

Pro Gradu –study

**Sex-biased dispersal and kin aggregation on leks in the black grouse (*Tetrao tetrix*)**

**Hanna Forss**



**University of Jyväskylä**

**Department of Ecological and Environmental Science**

**Evolutionary genetics**

**18.9.2007**

UNIVERSITY OF JYVÄSKYLÄ, Faculty of Science

Department of Ecological and Environmental Science

FORSS, H. : Sex-biased dispersal and kin aggregation on leks in black grouse (*Tetrao tetrix*)

Master of Science Thesis: 21 p.

Supervisors: Christophe Lebigre, Prof. Rauno Alatalo

Inspectors: Prof. Anneli Hoikkala, Prof. Rauno Alatalo

September 2007

---

Key Words: AMOVA, genetic structure, kin selection, microsatellite, philopatry, relatedness

## ABSTRACT

Dispersal is an important factor shaping the genetic structure of populations and frequently in the animals, dispersal is biased by sex. The philopatric sex, for instance, might have a lot to gain from staying close to its birth place. One reason for this could be cooperative behaviour (inclusive fitness), which is only possible in aggregations of kin. In some lekking bird species, possibility of kin selection having an effect on the evolution of leks, has been proposed. It would explain why low ranking males participate lekking aggregations, when their prospects of having any matings are low. Displaying together with a dominant related males, low ranking males would lure more females to the lek by increasing the size of the lek. This may benefit low ranking males indirectly as females prefer larger leks. This has proved out to be a controversial hypothesis, some species having supporting evidence for it and some not. In this thesis we used 13 microsatellite loci to find out whether black grouse females disperse more than males and whether black grouse males aggregate according to kin more than expected as an effect of males' philopatric behaviour. In an analysis of molecular variance (AMOVA) males had a stronger pattern of structuring than females did. This indicates that males are more philopatric in the black grouse. For males being philopatric might be beneficial in acquiring a territory from a lek. Females might benefit from dispersing by getting a possibility to compare males in different areas and by avoiding inbreeding. In a test using pairwise relatedness estimates calculated for each lek, five out of twelve leks differed significantly from the null expectation of zero. However, when testing these relatedness values against the relatedness calculated between lekking and non-lekking individuals, there were no differences. Thus, even though some degree of relatedness is apparent between males on a same lek, it is still uncertain whether black grouse males actively choose leks occupied by their relatives. The observed level of relatedness between males on leks could be explained by their philopatric behaviour. Moreover, to test the importance of kin selection having an effect on black grouse lek formation, the costs and benefits of aggregation need to be assessed. For males to successfully find leks with relatives they should have a mechanism to recognize kin. A mechanism to recognize related individuals that have not been raised in the same nest has not been found in birds. Since no consensus on the importance of kin selection on the evolution of lek aggregation has been reached, other explanations have to be considered. Also, the importance of kin selection may vary between the species.

JYVÄSKYLÄN YLIOPISTO, Matemaattis-luonnontieteellinen tiedekunta

Bio- ja ympäristötieteiden laitos  
Evoluutiogenetiikka

FORSS, H. : Teeren (*Tetrao tetrix*) sukupuoleen sitoutunut dispersaali ja sukulaiskoiraisten aggregoituminen soitimella

Pro Gradu –tutkielma: 21 s.

Työn ohjaajat: Christophe Lebigre, Prof. Rauno Alatalo

Tarkastajat: Prof. Anneli Hoikkala, Prof. Rauno Alatalo

Syyskuu 2007

---

Hakusanat: AMOVA, filopatria, geneettinen rakenne, mikrosatelliitti, sukulaisvalinta

## TIIVISTELMÄ

Dispersaali voi olla tärkeä tekijä populaatioiden geneettisen rakenteen muokkaajana. Eläinkunnassa on yleistä, että sukupuolet dispersoivat eri tavoin. Filopatriempimpi sukupuoli voi hyötyä monin tavoin synnyinseudulleen tai sen läheisyyteen jäämisestä. Yksi tällainen syy voi olla yhteistoiminta sukulaisten kanssa (inclusive fitness), joka on mahdollista ainoastaan sukulaisryhmissä. On ehdotettu, että joillain soivilla lintulajeilla sukulaisvalinnalla olisi vaikutusta soidinten muodostumisen evoluutioon. Hypoteesi selittäisi, miksi alhaisen statuksen koiraat osallistuvat soitimille, kun niiden mahdollisuudet päästä parittelemaan ovat pienet. Perustamalla reviirin samalle soitimelle dominoivan sukulaiskoiraan kanssa, ne houkuttelisivat lisää naaraita soitimelle. Tämä hyödyttäisi dominoivaa sukulaiskoiraista, mikä puolestaan epäsuorasti hyödyttäisi myös alhaisen statuksen koiraita. Tämä teoria on osoittautunut hyvin kiistanalaiseksi, sillä joillain lajeilla koiraat näyttäisivät aggregoituvan sukulaisten kanssa kun taas toisilla on osoitettu päinvastaista. Tässä tutkielmassa käytettiin 13 mikrosatelliitti-markkeria selvittämään dispersoivatko teerinaaraat enemmän kuin koiraat. Lisäksi tutkittiin aggregoituvatko teerikoiraat sukulaisuuden perusteella. Koirailta todettiin olevan voimakkaampi geneettinen rakenne kuin naarailla AMOVA:lla testattaessa. Tästä voidaan päätellä, että koiraat ovat filopatriempimpiä ja naaraat dispersoivat enemmän. Koiraille voi olla hyötyä filopatriesta käyttäytymisestä kun ne valtaavat reviiriä soitimella, koska se saattaa olla helpompaa tutussa ympäristössä. Naaraille taas dispersoiminen saattaa tuoda mahdollisuuden vertailla koiraita eri alueilta. Lisäksi naaraat voivat välttyä sukusiitokselta lisääntyessään. Viidellä soitimella kahdestatoista havaittiin koiraiden parittaisen sukulaisuusasteen olevan korkeampi kuin sattumalta voisi odottaa, kun sukulaisuusastetta testattiin nolla odotusarvoa (sukulaisuusaste=0) vastaan. Toisaalta, kun soidinten parittaisia sukulaisuusasteita testattiin soitimelle tulleiden ja muualle menneiden koiraiden välistä sukulaisuusastetta vastaan, eroa ei löytynyt. Koiraiden välillä näyttäisi siis olevan jonkin asteista sukulaisuutta, mutta on vielä epäselvää valitsevatko koiraat saman soitimen sukulaistensa kanssa. Tämän asteinen sukulaisuus koiraiden välillä voisi selittyä koiraiden filopatriisuudella. Aggregoitumisen hyödyt ja haitat tulisi selvittää, jotta saataisiin selville onko sukulaisvalinnalla vaikutusta teerikoiraisten aggregoitumiseen. Jotta koiraat löytäisivät sukulaistensa, niillä täytyisi olla mekanismi tunnistaa sukulaistensa muutenkin kuin samassa pesässä kasvamisen kautta. Tällaista sukulaisten tunnistusmekanismia ei linnuilla ole varmistettu. Koska konsensusta sukulaisvalinnan tärkeydestä soidinten muodostumiselle ei olla saavutettu, myös muita selityksiä täytyy harkita. On myös todennäköistä, että sukulaisvalinnan merkitys vaihtelee lajien välillä.

## Index

1. INTRODUCTION.....	5
1.1. Sex-biased dispersal.....	5
1.2. Kin selection on leks.....	6
1.3. Study.....	7
2. METHODS.....	8
2.1. Genotyping.....	8
2.2. Analysis.....	9
3. RESULTS.....	10
3.1. Sex-biased dispersal.....	12
3.2. Kin aggregation on leks.....	14
4. DISCUSSION.....	16
ACKNOWLEDGEMENTS.....	19
REFERENCES.....	19

## 1. INTRODUCTION

The study of the genetic structure of a population can reveal important information concerning the population studied. Usually structuring occurs between populations or demes on a large spatial scale because it is more difficult to be in contact with individuals living far (Beebee & Rowe 2004). On a small scale differentiation may occur in species with restricted dispersal abilities. As a consequence of restricted dispersal, individuals living close to each other may also be more related. This connection between dispersal patterns and degree of relatedness should be kept in mind when trying to understand kin aggregations.

### 1.1. Sex-biased dispersal

Dispersal can be defined as movement of individuals from an area to another. Two types of dispersal can be distinguished. First, natal dispersal where individuals move permanently away from the area they are born before reaching a mature age. Second, breeding dispersal where adult individuals disperse between breeding areas (Greenwood & Harvey 1982).

In many animal species one sex disperses more than the other (Beebee & Rowe 2004). Many explanations for this phenomenon have been proposed. First, sex-biased dispersal may act as a mechanism to avoid inbreeding. It has been shown that inbreeding is detrimental to offspring through increase of homozygosity at fitness related loci, mostly because recessive detrimental alleles become homozygous (Charlesworth & Charlesworth 1999; Keller & Waller 2002; Höglund *et al.* 2002). If one of the sexes disperses instead of staying in its natal area, chances of mating with a relative become smaller (Beebee & Rowe 2004). In a theoretical study Perrin and Mazalov (1999) investigated the importance of inbreeding avoidance in sex-biased dispersal. They show that inbreeding as the only reason for dispersal, should lead to complete philopatry of the other sex. Bechstein's bat (*Myotis bechsteinii*) seems to follow this rule. An extreme differentiation was found using mitochondrial microsatellite markers as contrast to weak structure in nuclear markers. This could indicate a complete female philopatry and male dispersal, inbreeding avoidance being the crucial factor behind this pattern (Kerth *et al.* 2002). However this is an extreme case since in most of the species both sexes disperse even when it is sex-biased (Perrin & Goudet 2001). To conclude, although inbreeding probably is an important factor, it may not be the only reason for dispersal in many cases.

Competition for resources may also affect dispersal patterns (Lambin *et al.* 2001), especially if an individual has to compete with its own relatives. Competition with kin may be costly because an individual is not only lowering its own fitness, but also the fitness of its relatives (Perrin & Goudet 2001). On the other hand, kin cooperation may enable individuals to be philopatric. There is probably a continuum of competitive and cooperative interactions among relatives that affect dispersal patterns (Lambin *et al.* 2001). But also, kin competition may reduce or even completely cancel out the benefits of kin cooperation (West *et al.* 2002). Perrin and Goudet (2001) have found in an empirical work that both sexes should disperse at even rates, when only the pure effects of kin competition are tested.

The role of mating systems seems also important, especially as determining which of the sexes is to disperse more. Two systems have been identified: resource-defense mating system and mate-defense mating system (Greenwood 1980). Resource-defense mating

systems are usually found in monogamous species in which males take part in the rearing of the young. It is important for a male to acquire and defend a territory, which may be easier in a familiar environment. Thus, it is more costly for a male to disperse. For females, on the other hand, it might be more beneficial to disperse since it allows them to evaluate and choose between resources defended by males and / or avoid inbreeding. This kind of behaviour is common in avian species (Clarke *et al.* 1997). In great reed warblers, it has been shown that philopatric males have a better lifetime fitness than males that dispersed (Hansson *et al.* 2004). Females on the other hand had best breeding success when they had dispersed intermediate distances. It may be possible that dispersing long distances is not favourable because of energetic costs or local maladaptations. In mate-defense mating system, the defensibility of females, not the resources, has been considered as the key element in males' reproductive success. Thus the distribution of females influences the dispersal of males. These species are usually polygynous and paternal investment is minimal. Rearing the offspring may be easier for females in a familiar surrounding. This is the case in many mammal species (Greenwood 1980). However, exceptions to this general rule can be found. For example in many species of the bird family *Anatidae*, males disperse more than females (Greenwood 1980; Clarke *et al.* 1997). It is also worth noticing that lekking species do not meet the conditions described by Greenwood (1980), as they are usually polygynous (Höglund & Alatalo 1995). In some lekking species, and in the black grouse (Caizergues & Ellison 2002), females seem to disperse more than males do, as in majority of the bird species. In some lekking species, however, the reversed pattern of sex-biased dispersal is observed. Such is the case in the lekking great bustard (*Otis tarda*) which has a pattern of male-biased dispersal (Alonso *et al.* 1998).

Because dispersing individuals carry their genes to a new areas, dispersal patterns have fundamental effects on the genetic structure of a population. When considering sex-biased dispersal system where, for example, females are the dispersing sex and males are philopatric, relatedness of males living close together should be higher than that of females. This should be seen as a genetic structure between groups of males from different areas. Females on the other hand should have a less clearcut relationship between relatedness and distance between individuals (Goudet *et al.* 2002, Beebee & Rowe 2004). Pierny *et al.* (2000) investigated the female mediated gene flow in the red grouse using the hypervariable 5' end of the mitochondrial control region. They found patterns of panmixia among the 14 populations studied. These results are in strong contrast to earlier results using hypervariable microsatellite markers where considerable levels of population structuring were resolved in the same set of populations (Pierny *et al.* 1998). Red grouse cocks are territorial and very philopatric. Females on the other hand, as the dispersing sex, prevent the divergence of female-mediated mitochondrial DNA.

Incomplete or sex-biased dispersal, leading to high levels of relatedness between neighbouring individuals can also facilitate social behaviour between related individuals and make kin selection possible in the more philopatric sex (Höglund *et al.* 1999; Hedrick 2000).

## 1.2. Kin selection on leks

Leks are aggregations where males defend a small territory and where females visit solely with the purpose of mating. Lekking species typically have a pattern of strong sexual selection where only a few dominant males have most of the copulations (Höglund & Alatalo 1995). This skew in mating success has led to the question of why subordinate males come to the lek at all. Since in most of the (lekking) bird species males tend to be philopatric and are thus likely to be related to other males living close to them, it has been

proposed that kin selection may have an effect on evolution of leks (Kokko & Lindström 1996). Females seem to prefer larger leks (Alatalo *et al.* 1992, Aspi & Hoffmann 1998) and low ranking males would boost their fitness indirectly by displaying next to a related dominant male (Kokko & Lindström 1996).

Selection that is driven by interactions between related individuals is called kin selection (Hedrick 2000). Because related individuals share a relatively high proportion of their genes, an individual can indirectly transmit its genes and enhance its own fitness by helping a relative to achieve a better reproductive success. The amount of help invested in a relatives' fitness should be the highest when the individual helped is a close relative, since the amount of mutual genes is also the highest. In other words what is known as Hamilton's rule: helping a relative pays off when degree of relatedness and benefit to recipient weight off the costs of helping (Stearns & Hoekstra 2000).

For kin selection to operate, a population must be structured into kin groups, such as families (Hedrick 2000). There is evidence showing that in some species leks are indeed composed of clusters of kin (Höglund *et al.* 1999, Shorey *et al.* 2000, Höglund & Shorey 2003). White-bearded manakin (*Manacus manacus*) males cluster according to their relatedness (Shorey *et al.* 2000). In addition, using two microsatellite loci, Höglund *et al.* (1999) showed that male black grouse (*Tetrao tetrix*) are genetically structured at the lek level, which may indicate that males are clustering with related males in leks. The same was detected in the capercaillie *Tetrao urogallus* (Regnault *et al.* 2006). Krakauer (2005) showed in his study with wild turkeys that indirect fitness benefits, as calculated by Hamilton's rule, outweigh the costs of helping a related male in lek display. Wild turkeys display in groups of two to four close relatives within a larger display aggregation and males lost from coalitions are never replaced by new males. Females also prefer males displaying in groups rather than individual males. Helper males don't reproduce themselves, so there is probably no queuing system in this species. On the other hand, in some lekking species researchers have failed to find any signs of related males being aggregated during lek display. In greater sage grouse (*Centrocercus urophasianus*), Gibson *et al.* (2005) found no evidence of clustering of related males. Also Loiselle *et al.* (2006) showed in four manakin species that males within leks were not more related than a random assortment of the males in the population. This implies that kin selection is unlikely to explain lek participation of low- ranking males in greater sage grouse or in some manakin species. Thus there is no consensus concerning kin selection as an explanation to why lekking males aggregate.

### 1.3. Study

The purpose of this study is to compare the genetic substructure of black grouse males and females using 12 autosomal and one sex-specific microsatellite marker. The study population is organized at several levels: in the study area there are 11 flocks which are further divided into 12 leks. Black grouse gather in flocks during winter and in spring these flocks disappear as males aggregate on leks.

We were interested to find answers to two questions: 1) do female black grouse disperse more than males, and 2) do male black grouse form kin aggregations on leks. This is the first step towards testing the possibility of kin selection on leks. 1) Males should have a stronger genetic structure than females if females disperse longer distances. If females disperse their  $F_{ST}$  should be close to zero and for males it should be higher if they are philopatric.  $F_{ST}$ -value is expected to be the higher the less the individuals disperse and vice versa.  $F_{ST}$ , the fixation index, is the probability that two alleles drawn from a

subpopulation are identical by descent. This probability is high when there is no gene flow between subpopulations and as a result, in the course of time, the populations are becoming more and more differentiated (Frankham *et al.* 2002). If there is a pattern of isolation by distance, the linearized  $F_{ST}$  values should have a positive correlation to the geographic distance between flocks of individuals. Thus, individuals living in flocks situated far from each other, should also be genetically distant (high  $F_{ST}$ ). Closely situated flocks, on the other hand, should have a greater amount of gene-flow between them and thus be genetically similar (small  $F_{ST}$ ). 2) If kin aggregation occurs, males gathering on a lek should be more related than expected by chance. If philopatric males stay in or close to their natal area they should be related to some degree. Thus the relatedness of males lekking on a same lek should be compared to the background level of relatedness among males from the same area when kin aggregation is tested.

The black grouse (*Tetrao tetrix*) is a northern Eurasian grouse species (Tetraoninae) living in moor and forest habitats. Females are brownish and much smaller than males, who have shining black plumage and have a lyre-like tail feathers (Siitari *et al.* 2007). The cocks defend a small territory on an open area called the lek. During the mating season, which lasts about a fortnight, females visit males displaying on leks and finally mate with one of them. Females usually prefer central males (Hovi *et al.* 1994) that are dominant in male-male contest (Alatalo *et al.* 1991) and mate once with one male in a breeding season (Lebigre *et al.* in press).

## 2. METHODS

### 2.1. Genotyping

Birds were captured in 2006 on 11 winter feeding sites in Central Finland (Table 1). 1 to 2 ml of blood was taken from the brachial vein and stored in 70% ethanol at 4°C. All birds were ringed with aluminium and colour rings for identification on the lek sites. During the mating season (late April- early May) birds were observed and their visits to lek sites were recorded in leks. A total of 609 birds (376 males and 233 females) was captured or observed displaying on leks during the mating season.

Table 1. The coordinates of capture sites according to the Finnish grid system, total number of birds captured and the numbers of males and females captured in winter 2006.

	coordinates		n° of birds captured	n° of males captured	n° of females captured
Teerisuo	6900300	3399550	44	23	21
Nyrölä	6914535	3420700	80	52	28
Lehtosuo	6907450	3394650	77	41	36
Kummunsuo	6917550	3384300	79	44	35
Koskenpää	6882800	3406300	77	48	29
Lauttasuo	6935550	3390550	27	20	7
Pirttilampi	6910000	3396100	12	7	5
Saarisuo	6915550	3395175	55	33	22
Palosuo	6921000	3411000	60	38	22
Pirttisuo	6940000	3397500	57	41	16
Pihtisuo	6932500	3390500	33	23	10



Genomic DNA was extracted using the BioSprint 15 DNA Blood Kit reagents (Quiagen, Ref. 940017) and a Kingfisher magnetic particle processor. Black grouse individuals were genotyped using 12 autosomal microsatellite markers (BG6, BG15, BG16, BG18, BG19, Piertney & Höglund 2001; TTT1, TTT2, TTD2, TTD3, Caizergues *et al.* 2001, TUD6, TUT3, TUT4; Segelbacher *et al.* 2000) and one sex-linked marker (BG10; Piertney & Höglund 2001). Microsatellites are selectively neutral co-dominant markers that are frequently used in studies of population structure and they can also be used to estimate the degree of relatedness between individuals.

PCR were done in 10 µl reaction volume containing 2 µl of DNA, 0.3 units of DNA polymerase (Biotools, Ref. 10.048), 50 mM KCl, 75 mM Tris HCl (pH 9.0), 20 mM  $(\text{NH}_4)_2\text{SO}_4$ , 100µM of dNTP, 10 pM of primer and from 1 to 2.5 mM of  $\text{MgCl}_2$  depending on the primer. We used fluorescently labelled forward primers to tag our PCR products (Applied Biosystems). Starting denaturation of the DNA was done at 94°C for 2 minutes. Amplifications were done with 30 cycles of denaturation at 94°C for 30 seconds, annealing temperature for 30 seconds, extension step at 72°C for 1 minute. Final elongation step was done at 74°C for 10 minutes.

DNA fragments were run on agarose gel electrophoresis, stained with ethidiumbromide or syber safe and visualized under UV-light. Microsatellite allele length polymorphism was scored in Prism<sup>®</sup> 3100 Applied BioSystems sequencer. Fragment size was assessed using a size standard GENESCAN LIZ. Genotypes were assigned using Gene Mapper v.3.7 (Applied Biosystems, Inc.).

## 2.2. Analysis

The software Arlequin 3.1 was used to examine indices of molecular diversity, Hardy-Weinberg equilibrium, linkage disequilibrium and the genetic structure. The indices of molecular diversity include the observed and expected heterozygosity and number of alleles per locus in each of the 11 flocks. Hardy-Weinberg equilibrium was calculated using a Markov chain with 1001000 steps for each locus in each flock. Hardy-Weinberg equilibrium can give information on whether the allelic frequencies in the population studied are in equilibrium or whether factors that change allelic frequencies in a population such as gene flow, genetic drift, mutation or selection are at work (Hedrick 2000). Linkage disequilibrium was tested for all pairs of flocks. Non-random association of alleles at different loci (linkage disequilibrium) can be due to physical location of the loci close to each other in the same chromosome but also due to for example recent mixing of different populations, selection, inbreeding or drift (Frankham *et al.* 2002, Hedrick 2000). Lehtosuo and Pirttilampi flocks were pooled together, because there were only few birds seen or captured in Pirttilampi and the distance between these two flocks is so short (only 4 km) that many birds fly from one flock to another.

The population subdivision was investigated using an analysis of molecular variance (AMOVA). In the analysis groups are defined to test a particular structure. The analysis partitions the total variance of allele frequencies into covariance components that are used to compute the classical fixation indices (Arlequin manual). When the AMOVA are performed to determine the percentage of variation among flocks, the genetic variation is divided into two groups (two hierarchical levels): the variation among flocks (the degree of differentiation between flocks;  $F_{ST}$ ) and the variation within flocks (the amount of variation inside the flocks). The significance of the  $F_{ST}$ -values was obtained by permutations of the haplotypes among flocks. An AMOVA with three hierarchical levels was used to determine differences between the sexes (male or female) and between age

groups (yearling or older) within the flocks. In this test the genetic variation is divided into three groups. First the variation among flocks, which is described above. Second to variation among sexes or age groups within flocks (the amount of differentiation between the sexes or age groups in different flocks;  $F_{SC}$ ). Third group of genetic variation is within the sexes or age groups. In the three level hierarchical design, the significance of the  $F_{SC}$ -values is tested by permutating the genotypes of individuals among age or sex groups within flocks. To investigate isolation by distance, the Mantel test option of Arlequin was used to assess the significance of the correlation between the geographical distance and Slatkin's linearized  $F_{ST}$  values (Slatkin 1995) indicating genetic distance. Linearized  $F_{ST}$  is a transformation of  $F_{ST}$  values ( $F_{ST}/(1 - F_{ST})$ ). It enables parametric statistical tests as it is normally distributed. Sex-linked locus BG10 was analysed separately with diploid data options for males and haploid options for females as in black grouse females are the heterogametic sex.

Kingroup 2.0 was used to investigate whether black grouse leks are kin groups. Parents and offsprings or full siblings' have a relatedness value  $r$  of 0.5 and half siblings of 0.25 and so forth as determined by mendelian inheritance in a diploid species. First we identified half and full siblings and parent-offspring relationships using 11 loci (BG16, BG18, BG15, BG19, BG6, TTT1, TTD2, TTD3, TUD6, TUT3 and TUT4). Pirtilampi lek was removed from the analysis as it had only four lekking males. Using these relatedness informations birds within every flock were assigned to kin groups. These birds were grouped in the analysis to correct the allele frequencies for the presence of relatives in the flock. Then an unbiased estimate of Queller & Goodnight pairwise relatedness ( $r$ ) was calculated for all pairs of individuals in each flock. A bias correction is recommended because bias in allele frequencies can result in an underestimate of the true relatedness of the individuals. Relatedness within leks was tested against null expectation of zero using one-sample  $t$ -test in SPSS 14.0. If black grouse males are philopatric they should have some level of relatedness within leks. For this reason lekking males' pairwise relatedness needs to be compared to the background level of relatedness between males from the same area. This background level of relatedness was calculated as the pairwise relatedness of lekking males and males found in winter flocks but not in corresponding leks (considered non-lekking). The relatedness within leks was tested against the background relatedness using binary logistic regression for each lek separately. These non-lekking males either lekked somewhere else or died during spring months before mating season started. If males aggregate according to their relatedness we should find a higher value of average relatedness within the lekking males compared to the average relatedness calculated between lekking and non-lekking males. If we make a simple example: males a1 and a2 are full brothers and males b1 and b2 are also full brothers. If males aggregate according to kin, male a1 and a2 should go to the same lek (they are considered lekking). The males b1 and b2 should also go to the same lek, but in this example we assume that they display on an unknown lek (they are thus considered non-lekking). Now we calculate the average relatedness for a1 and a2 which is  $r = 0.5$ . We compare this to the average relatedness calculated between lekking and non-lekking males: a1 and b1  $r = 0$ , a1 and b2  $r = 0$ , a2 and b1  $r = 0$  and a2 and b2  $r = 0$ , average  $r$  being 0. Thus, by testing the difference between the average relatedness of lekking males and the background level of relatedness we should be able to determine whether males aggregate with kin.

### 3.RESULTS

The number of alleles per locus ranged from 3-16 with an average of 7.7 (s.d. = 2.6). The expected heterozygosity per locus ranged from 0.09 to 0.89 with an average of 0.74

Table 2. The number of alleles (a), observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity and p-value for HW for every locus in all the study flock.

<b>Teerisuo</b>				<b>Nyrölä</b>				<b>Lehtosuo</b>				
locus	a	$H_O$	$H_E$	p(HW)	a	$H_O$	$H_E$	p(HW)	a	$H_O$	$H_E$	p(HW)
TTD2	3	0.14	0.13	1.00	4	0.15	0.14	1.00	5	0.20	0.19	0.40
TTD3	14	0.86	0.88	0.15	15	0.86	0.84	0.98	15	0.90	0.85	1.00
TUD6	6	0.86	0.74	0.15	5	0.64	0.70	0.55	7	0.65	0.73	0.24
TUT3	8	0.86	0.81	0.30	8	0.85	0.80	0.43	7	0.81	0.79	0.54
TUT4	8	0.84	0.81	0.99	7	0.73	0.77	0.45	9	0.82	0.80	0.38
BG16	7	0.91	0.80	0.34	7	0.82	0.80	0.76	6	0.86	0.76	0.01
BG18	9	0.80	0.84	0.83	9	0.81	0.81	0.33	8	0.82	0.83	0.47
BG15	5	0.77	0.72	0.66	6	0.79	0.77	0.58	7	0.64	0.75	0.19
BG19	7	0.77	0.79	0.51	7	0.75	0.79	0.04	9	0.84	0.79	0.20
BG6	11	0.75	0.77	0.31	12	0.83	0.79	0.22	11	0.83	0.80	0.70
TTT1	7	0.75	0.79	0.76	10	0.78	0.78	0.60	8	0.77	0.79	0.97
<b>Kummunsuo</b>				<b>Koskenpää</b>				<b>Lauttasuo</b>				
locus	a	$H_O$	$H_E$	p(HW)	a	$H_O$	$H_E$	p(HW)	a	$H_O$	$H_E$	p(HW)
TTD2	4	0.34	0.32	0.82	5	0.15	0.17	0.14	4	0.11	0.18	0.04
TTD3	16	0.87	0.89	0.47	16	0.88	0.89	0.18	12	0.89	0.88	0.58
TUD6	6	0.75	0.72	0.78	6	0.73	0.67	0.60	5	0.85	0.73	0.48
TUT3	6	0.81	0.77	0.94	7	0.90	0.81	0.04	7	0.89	0.81	0.00
TUT4	9	0.73	0.77	0.88	8	0.73	0.77	0.41	7	0.78	0.75	0.37
BG16	8	0.78	0.79	0.88	9	0.83	0.79	0.85	8	0.74	0.77	0.25
BG18	9	0.86	0.83	0.69	9	0.78	0.81	0.66	8	0.78	0.83	0.59
BG15	7	0.81	0.77	0.66	6	0.73	0.75	0.71	6	0.67	0.75	0.12
BG19	6	0.73	0.80	0.41	8	0.83	0.79	0.11	6	0.67	0.80	0.45
BG6	11	0.72	0.80	0.17	11	0.82	0.82	0.54	8	0.78	0.80	0.67
TTT1	7	0.80	0.81	0.64	8	0.84	0.80	1.00	8	0.63	0.82	0.00
<b>Pirttilampi</b>				<b>Saarisuo</b>				<b>Palosuo</b>				
locus	a	$H_O$	$H_E$	p(HW)	a	$H_O$	$H_E$	p(HW)	a	$H_O$	$H_E$	p(HW)
TTD2	2	0.09	0.09	1.00	4	0.20	0.20	0.26	5	0.17	0.16	1.00
TTD3	12	0.73	0.87	0.05	14	0.93	0.82	0.70	16	0.90	0.89	0.65
TUD6	4	0.73	0.74	0.26	6	0.75	0.70	0.61	5	0.68	0.72	0.90
TUT3	5	0.91	0.82	0.69	6	0.85	0.77	0.85	7	0.73	0.77	0.51
TUT4	6	0.73	0.77	0.88	8	0.82	0.81	0.12	8	0.82	0.79	0.89
BG16	5	0.92	0.80	0.68	7	0.83	0.78	0.91	7	0.83	0.81	0.25
BG18	7	0.92	0.85	0.95	9	0.91	0.83	0.47	10	0.85	0.84	0.99
BG15	5	0.82	0.61	0.74	7	0.71	0.73	0.28	5	0.80	0.77	0.72
BG19	6	0.91	0.81	0.84	7	0.76	0.81	0.25	5	0.80	0.77	0.56
BG6	6	0.91	0.81	1.00	11	0.82	0.80	0.53	10	0.78	0.81	0.20
TTT1	5	0.58	0.74	0.45	9	0.82	0.81	0.44	9	0.85	0.84	0.08
<b>Pirttisuo</b>				<b>Pihtisuo</b>								
locus	a	$H_O$	$H_E$	p(HW)	a	$H_O$	$H_E$	p(HW)				
TTD2	5	0.23	0.21	1.00	4	0.24	0.22	1.00				
TTD3	15	0.86	0.87	0.38	12	0.91	0.86	0.65				
TUD6	4	0.65	0.62	0.59	5	0.76	0.71	1.00				
TUT3	7	0.79	0.76	0.64	7	0.85	0.80	0.63				
TUT4	9	0.72	0.77	0.67	7	0.82	0.79	0.51				
BG16	7	0.86	0.80	0.47	7	0.79	0.77	0.39				
BG18	9	0.93	0.82	0.81	7	0.91	0.83	0.74				
BG15	6	0.75	0.77	0.14	5	0.73	0.75	0.40				
BG19	7	0.79	0.82	0.14	6	0.79	0.79	0.30				
BG6	11	0.75	0.77	0.15	11	0.85	0.84	0.26				
TTT1	8	0.86	0.84	0.99	9	0.76	0.83	0.60				

(s.d. = 0.17) (Table 2). After correction for multiple tests, no deviation from the Hardy-Weinberg expectations was found (Table 2). One pair of loci was in linkage disequilibrium in all the flocks (TTT2 and BG16). Locus TTT2 was removed from further analysis because presence of two loci linked together might bias the results and also does not give any additional information.

### 3.1. Sex-biased dispersal

Population differentiation for the whole data, males and females pooled, was very weak but significant ( $F_{ST} = 0.008$ ;  $p < 0.001$ ). There are, however, differences between males and females in the amount of differentiation nested in them among flocks (% variation among sexes within flocks:  $F_{SC} = 0.40$ ;  $p = 0.002$ ). For this reason, males and females were tested separately in further analysis. Some of the yearling birds in the data may have not yet dispersed. Thus, differences between age groups (yearling versus older birds) within flocks were looked at using an AMOVA with three hierarchical levels. For both males and females there were no differences between age groups within flocks (in males: % variation:  $F_{SC} = 0.18$ ;  $p = 0.244$ , in females: % of variation:  $F_{SC} = -0.22$ ;  $p = 0.766$ ). Some of the flocks with older females had a very few individuals (1-4). When removing these flocks from the analysis the result did not change (% of variation among sexes within flocks:  $F_{SC} = 0.01$ ;  $p = 0.457$ ). As there were no differences between yearlings and older individuals within flocks, age groups were pooled together for male-female comparisons. There is a significant structure in both males and females. However, males had a stronger genetic structure compared to females (males:  $F_{ST} = 0.014$ ;  $p < 0.001$ ; females:  $F_{ST} = 0.004$ ;  $p < 0.01$ ) (Table 3). Similar results were found with the sex-linked locus BG 10 (males:  $F_{ST} = 0.011$ ;  $p < 0.01$ , females:  $F_{ST} = 0.001$ ;  $p = 0.42$ ).

Table 3. Percentages of variation in AMOVA and their p-values in the together tested 11 loci and the separately tested locus BG10.

	source of variation	percentage of variation	p-value
all (11 loci)	among flocks	0.84	<0.001
	within flocks	99.16	<0.001
males (11 loci)	among flocks	1.44	<0.001
	within flocks	98.56	<0.001
females (11 loci)	among flocks	0.43	<0.01
	within flocks	99.57	<0.01
males (BG10)	among flocks	1.05	<0.01
	within flocks	98.95	<0.01
females (BG10)	among flocks	0.13	0.42 NS
	within flocks	99.87	0.42 NS

For both males and females there was a significant correlation between pairwise estimates of Slatkin's linearized  $F_{ST}$  with geographic distance (Mantel test: males  $r = 0.377$   $p = 0.029$ , females  $r = 0.468$   $p = 0.011$ ) (Table 4; Figure 1a and 1b). This indicates that there is a positive association between geographic and genetic distance: individuals sampled further apart have also a higher genetic distance between them.

Table 4. Slatkin linearized  $F_{ST}$  -values and corresponding  $F_{ST}$  p-values for all pairs of flocks. Number of permutations for  $F_{ST}$  p-values is 1023. Lehtosuo has Pirttilampi birds pooled in it.

MALES		<b>Fst p-value</b>									
		Teerisuo	Nyrölä	Lehtosuo	Kummunsuo	Koskenpää	Lauttasuo	Saarisuo	Palosuo	Pirttisuo	Pihtisuo
<b>Slatkin's linearized Fst</b>	Teerisuo	*	0.006	0.013	0.001	<0.001	NS	<0.001	0.001	0.001	0.032
	Nyrölä	0.011	*	<0.001	<0.001	<0.001	0.032	0.004	<0.001	<0.001	0.037
	Lehtosuo	0.009	0.019	*	<0.001	<0.001	0.027	0.001	<0.001	<0.001	0.019
	Kummunsuo	0.016	0.014	0.018	*	<0.001	NS	<0.001	0.018	<0.001	NS
	Koskenpää	0.020	0.021	0.019	0.022	*	0.003	<0.001	0.001	<0.001	0.003
	Lauttasuo	0.009	0.008	0.010	0.004	0.016	*	0.015	0.021	NS	NS
	Saarisuo	0.020	0.009	0.015	0.014	0.026	0.012	*	0.001	0.001	NS
	Palosuo	0.015	0.012	0.016	0.008	0.010	0.010	0.016	*	0.014	NS
	Pirttisuo	0.018	0.015	0.026	0.013	0.024	0.006	0.020	0.008	*	0.015
	Pihtisuo	0.010	0.007	0.009	0.007	0.016	0.003	0.007	0.006	0.009	*
FEMALES		<b>Fst p-value</b>									
		Teerisuo	Nyrölä	Lehtosuo	Kummunsuo	Koskenpää	Lauttasuo	Saarisuo	Palosuo	Pirttisuo	Pihtisuo
<b>Slatkin's linearized Fst</b>	Teerisuo	*	NS	NS	NS	NS	NS	NS	NS	NS	0.001
	Nyrölä	0.007	*	0.005	0.013	0.003	NS	0.008	NS	NS	0.037
	Lehtosuo	0.000	0.010	*	NS	0.040	NS	NS	NS	NS	NS
	Kummunsuo	0.003	0.010	0.002	*	0.044	NS	NS	NS	NS	0.031
	Koskenpää	0.002	0.012	0.007	0.006	*	NS	NS	NS	0.030	0.002
	Lauttasuo	0.000	0.003	0.000	0.004	0.007	*	NS	NS	NS	NS
	Saarisuo	0.001	0.014	0.000	0.000	0.006	0.000	*	NS	NS	NS
	Palosuo	0.000	0.004	0.000	0.000	0.006	0.000	0.000	*	NS	NS
	Pirttisuo	0.007	0.011	0.000	0.000	0.011	0.000	0.003	0.000	*	NS
	Pihtisuo	0.025	0.017	0.008	0.015	0.025	0.000	0.008	0.011	0.009	*

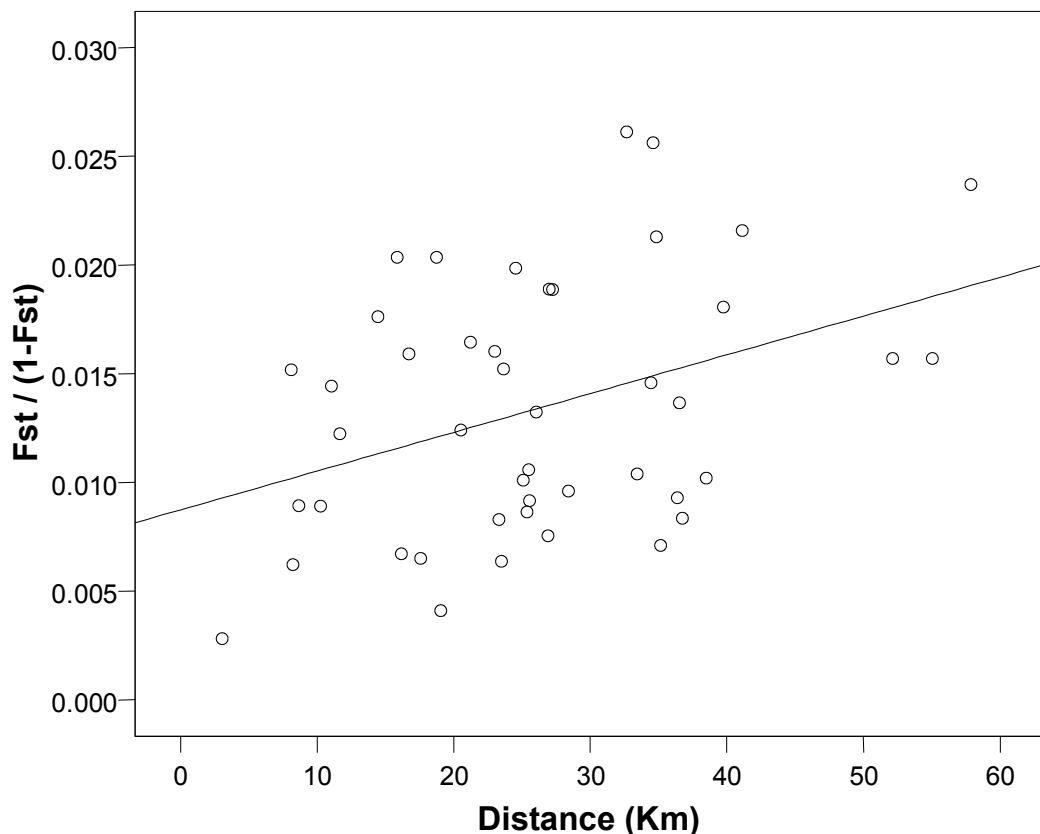


Figure 1a. Correlation of genetic (Slatkin's linearized  $F_{ST}$ ) and geographic distance in male black grouse.

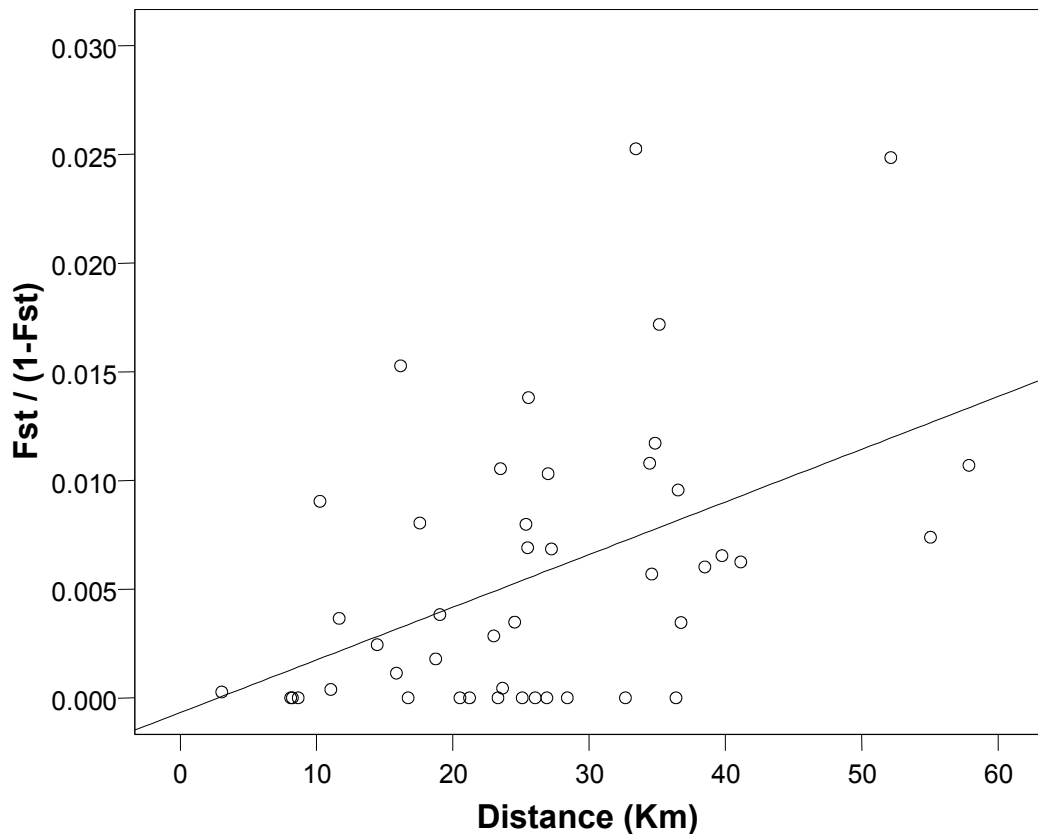


Figure 1b. Correlation of genetic (Slatkin's linearized  $F_{ST}$ ) and geographic distance in female black grouse.

### 3.2. Kin aggregation on leks

The average relatedness of males within leks varied between -0.017 (Lauttasuo) and 0.057 (Nyrölä b). Average relatedness differed from zero in five of the leks: Nyrölä b, Lehtosuo, Kummunsuo, Koskenpää and Saarisuo (Table 5). These leks have average relatedness ranging between 0.014 (Kummunsuo) and 0.057 (Nyrölä b). There was no effect of lek size on the average relatedness (Figure 3). Although the average relatedness of some leks differed from zero, none of the leks showed a significant difference when tested against the average relatedness calculated between lekking and non-lekking males within the corresponding flocks (Figure 2 and Table 6). This may indicate that black grouse males do not lek with kin more than expected by chance. The only comparison made between the pairwise relatedness values of two leks, “Nyrölä a” and “Nyrölä b”, has a significant difference (Table 6). However, when one considers the number of pairwise tests done in this analysis, the significance level of 0.05 may have to be lowered. This would make the comparison between “Nyrölä a” and “Nyrölä b” non-significant.

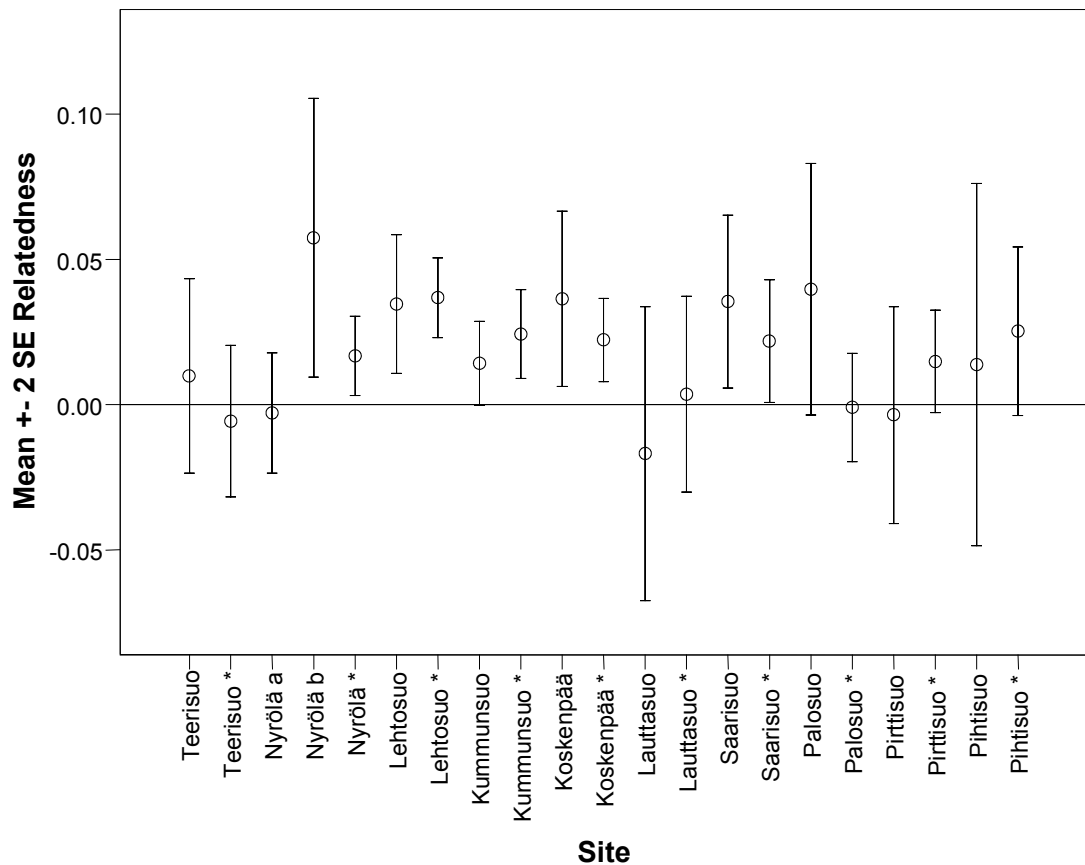


Figure 2. Pairwise estimates of relatedness ( $r$ ) for lekking males in the study leks (the first bars) and the pairwise estimates of relatedness calculated between lekking and non-lekking males in the flocks (after corresponding leks, marked with \*).

Table 5. Lekking males' average relatedness ( $r$ ) tested against null expectation of zero in one sample t-test.

lek	N	average $r$	one sample t-test (2-tailed)		
			t	df	p
Teerisuo	13	0.010	0.591	77	0.556 NS
Nyrölä a	24	-0.003	-0.278	275	0.781 NS
Nyrölä b	9	0.057	2.393	35	0.022 *
Lehtosuo	20	0.035	2.911	189	0.004 *
Pirttilampi	4	-0.008	-0.101	5	0.923 NS
Kummunsuo	35	0.014	1.967	594	0.05 *
Koskenpää	19	0.037	2.420	170	0.017 *
Lauttasuo	10	-0.017	-0.667	44	0.508 NS
Saarisuo	19	0.036	2.389	170	0.018 *
Palosuo	12	0.040	1.833	65	0.071 NS
Pirttisuo	13	-0.004	-0.189	77	0.851 NS
Pihtisuo	9	0.014	0.444	35	0.66 NS

Table 6. Binary logistic regression testing the pairwise relatedness of lekking males against pairwise relatedness between lekking and non lekking males relatedness.

lek	B	S.E.	Wald	df	p
Teersuo	-0.548	0.801	0.469	1	0.494 NS
Nyrölä a	0.637	0.409	2.429	1	0.119 NS
Nyrölä b	1.226	0.917	1.787	1	0.181 NS
Nyrölä a-b	1.992	1.000	3.969	1	0.046
Lehtosuo	0.078	0.491	0.025	1	0.874 NS
Kummunsuo	0.326	0.341	0.915	1	0.339 NS
Koskenpää	-0.439	0.480	0.836	1	0.361 NS
Lauttasuo	0.687	1.034	0.441	1	0.507 NS
Saarisuo	-0.413	0.541	0.583	1	0.445 NS
Palosuo	-1.413	0.791	3.192	1	0.074 NS
Pirttisuo	0.667	0.758	0.775	1	0.379 NS
Pihtisuo	0.376	1.073	0.123	1	0.726 NS

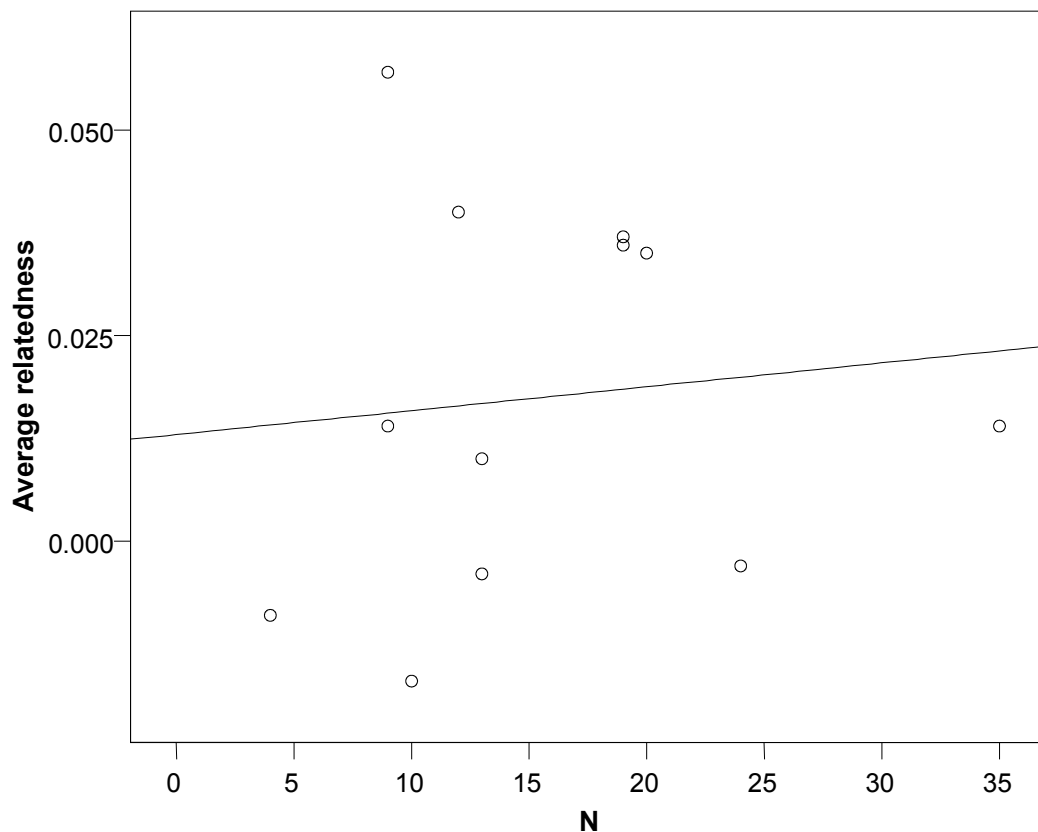


Figure 3. Effect of sample size (N) to average relatedness estimate in all the study leks.

#### 4. DISCUSSION

Males had a stronger genetic structure than females in the study population. This indicates that, in the black grouse, females are the more dispersing sex while males are philopatric or disperse only short distances. This pattern is supported by a radiotracking study in France (Caizergues & Ellison 2002), in this study population (unpublished data) and a previous genetic study (Höglund *et al.* 1999). It might be important for black grouse males to stay in their natal area to acquire and defend a territory during the mating seasons. Black grouse males do not defend resources as Greenwood's (1980) hypothesis predicts for avian males. But if the acquisition of a lek territory is easier in the natal area black grouse



males would still follow the basic idea behind Greenwoods hypothesis: the dispersing sex should be the one with less to lose from dispersing. For females, dispersal could be a way to see and compare more males from which to choose a mate. Besides, dispersal might be an important mechanism to avoid inbreeding. It has been shown that male black grouse with a lower mean heterozygosity also have a lower mating success (Höglund *et al.* 2002). Female black grouse usually mate only once with one male in a breeding season (Lebigre *et al.*, in press). Thus, if a female mates with a relative, her whole clutch may suffer from inbreeding depression. Thus dispersing may be a very efficient way of lowering the chance of having inbred offsprings. For males the risk of mating with a relative may not be as harmful. Males successful in mating probably father other broods as well lowering the disadvantage of some of them being inbred.

The estimates of  $F_{ST}$  found in both males and females may be considered to be low. However, one has to point out that since microsatellite-markers used are not sex-specific (like e.g. mitochondrial markers) they underestimate the amount of differentiation present in males compared to the amount of differentiation that would be given by a sex-linked marker. Every male studied has a mother that probably dispersed a longer distance than his father did. As half of the alleles carried by an individual come from each parent the gene flow caused by dispersing females dilutes the amount of differentiation in males. Also, the spatial scale of this study is relatively small; the average distance between flocks is 25.28 km (s.d. = 12.9) and the longest distance between two flocks is 59.91 km. As a result black grouse can easily fly from a near flock to another; females have been observed to disperse 20 to 30 km (Alatalo, personal communication). Thus, in a bigger scale we would have probably found a stronger structure.

Isolation by distance was found for both males and females. Individuals sampled far from each other were also found to be genetically distant and vice versa. Isolation by distance is a phenomenon caused by limited gene flow in distant subpopulations. Gene flow, in general, has the effect of homogenizing genetic variation between subgroups. If gene flow is restricted changes may start to take place between the subgroups due to genetic drift, selection or mutation (Hedrick 2000). The most important factor causing the divergence between far situated subpopulations is usually the genetic drift, as it is the only factor affecting the whole genome at the same time. Genetic drift is a random change in the allelic frequencies caused by sampling of gametes from generation to generation. The effect of drift is dependent on the effective population size (the number of individuals that actually reproduce). In small populations allelic frequencies can go through remarkable fluctuations due to drift, whereas in large populations only small changes may take place in each generation (Hedrick 2000). The restricted male dispersal and the strong mating skew should enforce the genetic drift in the black grouse. Also, the size of flocks varies: some flocks are small making the effects of drift stronger. Isolation by distance has been found also in other grouse species as for example in the capercaillie (Regnault *et al.* 2006).

As limited dispersal of black grouse males should lead to genetic structuring and related individuals living close to each other it is expected that some males displaying on the same lek are related. That is why it is important to show that males' choice of lek is not random if kin selection is to be tested. In some of the studied leks, the average relatedness differed from zero. But, there was no difference between the average relatedness of lekking males and the relatedness between lekking and non-lekking males. Therefore, males do not seem to prefer displaying with kin. This makes kin selection alone an improbable factor explaining the evolution of lekking in this species. The study by Höglund *et al.* (1999), however, found structuring at the lek level suggesting a high level of relatedness within leks. They interpret most of this structuring to be due to male philopatry but they leave the

possibility of kin selection open. Here we assessed the relatedness values and failed to find a pattern of higher relatedness within lekking males compared to the background level of relatedness in males.

There is a lot of controversy concerning kin selection as a driving force in evolution of lek formation. Some species seem to form kin aggregations at leks while in other species leks appear to be composed of unrelated individuals. However, kin aggregation does not by itself prove kin selection as there may be other factors explaining relatedness within male aggregations. There is only one study this far to show that kin selection may explain male cooperative courtship (Krakauer 2005). Could there be multiple explanations to explain lek aggregation in different species in some species kin selection being a driving force and in others not?

Active choice of lek based on kinship would demand for a mechanism to recognize kin. In many cooperatively breeding bird species kin recognition has been reported but it is based on associative learning, where kin association can be assessed by manipulation of non related individuals (Komdeur & Hatchwell 1999, Hatchwell *et al.* 2001, Komdeur *et al.* 2004, Sharp *et al.* 2005). Learning one's kin through association is only possible in the black grouse for full siblings born in the same nest, since chicks are reared by their mother alone. This is a small representation of all the possible relatives in an area (half and full siblings from the same father and the father himself). Kin recognition would have to be based on non associative learning cues (recognition alleles, phenotype matching) to be effective enough to influence lek evolution. In one study, peacocks lekked with relatives even when they had been raised in mixed family groups indicating a possibility for a non associative learning cue (Petrie *et al.* 1999). The mechanism behind this aggregation, however, is not reported.

The average relatedness calculated within lekking males may be diluted by presence of different families on the same lek. Thus, it would be interesting to test whether the males that are related at leks would cluster close to each other in the black grouse. Also, it may be beneficial for closely related males to form alliances by lekking side by side because then one side of their territories could be left with less attention (Alatalo, personal communication). If there is kin clustering, there should be a negative correlation between the distance of territories and the relatedness of the individuals. However, if there were a significant number of clusters of relatives present in leks it should have shown as an increase in the average relatedness of lekking males compared to background level of relatedness. Even if we would find some kin clustering on leks, it is not sure that low ranking males get considerable fitness benefits. These fitness benefits need to be calculated as well to test whether kin selection may work on leks.

The significant difference in relatedness between “Nyrölä a” and “Nyrölä b” calls for a closer consideration. “Nyrölä b” is a new sublek in this area, situated 200 metres from “Nyrölä a”. There is a road between the leks blocking the view, which probably had an effect on the formation of this sublek. “Nyrölä b” is composed of young males. In the year 2007 this lek disappeared after persisting for three years. Subleks are rare as typically leks have approximately 2 km distance between them. “Nyrölä b” shows the highest degree of relatedness of all the study leks. There may be benefits for young males to form new leks instead of displaying on big aggregations, as “Nyrölä a” is. On this new lek young males may be more successful in alluring females than on a big lek with many older males. On the other hand, why young males do not always establish new leks instead of participating existing leks if it were advantageous to them? One may also ask why did this new lek ceased to exist?

As kin selection does not seem to fully explain lek aggregation in the black grouse alternative hypotheses with direct fitness benefits for young males have to be considered. Several hypotheses have been proposed to explain lek evolution and many of them are not mutually exclusive. In the hot spot hypothesis females are thought to gather in higher densities in areas of high quality. Males are expected to follow females to these areas and this would lead to more males being aggregated in certain areas (Bradbury *et al.* 1986; Höglund & Alatalo 1995). The hotshot-theory predicts that attractive dominant males lure females effectively and the less attractive males would be tempted to come near these males and try to parasitize on the dominant males attractiveness (Beeher & Foster 1988; Höglund & Alatalo 1995). Jiguet and Bretagnolle (2006) showed in their study using decoys that hotshot males exist in little bustard, attracting both males and females. In the delayed benefits hypothesis young subordinate males come to the lek in the hope of future success as the dominant status is transferred to next generations of males (Kokko & Johnstone 1999). Also female preference for larger leks may explain lek aggregations. There is evidence for this in the black grouse (Alatalo *et al.* 1992). Larger leks have more female visits and copulations which lead to higher average male mating success.

Lowered risks of predation in lek aggregations have also been proposed a possible factor behind lek formation (Höglund & Alatalo 1995). In a lek composed of mixed grouse species, Gibson *et al.* (2002) showed that greater prairie chickens join leks of sharp-tailed grouse although this behaviour does not have an effect on the number of conspecific female visits. A likely explanation could be avoidance of predation in a bigger lek. The unpredictability of males' mating success or imperfect female choice may explain male participation as well. If females were to always accurately distinguish best males from lekking aggregations, it would leave subordinate males with no mating opportunities at all. If females make mistakes time to time, however, it would make participation of subordinate males more beneficial (Kokko 1997).

As a conclusion, the dispersal pattern of the black grouse seemed to follow the expected pattern of male philopatry and female dispersal. However, we failed to find any evidence that black grouse males would choose to display on leks where they have relatives. More studies have to be done to find out what factors are behind male aggregation and lek evolution in the black grouse and in other lekking species.

## ACKNOWLEDGEMENTS

I would like to thank my supervisors Christophe Lebigre for the huge amount of trouble he has seen guiding me and commenting my work, and Rauno Alatalo for his comments and ideas. I would also like to thank the laboratory personnel for guidance at the laboratory. Also, my husband Väinö Forss and my friend Mikko Ylilauri have helped me by reading through the thesis and commenting on its clarity and logic.

## REFERENCES

- Alatalo R.V., Höglund J. & Lundberg A. 1991. Lekking in black grouse: a test of male viability. *Nature* 352:155-156
- Alatalo R.V., Höglund J., Lundberg A. & Sutherland W. 1992. Evolution of black grouse leks: female preferences benefit males in larger leks. *Behav Ecol.* 3:53-59
- Alonso J.C., Martin E., Alonso J.A. & Morales M.B. 1998. Proximate and ultimate causes of natal dispersal in the great bustard *Otis tarda*. *Behav Ecol.* 9:243-252
- Aspi J. & Hoffmann A. 1998. Female encounter rates and fighting costs of males are associated with lek size in *Drosophila mycetophaga*. *Behav Ecol Sociobiol.* 42:163-169
- Beebe T. & Rowe G. 2004. An Introduction to Molecular Ecology. 1<sup>st</sup> edition. Oxford University Press. p.102-103, 117, 133

- Beeher B.M. & Foster M.S. 1988. Hotshots, hot spots and female preferences in the organization of lek mating systems. *Am Nat.* 131:203-219
- Bradbury J., Gibson R. & Tsai I.M. 1986. Hotspots and the dispersion of leks. *Anim Behav.* 34:1694-1709
- Caizergues A. & Ellison L. 2002. Natal dispersal and its consequences in Black Grouse *Tetrao tetrix*. *Ibis.* 144:478-487
- Caizergues A., Dubois S., Loiseau A., Mondor G. & Rasplus J.Y. (2001) Isolation and characterization of microsatellite loci in black grouse (*Tetrao tetrix*). *Mol Ecol Notes.* 1, 36-38.
- Charlesworth B. & Charlesworth D. 1999. The genetic basis of inbreeding depression. *Genet. Res., Camb.* 74:329-340
- Clarke A., Sæther B-E. & Røskaft E. 1997. Sex biases in avian dispersal: a reappraisal. *Oikos.* 79:429-438
- Frankham R., Ballou J.D. & Briscoe D.A. 2002. Introduction to conservation genetics. Cambridge University Press. p. 90, 324
- Gibson R.M., Aspbury A.S. & McDaniel L.L. 2002. Active formation of mixed-species grouse leks: a role of predation in lek evolution? *Proc. R. Soc. Lond. B.* 269:2503-2507
- Gibson R., Pires D., Delaney K. & Weyne R. 2005. Microsatellite DNA analysis shows that greater sage grouse leks are not kin groups. *Mol Ecol.* 14:4453-4459
- Goudet J., Perrin N. & Waser P. 2002. Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Mol Ecol.* 11:1103-1114
- Greenwood P. 1980. Mating systems, philopatry and dispersal in birds and mammals. *Anim Behav.* 28:1140-1162
- Greenwood P. & Harvey P. 1982. The natal and breeding dispersal of birds. *Ann. Rev. Ecol. Syst.* 13:1-21
- Hansson B., Bensch S. & Hasselquist D. 2004. Lifetime fitness of short- and long-distance dispersing great reed warblers. *Evolution.* 58:2546-2557
- Hedrick P.W. 2000. Genetics of Populations. 2<sup>nd</sup> edition. Jones et Bartlett Publishers. p. 49, 217, 265, 266, 299, 396
- Hatchwell B.J., Ross D.J., Fowlie M.K. & McGowan A. 2001: Kin discrimination in cooperatively breeding long-tailed tits. *Proc. R. Soc. Lond. B.* 268:885-890
- Hovi M., Alatalo R.V., Höglund J., Lundberg A. & Rintamäki P.T. 1994. Lek centre attracts black grouse females. *Proc. R. Soc. Lond. B.* 258:303-305
- Höglund J. & Alatalo R. 1995. Leks. Princeton University Press. USA p. 20-21, 52
- Höglund J. & Shorey L. 2003. Local genetic structure in a white-bearded manakin population. *Mol Ecol.* 12:2457-2463
- Höglund J., Alatalo R.V. Lundberg A., Rintamäki P.T. & Lindell J. 1999. Microsatellite markers reveal the potential for kin selection on black grouse leks. *Proc. R. Soc. Lond. B.* 266:813-816
- Höglund J., Pieltney S.B., Alatalo R.V., Lindell J., Lundberg A. & Rintamäki P.T. 2002. Inbreeding depression and male fitness in black grouse. *Proc. R. Soc. Lond. B.* 269:711-715
- Jiguet F. & Bretagnolle V. 2006. Manipulating Lek Size and Composition Using Decoys: An Experimental Investigation of Lek Evolution Models. *Am Nat.* 168:758-768
- Keller L.F. & Waller D.M. 2002. Inbreeding effects in wild populations. *Trends Ecol Evol.* 17:230-241
- Kerth G., Mayer F. & Petit E. 2002. Extreme sex-biased dispersal in the communally breeding, nonmigratory Bechstein's bat (*Myotis bechsteinii*). *Mol Ecol.* 11:1491-1498
- Kokko H. 1997. The Lekking Game: Can Female Choice Explain Aggregated Male Displays? *J. theor. Biol.* 187:57-64
- Kokko H. & Lindström J. 1996. Kin selection and the evolution of leks: whose success do young males maximize? *Proc. R. Soc. Lond. B.* 263:919-923
- Kokko H., & Johnstone R.A. 1999. Social queuing in animal societies: a dynamic model of reproductive skew. *Proc. R. Soc. Lond. B.* 266:571-578
- Komdeur J. & Hatchwell B.J. 1999. Kin recognition: function and mechanism in avian societies. *Trends Ecol Evol.* 14:237-241
- Komdeur J., Richardson D.S. & Burke T. 2004. Experimental evidence that kin

- discrimination in the Seychelles warbler is based on association and not on genetic relatedness. *Proc. R. Soc. Lond. B.* 271:963-969
- Krakauer A. 2005. Kin selection and cooperative courtship in wild turkeys. *Nature.* 434:69-72
- Lambin X., Aars J. & Piertney S. 2001. Dispersal, intraspecific competition, kin competition and kin facilitation: a review of the empirical evidence. In: Clobert J., Danchin E., Dhondt A.A. & Nichols J.D. (edited). *Dispersal.* Oxford University Press p.111
- Loiselle B.A., Ryder T.B., Durães R., Tori W., Blake J.G. & Parker P.G. 2006. Kin selection does not explain male aggregation at leks of 4 manakin species. *Behav Ecol.* 18:287-291.
- Perrin N. & Mazalov V. 1999. Dispersal and Inbreeding Avoidance. *Am Nat.* 154:282-292
- Perrin N. & Goudet J. 2001. Inbreeding, kinship, and the evolution of natal dispersal. In: Clobert J., Danchin E., Dhondt A.A. & Nichols J.D. (edited). *Dispersal.* Oxford University Press. Great Britain. p. 127-131, 134
- Petrie M., Krupa A. & Burke T. 1999. Peacocks lek with relatives even in the absence of social and environmental cues. *Nature.* 401:155-157
- Piertney S.B. & Höglund J. 2001. Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). *Mol Ecol Notes.* 1, 303-304.
- Piertney S., MacColl A., Bacon P & Dallas J. 1998. Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Mol Ecol.* 7:1645-1654
- Piertney S.B., MacColl A.D.C., Bacom P.J., Racey P.A., Lambin X. & Dallas J.F. 2000. Matrilineal genetic structure and female-mediated gene flow in red grouse (*Lagopus lagopus scoticus*): an analysis using mitochondrial DNA. *Evolution.* 54:279-289
- Regnault S., Christe P., Chapuisat M. & Fumagalli L. 2006. Genotyping faeces reveals facultative kin association on capercaillie's leks. *Conserv Genet.* 7:665-674
- Segelbacher G., Paxton R.J., Steinbrück G., Trontelj P. & Storch I. (2000) Characterization of microsatellites in capercaillie *Tetrao urogallus* (AVES). *Mol Ecol.* 9:1934-1935.
- Sharp S.P., McGowan A., Wood M.J. & Hatchwell B.J. 2005. Learned kin recognition cues in a social bird. *Nature.* 434:1127-1130
- Shorey L., Piertney S., Stone J. & Höglund J. 2000. Fine-scale genetic structuring on *Manacus manacus* leks. *Nature.* 408:352-353
- Siitari H., Alatalo R.V., Halme P., Buchanan K.L. & Kilpimaa J. 2007. Color signals in the Black Grouse (*Tetrao tetrix*): Signal Properties and Their Condition Dependency. *Am Nat.* 169:S81-S92
- Slatkin M. 1995. A Measure of Population Subdivision Based on Microsatellite Allele Frequencies. *Genetics* 139:457-462
- Stearns S.C. & Hoekstra R.F. 2000. *Evolution: an introduction.* 1<sup>st</sup> edition. Oxford University Press. p. 289
- West S., Pen I. & Griffin A. 2002. Cooperation and Competition Between Relatives. *Science.* 296:72-75