

Microsatellite markers reveal the potential for kin selection on black grouse leks

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The evolution of social behaviour has puzzled biologists since Darwin. Since Hamilton's theoretical work in the 1960s it has been realized that social behaviour may evolve through the effects of kinship. By helping relatives, an individual may pass on its genes despite negative effects on its own reproduction. Leks are groups of males that females visit primarily to mate. The selective advantage for males to join such social groups has been given much recent attention, but no clear picture has yet emerged. Here we show, using microsatellite analysis, that males but not females of a lekking bird (the black grouse, *Tetrao tetrix*) are genetically structured at the lek level. We interpret this structuring to be the effects of strong natal philopatry in males. This has the consequence that males on any specific lek should be more related than expected by chance as indicated by our genetic data. Our results thus suggest that kin selection is a factor that needs to be considered in the evolution and maintenance of the lek mating system in black grouse and sheds new light on models of lek evolution.

Keywords: leks; genetic structure; microsatellites; kin selection; social evolution

1. INTRODUCTION

Hamilton's theory of kin selection is generally accepted as one of the key elements in explaining social evolution (Hamilton 1964; Trivers 1985). Kin selection has been advocated in studies of why related individuals may sacrifice their own reproduction and instead help a relative. The main emphasis has been on 'helpers at the nest studies' in vertebrates (Emlen 1997) and studies of the evolution of eusociality in insects and vertebrates (Crozier & Pamilo 1996; Sherman *et al.* 1991). The benefit of sociality in all such systems is mediated through natural selection. However, recent theoretical work suggests kin selection may also apply when the benefit is mediated through sexual selection, e.g. lek mating (Kokko & Lindström 1996). Leks are aggregations of males which females visit primarily for the purpose of mating, and are rare in both vertebrate and invertebrate species (Höglund & Alatalo 1995). On leks, the distribution of matings is invariably highly skewed such that a few males reproduce while the majority never or only rarely do so (Höglund & Alatalo 1995). Therefore, a contentious issue has been why males that face meagre chances of reproduction should join the lek when it has been shown that joining leks is costly (Höglund *et al.* 1992; Aspi & Hoffman 1998). In many lekking species, females are choosy and prefer to mate in larger male aggregations rather than in smaller aggregations or with single males (Höglund & Alatalo 1995). Under such circumstances non-preferred males may

enhance their inclusive fitness (Hamilton 1964) by joining related preferred males (Kokko & Lindström 1996).

According to the kin-selection hypothesis for the evolution of leks, males establish themselves on a lek where the top male, in terms of reproductive success, is a close relative (Kokko & Lindström 1996). Given a female preference for aggregated males, increased lek size will attract more females (Alatalo *et al.* 1992; Höglund & Alatalo 1995; Widemo & Owens 1995). This will mainly benefit the top-ranking male (Alatalo *et al.* 1992). The newly established male, however, will indirectly pass on 'his' genes since he shares a certain proportion of his genome with the top male due to their relatedness. A firm prediction from this model of lek evolution is that males should be more related within than across leks. We set out to test this prediction in a wild population of black grouse near Jyväskylä, Finland.

Black grouse, and other grouse species, are known for their sex-biased natal dispersal, females being the dispersing sex (Dunn 1985; Willebrandt 1988; Small & Rusch 1989). During the non-breeding season, especially in midwinter, birds from nearby leks form mixed-sex winter feeding flocks that roost and forage together (Klaus *et al.* 1990). During the mating season males assemble at leks which females visit to mate (Höglund & Alatalo 1995). Females disperse from the winter flock in which they were born before their first breeding whereas males are almost invariably recruited to their natal winter flock (Willebrandt 1988). Since leks are formed by males from the same winter flock this has the consequence that males at the same lek might be close kin.

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2. METHODS

A total of 123 males and 110 females were captured on 12 leks or winter feeding sites within our 50 km × 50 km study area using cannon-nets or walk-in traps in 1989–1994 (see Alatalo *et al.* (1992) for details of the study area and population). Immediately after capture about 4 µl of blood was extracted from the wing vein and stored in EDTA-buffer, kept on ice and transferred to storage at –20 °C until analysis. Birds were colour-ringed and then released. Some females were equipped with radio-transmitters (Biotrack, Wareham, UK). During peak lekking activity, which lasts for about a fortnight at the turn of April–May annually, we observed the leks from nearby blinds and scored the presence of ringed and radio-tagged birds.

Microsatellites are neutral genetic markers suitable for revealing relatedness (Queller *et al.* 1993). We used two microsatellite markers originally cloned in red grouse (*Lagopus lagopus scoticus*) (Piertny & Dallas 1997) to detect genetic variation within and between leks, and genotyped 123 males and 110 females from five winter flocks and 15 leks (sequences of primers for PCR: LLST1 locus [(AAT)*n*], 5' primer, 5'-AAATTCTTT-TCTTGTGTTGATGAG-3', and 3' primer, 5'-TGAGGGTTATGACATTATTAGG-3'; LLST3 locus [(CAA)*n*], 5' primer, 5'-CCAATTATTTTGTCTTTTCATTTG-3', and 3' primer, 5'-CCAAGTTTCAAGTAACAGAAAAC-3'). After standard Chelex or phenol-chloroform extraction, the relevant DNA fragment was amplified using PCR. PCR was performed in a 10 µl reaction mixture containing 2 µl DNA extract, 0.4 µl of 2.5 mM dNTPs, 1 µl 10 × buffer [100 mM Tris HCl, pH 8.8, 500 mM KCl, 1% Triton X-100], 0.2 µl 50 mM MgCl₂, 0.31 µl 2U µl⁻¹ Dynazyme polymerase (Finnzymes Oy, Finland), 2.5 µl of each 1 µM primer. PCR profiles, LLST1: 3 min at 90 °C; 30 × 30 s at 90 °C, 30 s at 53 °C; 2 min at 72 °C, hold 4 °C; LLST3: 3 min at 90 °C; 20 × 30 s at 90 °C, 30 s at 55 °C touch down 0.5 °C, 30 s at 72 °C; 2 min at 72 °C, hold 4 °C. To check if we obtained approximately the predicted length of fragment a portion of the PCR products were analysed by electrophoresis on agarose gels, stained with ethidium bromide and visualized under UV light. Length polymorphism was detected by running ³³P end-labelled fragments on polyacrylamide gels and visualizing the fragments with autoradiography. Genotyping of each male was done by one of us who did not know to which lek specific birds belonged. A few individuals were run twice and were invariably assigned the same genotype. Genotype frequencies, deviation from Hardy–Weinberg and population subdivision were analysed using the randomization tests in GENEPOP 3.1, where the hypothesis of a random distribution of *k* different genotypes among *r* populations is tested analogous to Fisher's exact test on a 2 × 2 contingency table extended to a *r* × *k* contingency table (Raymond & Rousset 1995). Furthermore, we tested the population genetic structure using the hierarchical analysis of molecular variance (AMOVA) in Arlequin 1.1 (Schneider *et al.* 1997) using three levels of population structure: total population, winter flocks and leks.

3. RESULTS

For the locus LLST1, we found four alleles and nine genotypes and for the locus LLST3 we found two alleles and three genotypes. Genotype frequencies did not deviate significantly from Hardy–Weinberg (LLST1: Fisher's test, $\chi^2 = 3.7$, d.f. = 14, $p = 0.997$; LLST3: Fisher's

Table 1. *Analysis of molecular variance (AMOVA) based on the number of different alleles among microsatellite alleles*

(Significance tests were based on 1023 permutations. AMOVA based on the sum of squared size differences yielded almost identical *p*-values (not shown).)

source of variation	d.f.	sum of squares	variance components	percentage of variation
among winter flocks	4	4.39	0.018 V_a	4.18
among leks within winter flocks	9	3.79	0.000 V_b	0.0
within leks	450	190.85	0.424 V_c	95.87
total	463	199.03	0.44	

Fixation indices: $F_{SC} = 0.000$; $F_{ST} = 0.041$; $F_{CT} = 0.042$.

V_c and F_{ST} : p (random value ≤ observed value) = 0.0088 ± 0.0029.

V_b and F_{SC} : p (random value ≥ observed value) = 0.2952 ± 0.0150.

V_a and F_{CT} : p (random value ≥ observed value) = 0.1388 ± 0.0122.

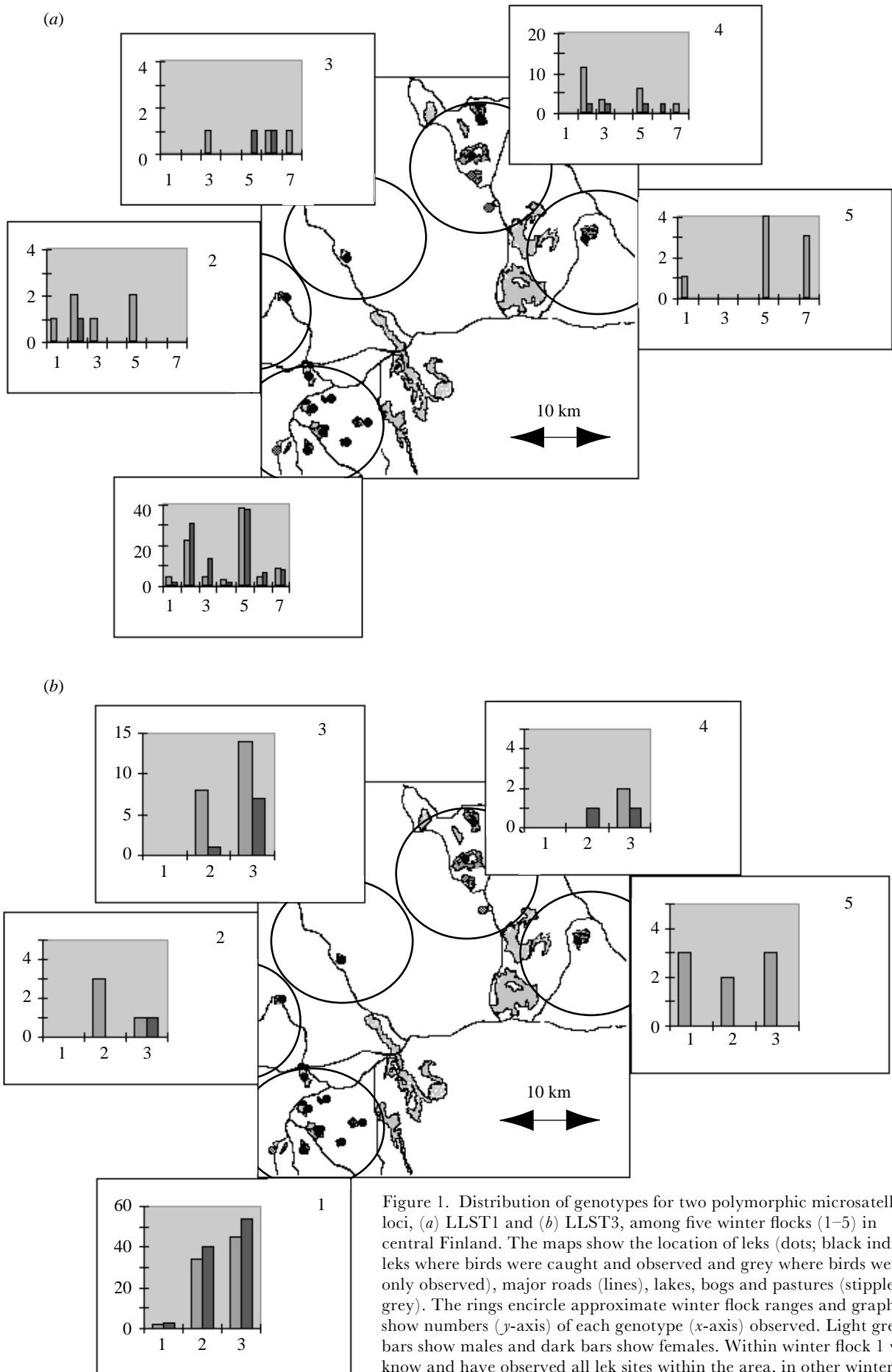
test, $\chi^2 = 6.8$, d.f. = 10, $p = 0.747$). For the sexes combined, most of the variation in the microsatellite loci was due to variation within leks. In AMOVA we found no significant effect among winter flocks or of leks among winter flocks (table 1). A significant value of F_{ST} indicated limited gene flow between winter flocks and leks.

For males, but not females, the population was found to be genetically structured among the five winter flocks (figure 1). The overall pattern of genotypic distribution for males was significantly deviant from random (*G*-tests and Fisher's combined probability, $\chi^2 = 14.84$, d.f. = 4, $p = 0.005$). Conversely, for females the pattern was not deviant from random (*G*-tests and Fisher's combined probability, $\chi^2 = 4.96$, d.f. = 4, $p = 0.34$). For winter flock 1, of which we have a complete knowledge of all leks used by the birds, male genotypic differentiation was significant among the seven leks (*G*-tests and Fisher's combined probability, $\chi^2 = 13.04$, d.f. = 4, $p = 0.011$. For locus 1 the distribution was non-random: *G*-like test, $p = 0.0128$, s.e. = 0.0054; but for locus 2 the distribution was not deviant from random: *G*-like test, $p = 0.1155$, s.e. = 0.0089).

It could be argued that since one site in this sample (winter flock 1 and lek 1) contained a large proportion of the total number of sampled individuals, this lek might determine the genotypes that will be used in the randomization procedure and therefore increase the apparent power of the test. We therefore reanalysed the data when removing winter flock 1. Males were significantly differentiated even within this limited sample ($\chi^2 = 18.8$, d.f. = 3, $p = 0.0001$).

4. DISCUSSION

We ascribe the observed distribution of genotypes mostly to effects of sex-biased dispersal with strong natal philopatry in males, which should have the consequence that males belonging to the same winter flock are more closely related than expected by chance. However, males within winter flock 1 showed genetic differentiation at the lek level, suggesting that the distribution of genotypes is not entirely due to dispersal patterns. Genetic differentiation between leks at this local scale suggests



that some active kin-recognition mechanism may be at work. What this mechanism might be, if it exists, is still unknown.

Sex-biased natal dispersal with males being the philopatric sex seems to be the common pattern within all grouse, including non-lekking species, and indeed within all birds (Herzog & Keppie 1980; Jamieson & Zwickel 1983; Small & Rusch 1989). We would thus expect that males in a local area in any bird species should show genetic structuring and we therefore do not argue that male-biased dispersal and kin selection is the sole explanation for why leks have evolved in some species and not in others.

The only other study that has looked at relatedness in a lekking organism that we are aware of failed to find any evidence that α and β males of long-tailed manakins (*Chiroxiphia linearis*) were close kin (McDonald & Potts 1994). However, in long-tailed manakins, leks consist of two males (α and β) that coordinate their display, with one male performing almost all copulations and a queue of males that never or seldomly appear at the lek (McDonald 1990). The nature of leks in manakins and grouse therefore appears quite different and increasing lek size does not seem to attract more females in manakins as it does in grouse.

Our results show, in accordance with the kin selection hypothesis (Kokko & Lindström 1996), that male black grouse within leks indeed are more related than expected by chance. This finding has profound implications for our understanding of the evolution and maintenance of the lek mating system. First, optimality and game-theory models have been applied to predict what lek a male of a given quality should attend. Such models all use individual optimization of fitness (Höglund & Alatalo 1995; Sutherland 1996; Widemo & Owens 1995). As a consequence of our results, future models have to be complemented with the effects of kinship (Kokko & Lindström 1996). Second, our results also suggest an answer to why males with meagre chances of their own reproduction, despite firm evidence that joining leks is costly (Höglund *et al.* 1992), should join leks. A way to look at the lek considering these new findings is to regard male aggregations (at least in the black grouse) as groups of close kin that are cooperating to attract females (Alatalo *et al.* 1992). Even though not all males reproduce directly, non-reproducing males still transfer genes indirectly by adding attractiveness to the lek that boosts the reproductive success of a close kin.

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