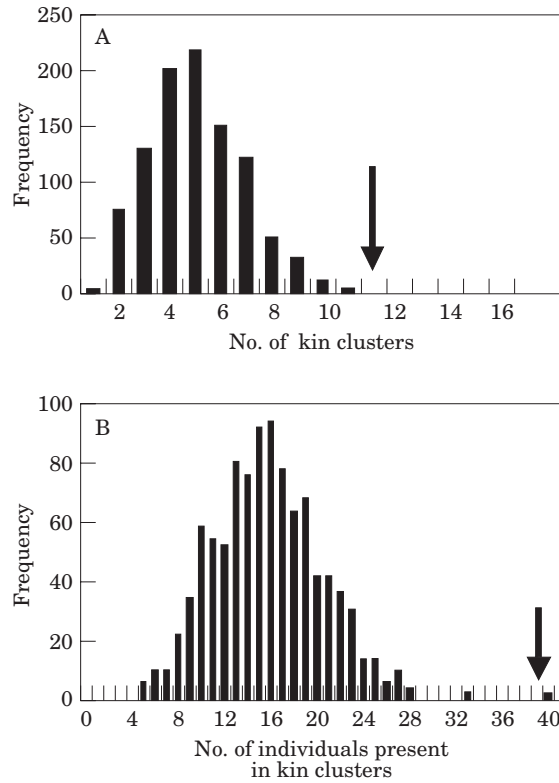


Figure 5. Frequency distribution of (A) the number of kin clusters, and (B) the number of individuals present in kin groups generated after random mixing of all territory holding birds from the autumn. The actual observed values are indicated by the arrow.

newly recruited birds were shuffled among the newly formed territories (frequency distributions not shown).

When the Chesser & Baker (1996) model is parameterized with the actual number of male kin clusters present at Glas Choille, asymptotic  $F_{IS}$  and  $F_{ST}$  values of  $-0.002$  and  $0.105$  are reached respectively. The change in simulated  $F_{IS}$  when a finite sized population of 100 males becomes dominated by fewer kin clusters with various levels of female dispersal is shown in Figure 6.  $F_{IS}$  values increased relatively little as the number of male kin clusters was reduced, and this certainly had considerably less effect than reducing the amount of female dispersal.

#### DISCUSSION

The use of hypervariable microsatellite markers has allowed us to view the relatedness structure and hence social organisation of red grouse with an extremely high level of resolution. Putative family associations have been described in grouse previously (Watson *et al.*, 1994) using behavioural observation, but this was highly labour intensive and in several cases had to infer the relationship between individuals

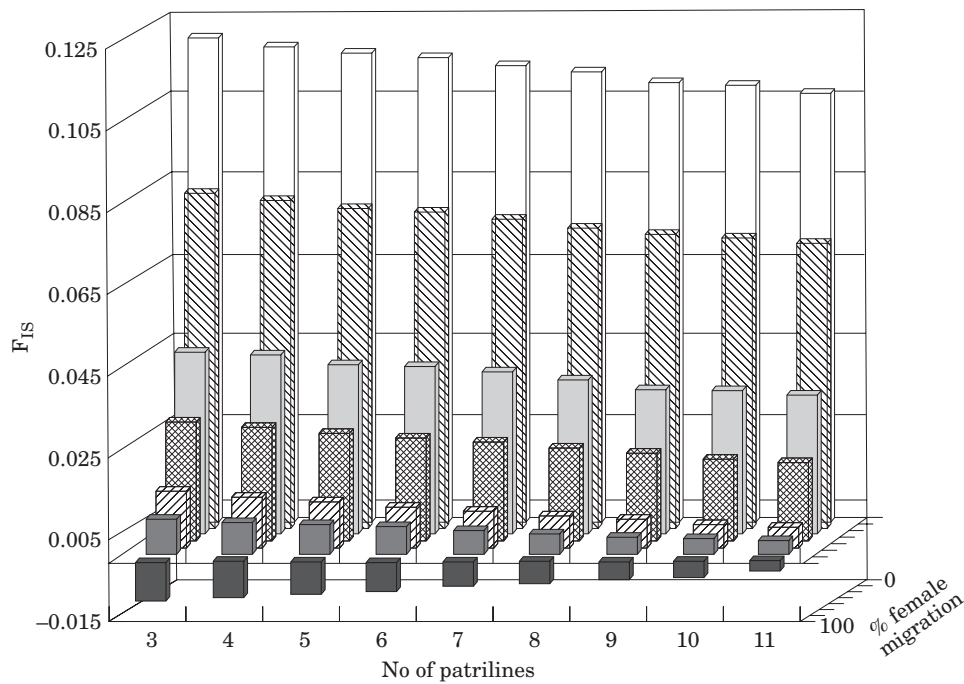


Figure 6. Simulated  $F_{IS}$  values for a fixed size population when dominated by different numbers of patriline and with various levels of female mediated gene flow. Additional parameters used in the model are 5% extra-pair mating, mean clutch size of four individuals and a variance of 2, plus 5% male immigration (see Piertney *et al.*, 1998; MacColl, 1998).

based on association, rather than by examining genetic relatedness explicitly. Our data highlight that the philopatric behaviour of male red grouse not only limits genetically effective dispersal between areas (Piertney *et al.*, 1998), but also promotes the formation of significant numbers of kin clusters within local populations. Several clusters of first-order relatives were identified at Glas Choille prior to chick hatching, and these increased both in size and in number the following autumn. These increases were associated with new recruits establishing in close proximity to first-order relatives, rather than a re-shuffling of previously established males. The presence of kin clusters resulted in the overall relatedness between neighbours being greater than between non-neighbours, though this effect was most pronounced in the autumn. Presumably, a smaller difference in the relatedness of neighbours and non-neighbours in the spring was largely due to the combined effects of: (1) over-winter mortality and subsequent replacement by unrelated immigrant individuals, and (2) subtle changes in the position of territories over the winter such that first-order relatives were no longer in territories that we defined as neighbouring.

Prevalent kin clustering can potentially have considerable demographic and population genetic consequences. Traditionally it has always been assumed that the primary effect of kin clustering would be to increase the potential for inbreeding. Since Lincoln (1934), it has been emphasized that the dispersal of offspring from their natal area functions as an inbreeding avoidance mechanism. In red grouse, where a proportion of individuals remain in close proximity to their parents,

inbreeding may prove prevalent, leading to high levels of within-population homozygosity correlated with a loss of individual fitness. However, such a premise ignores the fact that sex-biased dispersal can prevent incestuous matings. The maintenance of kin groups dominated by particular patriline will tend to retain particular alleles within populations, leading to rapid allelic coancestry, but this can accrue in the complete absence of inbreeding. Recently, considerable emphasis has been placed on explicitly modelling the effect of social organization and various behavioural processes such as philopatry on parameters that reflect genetic structure (deJong *et al.*, 1994; Chesser & Baker, 1996). Here we used the Chesser & Baker (1996) model to explore how the reproductive dominance of a small number of patriline will affect  $F_{IS}$  and  $F_{ST}$ . When the model is parameterized with data on the actual extent of kin clustering observed at Glas Choille, the  $F_{IS}$  and  $F_{ST}$  values produced ( $-0.002$  and  $0.105$  respectively) correspond sufficiently closely to the estimates derived from empirical data ( $-0.007$  and  $0.086$ ; Piertney *et al.*, 1998) to suggest that the model is informative. A small, non-significant, negative  $F_{IS}$  value highlights that the formation and retention of male kin groups within populations does not result in a loss of genetic variation, and any concomitant inbreeding effects. Indeed, the extent of female mediated gene flow is sufficient to maintain an excess of heterozygote genotypes. This effect is common when sex biased dispersal is prevalent (Prout, 1981), and is a product of differences in the allele frequency composition of males and females. A slight excess of heterozygotes within populations will also have the effect of protecting slightly deleterious recessive alleles, and hence will probably impart a higher genetic load than a random mating population in Hardy-Weinberg proportions (deJong *et al.*, 1994). Varying the number of patriline in the simulation study has relatively little effect on the resultant  $F_{IS}$  value that is generated, highlighting that female dispersal will augment allelic variation irrespective of the degree of male relatedness. The change in  $F_{IS}$  when female dispersal is reduced is considerably greater than the effect of manipulating patriline.

It has been suggested that kin clustering may also have an effect on grouse demography by influencing several aspects of population dynamics. First, as male recruitment is the main demographic determinant of population change in both sexes between years (Moss & Watson, 1991), philopatric dispersal, minimal immigration and extensive kin clustering means the reproductive potential of the population, defined as the number of males that recruit, is governed by the success of the locally reared cohort. Secondly, it has been shown that an individual's probability of recruitment increases with the number of close relatives already established in its paternal kin cluster (MacColl, 1998). As such, those patriline that form the largest kin groups will enhance the probability of recruitment of their own kin. Thirdly, it has been suggested that kin clustering may provide a mechanism that can drive the temporally unstable population dynamics characteristic of grouse populations. Many grouse populations undergo cyclic fluctuations in abundance, though cycle periodicity, amplitude and the specific trajectories of the incline and decline phases vary. Traditionally, ecologists have invoked extrinsic trophic interactions, such as predator-prey or host-pathogen effects for producing the delayed density dependence in the rate of population change that is necessary for cycles to occur. However, in grouse such factors do not appear completely appropriate for all populations; some grouse populations cycle under low parasite burden and predation pressure. Social and intrinsic mechanisms have generally been deemed

unlikely to generate cycles (Stenseth & Ims, 1993), though as discussed by Matthiopoulos *et al.* (1998), this may reflect a lack of parameterized models highlighting how intrinsic mechanisms may work, rather than explicit empirical falsification. Mountford *et al.* (1990) produced an illustrative conceptual model to highlight how grouse cycles could be driven by philopatric cocks and be explained by the joint action of territory size and differential behaviour between kin and non-kin. They suggested that at low density established males have large territories, making it easy to facilitate the recruitment of sons as neighbours, thus forming small kin clusters. Positive feedback in successive years will cause kin groups to proliferate and density to increase. When the carrying capacity of the moor is reached, recruitment is depressed to a level that cannot compensate for natural mortality. Density will start to decrease, and recruitment will remain low as kin clusters break up. Only when the number of territory holding birds is small enough for facilitated recruitment to begin again will the cycle start over. These ideas have been expressed in more mechanistic models parameterized with data from actual populations (Hendry *et al.*, 1998; Matthiopoulos *et al.*, 1998), and these do produce cyclic dynamics resembling natural grouse cycles. Testing these ideas explicitly requires an ability to map the formation and maintenance of kin clusters over the course of a complete cycle, a process that would be impractical if dependent on behavioural observation alone. Here we have highlighted that such a process is possible if highly variable molecular polymorphisms are utilized. Moreover, we have confirmed one of the central assumptions of the kin selection models, namely that philopatry and nepotism result in the formation of kin clusters.

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