Cristophe Lebigre

Mating Behaviour of the Black Grouse

Genetic Characteristics and Physiological Consequences

JYVÄSKYLÄN YLIOPISTO

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To Christelle,
to my family

ABSTRACT

Leks are clusters of males visited by females for the sole purpose of mating. As males do not provide any parental care, females mostly gain indirect fitness benefits from their mate choice. In the lekking black grouse (*Tetrao tetrix*), previous studies have reported extreme mating biases and a strong female choice which might exert a directional selection on the traits underlying male performance. To understand the maintenance of this mating system, we studied the mating behaviour of black grouse using molecular markers and tested whether male performance was related to several physiological factors. Most females mated with lekking males. There was a strong consistency between observed copulations and true paternity. Most females were seen mating only once with a single male and multiply-sired broods were rare. Hence, lek observations are reliable estimates of male reproductive success and female choice. Black grouse females did not discriminate against relatives during mate choice, but female-biased dispersal might efficiently decrease the likelihood of mating with close relatives. Because females prefer large aggregations, males may gain indirect benefits by settling on leks where relatives are displaying. However, most lekking males were unrelated and did not choose to display with or close to relatives. Therefore, the indirect fitness benefits associated with the lekking behaviour are probably limited. Stress may affect male fitness through the re-allocation of the energy necessary for the stress response from other functions. However, a stress indicator was not related to male activity, mating success or survival. Thus, the measured stress levels did not have strong deleterious effects on male fitness. Lek activity was not related to changes in parasite load or other health measurements. However, the most successful males lost more weight than subordinate males suggesting energetic costs of the lek display.

Keywords: Condition; inbreeding; kin selection; mating system; stress.

Lebigre Christophe, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland

Author's address   Lebigre Christophe
                          Department of Biological and Environmental Science
                          P.O. Box 35
                          FI-40014 University of Jyväskylä
                          Finland
                          lebigre@jyu.fi

Supervisors   Academy Professor Rauno Alatalo
                          Department of Biological and Environmental Science
                          P.O. Box 35
                          FI-40014 University of Jyväskylä
                          Finland

                          Docent Heli Siitari
                          Department of Biological and Environmental Science
                          P.O. Box 35
                          FI-40014 University of Jyväskylä
                          Finland

Reviewers   Academy Professor Juha Merilä
                          Department of Biological and Environmental Science
                          P.O. Box 65
                          FI-00014 University of Helsinki
                          Finland

                          Docent Jouni Aspi
                          Department of Biology
                          P.O. Box 3000
                          FI-90014 University of Oulu
                          Finland

Opponent   Professor Jan Lifjeld
                          Natural History Museum
                          University of Oslo
                          Department of Zoology
                          P.O. Box 1172
                          NO-0318 Oslo
                          Norway

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals. I have been involved in planning, collecting and analysing the data in all manuscripts. I am the main author of all the papers.

I   Lebigre, C., Alatalo, R. V., Siitari, H. & Parri, S. 2007. Restrictive mating by females on black grouse leks. Molecular Ecology 16: 4380-4389.

II Lebigre, C., Alatalo, R. V. & Siitari, H. 2008. Female-biased dispersal alone can reduce the occurrence of inbreeding in black grouse (*Tetrao tetrix*). Manuscript.

III Lebigre, C., Alatalo, R. V., Forss, H. E. & Siitari, H. 2008. Low levels of relatedness on black grouse leks despite male philopatry. Molecular Ecology 17: 4512-4521.

IV Lebigre, C., Alatalo, R. V., Kilpimaa, J., Siitari, H. & Staszewski, V. 2008. Current levels of stress are not related to short-term measures of male fitness in the lekking black grouse. Manuscript.

V   Lebigre, C., Alatalo, R. V. & Siitari, H. 2008. Changes in physiological indicators of condition and male performance in black grouse. Manuscript.

1 INTRODUCTION

1.1 Sexual selection

Why do males of some species express conspicuous ornaments while the expression of such traits might expose them to higher predation risks? Darwin (1871) suggested that a second type of selection could explain sexual dimorphism, a selection not based on environmental or biological factors (natural selection) but on intraspecific competition leading to variation in individual mating success: sexual selection. Females are usually considered to be the limiting sex because their initial investment in reproduction (pregnancy, production of nutrient-rich eggs) might be greater than males' investment (Bateman 1948; Trivers 1972). Therefore, females may maximize their reproductive success by mating with high quality males, while males may increase their fitness by fertilizing many females (Andersson 1994). Although intraspecific competition can occur through male-male competition or female choice, a common practice has been to associate sexual selection with female choice, which might have evolved in two ways. First, a runaway process can explain the female preference for male traits (Fisher 1930). Females may improve their offspring fitness by preferring male traits favoured by natural selection. As the female preference increases in frequency, the attractiveness for the trait increases as well, leading to a positive feedback loop between female preference and male trait that become genetically correlated (Lande 1981). Second, choosy females may use male ornaments as reliable cues of their underlying quality which could be transmitted to their offspring (Zahavi 1977; Grafen 1990). This is because in many species, the preferred males are more vigorous (Jennions et al. 2001) and produce offspring of higher viability (Møller and Alatalo 1999) than non preferred males.

One way or the other, the main consequence of sexual selection is that males with certain traits may gain more matings and/or survive better than others, leading to a larger variance in the reproductive success of males than females. Therefore, it is crucial to determine the importance of indirect selection

on female choice. Lek mating systems provide a good opportunity to study the indirect benefits associated with female mate choice because in these species there is no paternal care of the offspring and only a few males are very successful while most males will never mate (Höglund and Alatalo 1995).

1.2 The evolution of leks

Lekking is a rare, but taxonomically widespread mating system that has probably evolved several times independently (Höglund and Alatalo 1995). The main characteristics of lek-based mating systems are:
  - male clusters on relatively stable arenas to acquire or defend a territory
  - no resources are available on male territories, thus, the females visit the leks with the sole purpose of mating
  - males cannot force copulations, female mate choice is relatively free
  - males do not provide any parental care

The nature of leks is very diverse: male aggregation can range from from classical/clumped to exploded leks where males are only in aural contact, while cooperation can range from no cooperation to the remarkably cooperative display of some manakin species. However, the (presumably) intense direct male-male competition and the (usually) highly skewed male mating success in lekking species suggest that this mating system may be a product of sexual selection (Höglund and Alatalo 1995).

As male lekking behaviour induces some fitness costs (increased competition, lower survival, overall small probability of mating), male aggregations might evolve and be maintained through several direct fitness benefits. First, if there is variation in female density, males might optimize their encounter rate with females by clustering where female density is the highest (hotspot model; Bradbury and Gibson 1983; Bradbury et al. 1986). Second, some males might be more attractive than others. Therefore, subordinates might increase their chances of mating by establishing a territory close to these very attractive males (hotshot model; Beehler and Foster 1988). Third, females might simply prefer clusters of males, making solitary males less successful than males displaying on leks (female preference model; Bradbury 1981). Fourth, males may become successful after several years of lek attendance. Hence, subordinates might have direct fitness benefits later when achieving a higher rank in the hierarchy (delayed benefits model; Kokko and Johnston 1999; McDonald and Potts 1994). Finally, the male clustered display might have evolved in response to non-reproductive factors such as predation (Boyko et al. 2004). If females prefer males displaying on large aggregates, subordinate males (whose prospects of mating are low) can gain indirect fitness benefits. This is because by joining a lek on which relatives are displaying subordinates could increase the number of females visiting the lek and thus enhance the mating success of relatives (kin selection model; Hamilton 1964; Kokko and Lindström 1996).

1.3   The lek paradox and grouse studies

Leks are characterised by a lack of paternal care, and therefore females might gain mostly indirect benefits from their choice. The strong male mating skew suggests that female choice may exert a directional selection on preferred male traits. Hence, there should be a depletion of the genetic variance underlying male traits (Falconer and Mackay 1996) and the genetic benefits associated with female choice might disappear. Yet, directional female choice is still effective, suggesting that females may gain some benefits from their mate choice. This process is called the "lek paradox". Two main possibilities might explain the maintenance of genetic variance in fitness-related traits. First, the optimal phenotype varies in space and time, leading to a fluctuating selection that could prevent the fixation of alleles (e.g. Chaine and Lyon 2008). Second, it is very likely that many genes with small additive effects underlie the elaboration of condition-dependent traits. The genetic variance may be maintained because the large number of loci under selection offers a large mutational target and might be more resilient to the fixation of beneficial alleles (Tomkins et al. 2004). Condition is best seen as a summary of characteristics that reflect the general health and vigour of individuals (Andersson 1994). The concept of condition is difficult to use because it has both phenotypic and genetic components (Merilä 1996). Thus, in the context of sexual selection it is necessary to prove that (i) females use condition traits and (ii) have genetic benefits associated with their mate choice.

The study of black grouse in Central Finland was started in 1987 by Professor Rauno Alatalo with the aim of investigating the lekking behaviour of male black grouse. Black grouse have extreme mating biases and several ornaments that might serve as indicators of male condition (Alatalo et al. 1992; Höglund and Alatalo 1995; Rintamäki et al. 2001). Females prefer to mate with males that display on larger aggregations (Alatalo et al. 1992) and males may optimize the lek upon which they settle according to their own prospects of mating (Alatalo et al. 1992). The choosy females display a "best of *n*" strategy: they visit several males and mate usually only once with one of them (Rintamäki et al. 1995b; Alatalo et al. 1996a). Males cannot force females to copulate, therefore, females can choose their mate from all lekking males (Alatalo et al. 1996a). Female-female aggression is frequent, indicating that females might have some constraints in the timing of mating or mate choice (Karvonen et al. 2000).

The most successful males are dominant, have a high fighting frequency and defend large central territories (Alatalo et al. 1991; Hovi et al. 1994). However, it is unclear whether females prefer males that defend central territories or central territories *per se*: experiments suggest that male aggregates attract females more easily (Hovi et al. 1994), but observations suggest that males cluster around the most attractive males in order to increase their mating opportunities (hotspot model; Rintamäki et al. 1995a). The male territory

position may reflect past investments in reproduction (Kokko et al. 1999), as it takes several years to reach a central position (Kokko et al. 1998) and males have to display every autumn in addition to the mating season (Rintamäki et al. 1999). The successful males are usually three to five years old and are more likely to survive until the next winter (Alatalo et al. unpublished data; Alatalo et al. 1991). This suggests that females may gain good genes to improve the viability of their offspring (Møller and Alatalo 1999). The mating success of the top males in one year is only slightly correlated the mating success the following year. Hence, males do not necessarily retain their dominance status from year to year (Rintamäki et al. 1995a). In addition to lekking behaviour, males also have several morphological ornaments that could be important for female choice: the red eye-combs (Rintamäki et al. 2000), the size of the tail feather (affected by the number of microfilaria parasites; Höglund et al. 1992), or the blue coloration of the breast feathers (Siitari et al. 2007). The alteration of the tail feathers affected the attractiveness of males that displayed on the outside of the lek but not the attractiveness of central males (Höglund et al. 1994). Hence, ornaments may only provide some additional information to females who mostly base their mate choice on territorial and behavioural traits. To stress the importance of male display characteristics, males with higher levels of testosterone displayed on central territories and therefore, had a higher mating success than less central males (Alatalo et al. 1996b).

1.4 Aims

The aims of this thesis were to use genetic markers to understand the mating behaviour of black grouse and to test the relationship between male performance and some physiological factors. We first determined the levels of gene flow over the entire study area. Using a parentage analysis, we then determined whether females really mate on leks and assessed the occurrence of multiply sired broods. We tested whether females actively discriminated against relatives when choosing their mates or whether female-biased dispersal was a sufficient mechanism to decrease the risks of inbreeding. The formation of kin structures can arise either through philopatry or through an active choice of displaying with relatives. Hence, we tested whether these two mechanisms could induce the formation of kin groups on leks as expected by the kin selection hypothesis. In order to understand the factors affecting male performance, we tested whether an indicator of stress could be related to male morphological or behavioural traits. If the stress response is energetically costly, there could be trade-offs between the actual stress level and future male reproductive success or survival. Then, we examined the changes in several physiological parameters that have been suggested to be related to condition. By studying the changes in these measures of condition and relating them to male performance, one could estimate to which extent the lek display is costly.

2 MATERIALS AND METHODS

2.1 Captures and handling

Black grouse form relatively stable winter flocks between November and April, roosting and foraging together. These flocks mainly consist of older males (lekking in the area), and yearling males hatched in the surroundings. Females occasionally join the flocks, coming from their nest sites (where they stay most of the time) or from some other area (dispersal). Black grouse were captured from 2001 onwards at one to twelve study sites. In late December, feeders filled with oat seeds were placed close to known lekking sites. Few weeks later, birds were captured using walk-in traps. Subsequently, trappings were carried out at each site approximately once a week. The feeders were covered with snow and the only seeds available were placed under the traps. All the traps were connected to a single string, which unleashed the traps simultaneously. Thereby, up to 20 grouse could be captured in a single attempt.

After the capture, the birds were kept under the traps covered with a dark cloth to keep them calm. Birds were captured in the spring using the same traps baited with willow catkins, but with an automatic set-up. When entering a trap, the bird released the strings keeping the trap open. Traps were placed a few days after the mating season on the male territories and checked every day subsequently. Each captured bird was first marked with an aluminium ring having a unique number and a combination of colour rings for lek identification. The birds were then weighed (to the nearest 10 g) using a Pesola spring balance (IV, V). The size of sternum, wing, and tarsus was measured with an accuracy of 1.0 mm. The birds were aged as yearling or older according to plumage characteristics: the outermost primaries are not moulted in yearlings (Helminen 1963). A blood sample (1 to 2 ml) was taken from the brachial vein using a heparinised syringe, transferred to an Eppendorf tube and kept at 4°C. A small drop of blood was smeared on two slides for leukocyte counts (IV). Two capillary tubes were filled with blood (75 µl) for the blood parasite counts and the measurement of hematocrit (V). Females were equipped

with radio-transmitters (Holohil or Televilt). Finally, the size of the eye combs was measured as follow: both sides of the head were recorded with a digital video camera in front of a ruler. Pictures from this video were subsequently taken and the size of each eye comb (cm^2) was estimated using the software Image Pro Plus™ (v 4.0). The mean value of the two measures was calculated and used as a measure of the size of the eye combs (IV).

The handling of each bird lasted approximately half an hour. As birds were handled sequentially, some of them might have spent several hours under the traps before they were handled. Captures were stopped in late March-early April because the winter flocks split, males gathering on their display grounds (the lek territories) and females visiting their nest sites.

2.2 Lek observations

The lekking season takes place between mid-April and mid-June. Males gather every morning and every evening to display on their territories. However, the mating season lasts a few days at the end of April and early May during which male activity is very intense. Lek observations were carried out from hides during the most active period of the lekking season (end of April, early May). Markings were placed every 10 m on the leks to facilitate the localization of the males. Male activity was recorded on a regular basis on behavioural maps. The black grouse display consists of a complex male courtship directed towards females and/or other males (Rintamäki et al. 1995a). Male display was categorized as inactive (tail down), rookooing (the most common vocalization), hissing (occasional loud scream), or fighting (with several degrees of fighting from movements without contact to violent interactions) (IV). The centre of the male's territory was estimated using the median of all the X and Y coordinates of the behavioural maps (III). The centre of the lek was obtained by calculating the median of all coordinates from all the males on a given site. It was then possible to estimate the distance between the lek centre and a male's territory (I, IV, V). Male attendance at the lek was estimated from the number of maps drawn with a given male relative to the most present male (who has an attendance of 1) (I, IV, V). Male mating success was estimated from the observed copulations, during which a male would jump on a female's back, conspicuously flapping his wings. Females start laying their eggs soon after the mating season. Their nest sites were localized and the hatching date of the chicks was estimated by floating the eggs in warm water (I).

2.3 Lab procedures

Only the ring number was used when measuring all of the physiological measures. Therefore, the most lab analyses were carried out without knowledge of sampling site, sampling date, or phenotype of the individual measured.

2.3.1 Parasite counts and hematocrit

In the evening, after each trapping day, the capillary tubes were centrifuged at 12,000 rpm for 5 minutes. The extra cellular blood parasites were counted at the interface between the red blood cells and plasma. Using a light microscope (400 times magnification), three parasites were identified: Microfilaria, *Trypanosoma* spp. and *Leukocytozoon* spp. The number of these parasites was estimated by rotating the capillary. All analyses were restricted to Microfilaria (see Höglund et al. 1992) and *Trypanosoma* spp. (see e.g. Rätti et al. 1993), as they were the most common parasites (V). Capillaries were also used to determine the hematocrit levels of the birds by measuring the relative amount of red blood cells in the total volume of blood sampled. This haematological parameter has been widely used as an indicator of either physical activity or anaemia (V).

2.3.2 Leucocyte counts

The blood smears were air dried in the field. In the lab, they were fixed in 99% ethanol and stained using the May-Grunwald-Giemsa method (Ash and Orihel 1987). In total, 100 leukocytes were counted by scanning the smears at 1000 times magnification under a light microscope. The leukocytes were identified as heterophils, lymphocytes, macrophages, thrombocytes, and eosinophiles (IV).

2.3.3 Plasma measurements

The Eppendorf tubes were centrifuged for 5 minutes at 12,000 rpm to separate plasma and red blood cells. The plasma was subsequently transferred into several tubes and kept until analysis at either -20°C or -80°C. To determine the concentration of carotenoids contained in 35 µl of plasma, proteins were first precipitated by adding 200 µl of acetone. Then, the plasma was centrifuged at 16,000 rpm for 5 minutes. Subsequently, the supernatant was transferred to a microcuvette and its absorbance was read using a spectrophotometer (Beckmann DU 640) (see Tella et al. 1998). The measures were carried out at 476 nm and compared to a blank measure of acetone alone. The carotenoid concentration was determined by averaging 5 measures of the same sample (V).

Antibodies or immunoglobulins (Igs) are part of the humoral immune response. More specific than the cell mediated response, immunoglobulins are produced when the host detects foreign material, producing specific antibodies to the antigen. The total concentration of plasma immunoglobulins (IgG) was measured using the ELISA (Enzyme Linked Immuno Assay) method (V). ELISA

plates (Nunc™ Immunoplate) were coated with anti-chicken IgG (Sigma C-6409) and incubated at +4°C overnight. After emptying, the wells were masked with 1% BSA-PBS (Roche diagnostics) for 1 hour and then washed 3 times with 200 µl of PBS-Tween. The samples, their replicates and standards (50 µl/well) were used at the dilution 1:40,000 in 1% BSA-PBS and added to the wells. A mixture of plasma from all individuals measured was used as a standard and given an arbitrary concentration of 10^6. Samples and standards were incubated for 3 hours at room temperature. The plates were washed again (3 times 200 µl with PBS-Tween), and an alkaline phosphatase conjugated antibody (Sigma A-9171 anti chicken IgG diluted with 1% BSA-PBS) was then added to the wells and incubated overnight at +4°C. The wells were then washed 3 times (400 µl with PBS-Tween) and an alkaline phosphatase substrate PNPP (p-nitrophenyl phosphate, Sigma 104® phosphatase substrate) in 1M diethanol amine buffer (1 mg/ml) was distributed in each well (50 µl/well). The absorbance of each well was read after 45 minutes at 405 nm using a spectrophotometer.

2.3.4 Feather colouration

A few breast feathers were cut for colour analyses using a spectroradiometer GS3100 (EG & G Gamma Scientific, San Diego, CA) (IV). The colour spectra were recorded three times per sample over a range of 320 to 700 nm in steps of 5 nm. The estimated spectra were calculated relative to a 99% white reflectance standard (more details in Siitari et al. 2007).

2.3.5 Genotyping

After centrifugation, a part of the red blood cells was kept at 4°C in 70% ethanol. The genomic DNA was extracted from these samples. A subset of birds was tested for 14 microsatellite primers designed for black grouse (BG6, BG10, BG15, BG16, BG18, BG19, BG20, Piertney and Höglund 2001; TTT1, TTT2, TTD2, TTD3, Caizergues et al. 2001) and capercaillie (TUD6, TUT3, TUT4; Segelbacher et al. 2000). However, two loci were removed from the analyses because of linkage disequilibrium (BG20 and TTT2 linked with BG6 and BG18 respectively). One locus was sex-linked (BG10) and was therefore used to confirm the parentage analyses only. Thus, all the birds (hatchlings, adult males and females) included in the analyses were genotyped at 12 loci (11 autosomal and 1 sex-linked loci) (I, II, III). None of the autosomal loci differed significantly from the Hardy-Weinberg expectations within and across flocks (only a slight heterozygote deficit was found at two loci). Polymerase chain reactions (PCR) were carried out in 10 µl reaction volumes containing 2 µL of DNA (average concentration = 38.2 $ng.µl^{-1}$), 0.3 units of DNA polymerase (Biotools, Ref. 10.048), 50 mM KCl, 75 mM Tris HCl (pH 9.0), 20 mM $(NH_4)_2SO_4$, 100 µM of dNTP, 10 pM of primer and from 1 to 2.5 mM of $MgCl_2$ depending on the primer used. After the denaturation of DNA (94°C for 2 minutes), amplifications were done with 30 cycles of denaturation at 94°C for 30 seconds, annealing temperature for 30 seconds, and an extension step at 72°C for 1

minute. An additional elongation step was done at the end of the PCR at 74°C for 10 minutes. The scoring of the alleles was conducted using labelled forward primers provided by Applied BioSystems (dye labels: 6FAM, NED, PET and VIC). The genotypes were analysed using a Prism® 3100 Applied BioSystems sequencer and the software GeneMapper version 3.7.

2.4 Statistical analyses

The genetic variation among study sites and deviation from genetically random mating were tested using Weir and Cockerham's statistics (Theta for F_{ST} and f for F_{IS}) implemented in FSTAT 2.9.3 (Goudet 2001). The effect of the geographical distance on the genetic differentiation between pairs of sites was tested using a Mantel test implemented in Arlequin v. 3.1 (Excoffier et al. 2005). The degree of sex-biased dispersal was estimated by comparing the genetic structure of males and females with the biased dispersal option of FSTAT 2.9.3 (Goudet et al. 2002). The fathers of the hatchlings were identified using the likelihood based method implemented in CERVUS 2.0 (Marshall et al. 1998) for the data collected before 2005 and CERVUS 3.0 afterwards (Kalinowski et al. 2003). The relatedness within groups and between pairs of birds was estimated using the software RELATEDNESS 5.0.8 (Queller & Goodnight 1989). Within leks, we examined whether there were kin clusters by using spatial autocorrelations implemented in GENALEX 6.0 (Peakall & Smouse 2006). The software Poptools v. 2.7 and v. 3.0.3 (Hood 2007) was used to conduct the randomization tests. Finally, all the standard statistical tests were carried out using SPSS 11.5 or 15.0.

3 RESULTS AND DISCUSSION

3.1 Gene flow and dispersal (II, III)

In animals, the only means for gene flow is dispersal (the movement of individuals from their natal area to other areas), and in birds, dispersal is usually female-biased (Greenwood 1980). Males are either philopatric (remain in their natal area) or disperse short distances. Because there is some variation in dispersal propensity and distance, birds form subgroups of varying degrees of relatedness. Consequently, defining a population is not easy in nature because population differentiation ranges from complete isolation to panmixia along a continuum. Estimating the levels of gene flow between study sites is important to determine whether they form a single (genetically) randomly mating unit or whether they should be considered separately.

In 2006, there was a small significant differentiation among nine of the study sites and most pairwise comparisons indicated genetic differences. In the same study population but over a smaller spatial scale, a previous study has reported a larger genetic structure (Höglund et al. 1999). Our results are therefore surprising because by working at a larger spatial scale, the genetic structure should have been larger than the one previously reported. It has already been reported that the genetic structure can vary between years (e.g. Nussey et al. 2005; Piertney et al. 2008). In the late 90's, the number of black grouse was strongly decreasing but stabilized and started to increase again (Ludwig 2007). Thus it is possible that this increase in population size might have affected levels of gene flow, since more individuals could disperse and reproduce successfully, leading to a genetic homogenization. There was a significant positive relationship between the geographic distances among sites and the genetic structure, suggesting that gene flow was stronger between closer sites. Consistent with previous studies reporting female-biased dispersal in black grouse (Höglund et al. 1999; Caizergues and Ellison 2002; Warren and Baines 2002; Marjakangas and Kiviniemi 2005), there was a stronger genetic structure among males than females suggesting that males tend to stay close to

their natal area while the dispersal propensity and distance of females may be higher. Furthermore, within five of the study sites, males were on average more related to each other than females.

There are several definitions for the concept of population (Waples and Gaggiotti 2006), but the classical views emphasize demographic connectivity (ecological paradigm) or reproductive cohesion (evolutionary paradigm). In spite of the strong male mating skew, the sites (separated by a maximum of 60 km) did not form genetically distinct units, and no deviation from random mating could be found. Furthermore, all the flocks have similar demographic changes and are usually pooled within one geographical area in population dynamics studies. Hence, the study sites probably belong to a large continuous population and there are no strong indications that they are evolutionarily distinct. Nevertheless, the asymmetric gene flow associated with sex-biased dispersal might potentially lead to the formation of kin groups in males and decreased inbreeding risks associated with female dispersal.

3.2 Female mating behaviour (I, II)

Because female choice is not constrained by pairing opportunities and the intense male-male competition may allow the direct assessment of male relative quality, it has long been thought that polyandry might be rare in lekking species (Birkhead and Møller 1992; Höglund and Alatalo 1995). However, polyandry occurs in all lekking species studied and might even to be common in some species (cock-of-the-rock *Rupicola rupicola*, Trail 1985; peafowl *Pavo cristatus*, Petrie et al. 1992; great snipe *Gallinago media*, Fiske and Kålås 1995; buff-breasted sandpiper *Tryngites subruficollis*, Lanctot et al. 1997; ruff *Philomachus pugnax*, Lank et al. 2002; sage grouse *Centrocercus urophasianus*, Semple et al. 2001 but see Gibson et al. 1991). In black grouse, lek observations suggest that most females usually mate once with a single male, while repeated and multiple matings are rare (Alatalo et al. 1996a). However, the occurrence of polyandry might be underestimated if females mate on several leks or outside the leks (e.g. Lanctot et al. 1997). This uncertainty in female mating behaviour may in turn affect estimates of male mating success and female mate choice.

Using a parentage analysis, we determined that most broods were sired by males that displayed on leks. Furthermore, almost all males seen mounting females sired the entire broods even when neighbouring males tried to disturb the copulation. Furthermore, multiply sired-broods were very rare. This single copulation was sufficient to fertilize the whole clutch, as the fertilization of the eggs was close to 99%. A few females were observed mating with multiple males, but the resulting broods were not necessarily sired by several males, suggesting that a few copulations were not successful and females had to mate several times. Hence, black grouse females might be able to ascertain whether sperm has been transferred or it might be that males and females have a very high fertility. This remarkable mating behaviour might either be due to high

costs of multiple mating (increased risks of sexually transmitted disease or predation) or simply females do not need to copulate several times if one insemination is sufficient to fertilize the eggs and if the best male is available. It has been previously shown that most of the variation in the rate of multiple mating occurs among families or orders but not among closely related species (Griffith et al. 2002). Therefore, if polyandry is rare in grouse *Tetraoninae*, polyandry might be rare in lekking grouse (a similar reasoning might explain why lekking waders have relatively high rates of multiple paternity) (Griffith et al. 2002).

One potential benefit of polyandry might be to reduce inbreeding if females are paired with related males (e.g. Foerster et al. 2003). Inbred individuals have usually reduced fitness (inbreeding depression; Crnokrak and Roff 1999; Keller and Waller 2002). These fitness costs might have induced the evolution of inbreeding avoidance mechanisms if the costs of tolerating inbreeding are higher than the costs of avoiding inbreeding. It is usually suggested that inbreeding risks can be minimized either through kin discrimination or sex-biased dispersal. In black grouse, it has previously been suggested that inbreeding might affect some components of male fitness (Höglund et al. 2002). Furthermore, characteristics of this species might expose individuals to high risks of inbreeding. Very successful males are usually more viable (Alatalo et al. 1991). Hence, the pool of potential mates might be comprised of a female's close relatives. As females usually copulate with only one male, the entire brood might suffer from inbreeding depression.

The relatedness between mates was low and not significantly different from zero. Females did not seem to actively avoid mating with relatives. As a consequence, any increase in the mean relatedness of the females to the males tended to lead to an increase in the occurrence of inbreeding. In birds, two mechanisms of kin recognition have been described: associative learning and phenotype matching (Komdeur and Hatchwell 1999; Hauber and Sherman 2001). Associative learning in black grouse is only possible for chicks within a brood. As only females rear the brood, young females will only be familiarized with their brothers, but not necessarily with their fathers and half brothers. Therefore, this mechanism of kin recognition is unlikely in our study species. On the other hand, phenotype matching is possible, with heritable traits such as black spots of the white tail feathers (Alatalo et al. unpublished data). However, even if females were able to discriminate against kin, they do not seem to do so. Hence female-biased dispersal might be a more likely means of reducing the risks of inbreeding. Using females with known parents hatched in the vicinity of the study sites, we examined what would happen the following year if they would have joined their parental flock (philopatry), or other studied flocks (dispersal). We estimated that philopatry would expose females to higher risks of mating with relatives. The observed breeding females had lower relatedness to males than "philopatric" hatchlings, suggesting that dispersal alone might explain the observed patterns of relatedness between mates. Dispersal has probably evolved in response to several factors such as kin or resource competition, and spatial or temporal variability (Clobert et al. 2001). Due to

these reasons, dispersal has not evolved solely as a means to avoid inbreeding. However, the decrease in the risk of inbreeding is an important additional benefit that should not be neglected when assessing the benefits associated to dispersal.

3.3   Male settlement decisions (III)

Within each lek, a limited number of males (one or few) attract all of the females, while many males have no matings at all. Due to this mating skew, lekking males have different fitness payoffs (Höglund and Alatalo 1995). Therefore, understanding of the maintenance of male aggregates requires the estimation of the benefits associated with male behaviour. One possible explanation is that subordinate males could gain some indirect fitness benefits (Kokko and Lindström 1996). If females prefer large aggregations, males that join a lek on which relatives are displaying might attract more females to the lek. Thereby, subordinates may increase the mating opportunities of their kin (Petrie et al. 1999; Höglund et al. 1999). The formation of these kin groups might arise either passively if males are philopatric, or actively if there are kin recognition mechanisms. In black grouse, both female preference for large aggregations and male philopatry have been reported (Alatalo et al. 1992; Hovi et al. 1994; Caizergues and Ellison 2002). Furthermore, a genetic structure at the lek level has been previously reported which suggests that lekking males could be related (Höglund et al. 1999).

Although there was a genetic heterogeneity among leks due to male philopatry, the mean relatedness of the males displaying on each lek was not significantly different from zero. Many unrelated and a few related males were displaying on the same lek. This result is probably due to two main reasons. Firstly, the very successful males are more viable but do not necessarily retain their dominance status, since male mating success from one year to another is only weakly correlated (Rintamäki et al. 1995a). If the successful males are unrelated, this rapid turnover might explain the low observed average relatedness on the leks. Secondly, males might join a flock or display with unrelated birds if males disperse even short distances (Caizergues and Ellison 2002), or if the nest sites of the females are far from the leks where they mated. These results indicate that black grouse leks are not kin groups and the indirect fitness benefits associated with male lekking behaviour are probably limited. In black grouse, subordinate males tended to cluster around very attractive males (hotshot model; Rintamäki et al. 1995a) and females were attracted by clusters of males (female preference model; Hovi et al. 1994). Furthermore, it has also been suggested that females prefer male traits associated with lifetime performance (Kokko et al. 1999). Therefore, if males are to maximize their current and future reproductive success, they have no choice but to join a lek.

It has been suggested that males could have direct benefits when lekking close to relatives, if dominant males facilitate the establishment of relatives in

neighbouring territories by being less aggressive towards them than towards unrelated neighbours (Sæther 2002). However, males did not choose to actively display with and close to relatives, making this process very unlikely in black grouse. Male territory position is partly determined by previous mating success: successful males normally stay on their territories, unsuccessful males shift to a new spot and males that have just joined the lek defend territories far from the lek centre (Kokko et al. 1998). This inertia might therefore prevent dominant males from favouring kin establishment.

3.4 Physiological consequences of male performance

Organisms need a relatively steady supply of energy to sustain physiological functions. Because of limited resource reserves, the evolution of costly behavioural and morphological ornaments is subject to trade-offs with other life history traits (Stearns 1992). Males of better quality might have lower costs associated with the elaboration and maintenance of these condition-dependent traits. Therefore, females might use the expression of male traits to determine their underlying heritable quality (Zahavi 1977; Grafen 1990). In black grouse, the most successful males are three to five years old, and in yearling males, dominance is negatively related to lifetime mating success due to a shorter lifespan (Alatalo et al. unpublished manuscript). We tested whether male performance was related to differences in their stress levels (IV) and to changes in several physiological parameters that may reflect current health and vigour (V).

3.4.1 Male lekking performance

Black grouse males have to express a variety of morphological and behavioural traits that are important for female choice. The results of a principal component analysis (PCA) with seven traits (male body mass, territory centrality, lek attendance, fighting frequency, length of the tail feathers, blue colouration of the breast feathers and size of the eye-combs) indicated that morphological ornaments (independent of body mass) and behavioural traits were partly independent (IV). Only the behavioural traits were clearly related to male mating success. It has already been shown that male ornaments are condition dependent and related to male mating success (tail feather, Höglund et al. 1992; blue colouration, Siitari et al. 2007; eye comb size, Rintamäki et al. 2000). However, it seems that the effect of the size of the ornaments (independent of male body mass) on male mating success is limited compared to the lekking display (Fiske et al. 1998). This analysis was carried out only among older males and the morphological traits of yearling males differed in almost every respect. Females normally avoid mating with yearling males. Thus, male ornaments could provide reliable cues of male age so that females could discriminate against very active yearling males of uncertain quality.

3.4.2 Stress and male performance (IV)

Under adverse conditions, organisms utilise various behavioural and physiological means to reduce the deleterious effects of a stressor. This process (stress response) could affect individual fitness directly through changes in food intake and indirectly through the re-allocation of energy resources from other functions (Greenberg et al. 2002), or the immunosuppressive effects of stress on the expression of secondary sex traits (Apanius 1998; Råberg et al. 1998). The stress indicator used here (the heterophil/lymphocyte ratio) adequately measured both acute and chronic stress as it increased within a few hours during the handling of the birds (acute stress) and before the mating season (more chronic stress). However, there was no relationship between this stress indicator and male lekking performance, mating success or survival. Dominant and subordinate males had similar levels of stress, either because male-male competition did not induce an increased stress response or because we might not be able to detect differences in stress levels. It has been previously suggested that when fighting, males have increased stress levels. But after fighting, subordinates have a sustained level of stress whereas dominant males have a rapid decrease in their stress levels (Summers 2002). As dominant males have a higher fighting frequency (Alatalo et al. 1991), we might not observe any relationship between male dominance and their stress levels.

3.4.3 Changes in physiological indicators of condition (V)

Here we used the first axis of a PCA with male mating success, territory centrality and lek attendance to estimate male lekking performance. Males captured a few days after the mating season had a significantly lower body mass compared to winter measures. As *Tetraoninae* do not rely on accumulated fat reserves (e.g. Delahay and Moss 1996), changes in body mass probably indicated changes in hydration or in the protein content of tissues. The most active and successful males lost more weight than other males, which suggested that there was an increased energy expenditure due to the lekking display. These do not represent evolutionary costs because males that have a better underlying quality may be more likely to survive until the next winter (Alatalo et al. 1991). However, some males may not be able to cope with decreased body mass and might therefore adjust their lek display according to their current condition.

Over the same period, there was a significant increase in parasite load. These changes were probably due to changes in the condition of their hosts associated with the mating season as well as the increased presence of their vector (Altizer et al. 2006). The changes in parasite load were not related to male lekking performance, which indicated that dominant and subordinate males had a similar parasite load. Black grouse males with a large number of microfilaria larvae had shorter tail feathers and duller blue colouration of breast feathers, suggesting some condition dependence (Höglund et al. 1992; Siitari et al. unpublished data). Hence, parasites may affect other components of male

condition during the moult (taking place a few weeks after the mating season) and might be critical for their mating success in the following year.

In spite of the increased parasite number and prevalence, there were no changes in total plasma immunoglobulin concentrations between winter and spring captures. Three explanations are possible. First, the humoral immune response might not be the most efficient against the parasites studied here. Second, the target of the antibodies (but not their total concentration) may have differed between the two captures. As we did not measure different antibody subtypes or their specificity, parasites or disease may have induced the production of antibodies that were not able to identify. Alternatively, the birds may simply not be able to produce an immune response if there are high costs associated with the lekking display (Zuk & Stoehr 2002).

Carotenoids are pigments acquired by animals only through their diet. The carotenoids are widely used measurements because of their limited availability and their allocation to competing functions such as the expression of sexual coloration (e.g. in feathers of bright birds; Hill and McGraw 2006) and in immune function (Møller *et al.* 2000; Blount *et al.* 2003; Faivre *et al.* 2003; McGraw and Ardia 2003). The carotenoid concentration increased significantly between winter and spring captures very probably due to changes in the quality and diversity of food available. There was no relationship between changes in carotenoids, plasma immunoglobulins or parasite load. This suggests that carotenoids may affect other components of the immune system that are not related to antibody production or resistance against the two parasites considered in this study. Furthermore, there was no difference between the dominant and subordinate males in carotenoid changes or concentration. Lekking black grouse males form small flocks that forage and roost together. Therefore, there may be no difference in the quantity and quality of food intake between the males.

4 CONCLUSIONS

In spite of the directional selection exerted by females on the traits underlying male performance, female choice towards a few dominant males still prevails suggesting that females may still gain indirect (genetic) benefits from their mate choice. But what are the factors explaining the maintenance of this mating system?

The genetic variance is not increased through polyandry because of its rare occurrence. Therefore, the observed male mating skew estimated from lek observations accurately reflects the variation in male reproductive success. Because the most successful males are more viable, inbreeding might be frequent if there is no inbreeding avoidance behaviour. If most matings occurred between unrelated birds, it was only due to female-biased dispersal which associated with male (partial) philopatry might have effectively reduced the risks of inbreeding. Females did not actively discriminate against relatives during their mate choice. Hence, female mating behaviour is consistent with the idea that female choice exerts a directional selection on some male traits.

Males might gain mostly direct benefits from their mating behaviour: the increase of their current and future mating chances. A few relatives and many unrelated males were displaying on the same lek. Therefore, only a few males might gain some indirect fitness benefits by aggregating on the lek with relatives. The most dominant and successful males were not more stressed than subordinates. However, the lek display induced increased energetic expenditure as very active males lost more weight. Hence, male body condition might be a major factor underlying male lekking performance. The use of condition-dependent signals by females may allow a slower depletion of genetic variance. Yet, it is now necessary to estimate simultaneously all direct and indirect male fitness payoffs over their lifetime. Furthermore, it remains unclear whether female choice is directly based on condition-dependent traits, or whether within a cohort there is a disappearance of poor condition individuals over time. Only a longitudinal study following individuals throughout their lifetime could estimate accurately the current and future fitness payoffs associated with male and female mating behaviours.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Teeren pariutumiskäyttäytyminen: geneettiset tekijät ja fysiologiset seuraukset

Soidin on pariutumisjärjestelmä, jossa koiraat kokoontuvat vakituisille, toisiaan lähellä oleville reviireilleen, jotka yhdessä muodostavat soitimen. Soitimelle on tyypillistä, että naaraat vierailevat soitimella tarkoituksenaan ainoastaan pariutuminen tietyn koiraan kanssa. Lisäksi koiras ei puolusta reviirillään mitään resursseja (kuten esimerkiksi ruokaa). Naaraan valinta on vapaa, joten koiraat eivät voi pakottaa naarasta parittelemaan kanssaan, ja naaras huolehtii jälkeläishoidosta yksin. Täten naaraat saavat valinnastaan ainoastaan epäsuoria, geneettisiä, hyötyjä. Aikaisemmat tutkimukset teerellä (*Tetrao tetrix*) ovat osoittaneet suuria eroja koiraiden pariutumismenestyksessä ja voimakasta naaraiden valikoivuutta koiraiden suhteen. Tämän tyyppinen tiettyjen koiraiden suosiminen eli vinoutunut valintapaine saattaa johtaa tiettyjen ominaisuuksien geneettisen monimuotoisuuden pienenemiseen soitimella. Tämän vuoksi ymmärtääkseni, miten soidin pariutumisjärjestelmänä on säilynyt aikojen kuluessa, käytin soidinten tarkkailujen lisäksi molekyyligeneettisiä menetelmiä tutkiakseni teeren pariutumiskäyttäytymistä (poikueiden isyyksiä, talviparven ja soitimella esiintyvien yksilöiden sukulaisuussuhteita). Lisäksi tutkin fysiologisin menetelmin soidinkäyttäytymisen kustannuksia koiraille.

Poikueiden isyysanalyysissä paljastui, että naaraat valikoivat poikueidensa isiksi koiraita, joilla oli soitimella oma reviiri. Naaraat parittelivat koiraan kanssa vain yhden kerran, ja yksi koiras hedelmöitti yleensä kaikki pesyeen poikaset. Täten soitimen aikana tehdyt paritteluhavainnot olivat yhteneviä geneettisin menetelmin saatujen tulosten kanssa. Geneettiset menetelmät toki paljastivat enemmän isyyksiä kuin pelkät näköhavainnot, sillä osa paritteluista jäi soitimen aikana havaitsematta. Teerinaaraat eivät varsinaisesti välttäneet pariutumasta sukulaiskoiraan kanssa, mutta tällä lajilla, kuten yleisesti lintulajeilla esiintyvä naaraspainotteinen levittäytyminen voi olla tehokas keino välttää pariutumista sukulaiskoiraan kanssa. Koiraat sen sijaan ovat filopatrisia, eli koirasjälkeläiset jäävät synnyinseuduilleen loppuiäkseen, eivätkä levittäydy ainakaan kauas synnyinseuduiltaan.

Koska teerinaaraat ovat mieltyneitä soitimen koirasryppäisiin, koiraat voivat saavuttaa epäsuoria hyötyjä asettautumalla niille soitimille, joilla heidän omat sukulaisensa ovat esiintymässä. Tutkimukseni kuitenkin osoitti, että suurin osa soitimella esiintyvistä koiraista ei ollut läheisiä sukulaisia keskenään. Tutkittaessa soitimen sisäistä rakennetta havaittiin, etteivät koiraat valikoineet paikkaansa läheltä sukulaisiaan. Tästä päättelin, että koiraiden filopatria ei ole tarpeeksi voimakasta johtaakseen (läheisiin) sukulaisrakenteisiin soitimen

evoluutiossa. Sen sijaan soitimella vuosittain aktiivisesti esiintyvät koiraat parantavat omaa senhetkistä ja tulevaa pariutumismahdollisuuttaan.

Ymmärtääkseni koiraan soidinkäyttäytymistä pariutumisaikana, tutkin stressin ja eräiden fysiologisten muuttujien vaikutusta soidinkäyttäytymiseen. Vaste stressiin voi olla energeettinen kustannus koiraalle, jolloin voi muodostua kompromissitilanteita stressivasteen, lisääntymismenestyksen ja henkiinjäämisen välille. Kuitenkaan käyttämäni stressi-indikaattori, valkosolujen heterofiili-lymfosyytti–suhde, ei ollut suhteessa koiraan aktiivisuuteen soitimen aikana eikä koiraan pariutumismenetykseen tai hengissä säilymiseen. Koiraiden loismäärät kasvoivat kevään kuluessa, mutta tämä nousu, kuten muutokset muissakaan käytetyissä terveysmittareissa (mm. plasman karotenoidipitoisuus, kokonaisvasta-aineiden määrät) eivät olleet suhteessa koiraan lisääntymismenestykseen. Parhaiten menestyneet koiraat menettivät sen sijaan eniten painoa kevään kuluessa. Teeri ei monien muiden lajien tapaan, kerää rasvavarastoja kehoonsa. Täten painon lasku on suoraan pois koiraan lihasmassasta tai nestetasapainosta, josta taas voi olla seurauksia soitimen jälkeen, jolloin koiraiden hengissäsäilyminen on riskialteinta, ja niiden on kestettävä sulkasadon johtuvat energeettiset kustannukset.

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