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Genetic studies of black grouse with special reference to conservation biology: a review

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A b s t r a c t . In this paper I review genetic studies of black grouse to date. The topics cover different areas such as reproductive biology, mating system and, more recently, conservation genetics. The accumulated evidence shows that in the western part of the range of the species, populations are genetically differentiated. Furthermore, small and isolated populations have lost genetic variation due to increased levels of inbreeding and genetic drift. So far the vast majority of studies have been based on microsatellites. More recent investigations have employed sequence data as well as methods to study quantitative trait variation. These latter studies may aid in resolving the issue of whether local populations show any evidence of being adapted to local conditions. This is an important consideration in conservation biology since it determines the extent to which populations are interchangeable and which populations should be used for restocking if such are considered of value.

Key words: genetic variation, allozymes, microsatellites, mtDNA, population fragmentation

Introduction

Black grouse populations in the western part of the species the range have become fragmented and reduced in numbers (S t o r c h 2000). Within the last decades there are at least two documented cases of extinction (H a n c o c k et al. 1999, H o l s t - J ø r g e n s e n 2000) and several still extant populations are so small and reduced in numbers that their persistence is severely threatened. In order to reverse the trend, it is important to study factors that may contribute to local extinction and to suggest countermeasures that may ensure the species survival both locally and globally. Population genetic considerations are among several factors that may contribute to prevent the extinction of species (F r a n k h a m et al. 2002).

Apart from one pioneering allozyme study (S c h r e i b e r et al. 1998), black grouse (*Tetrao tetrix*) have not until recently been subjected to population genetic investigations. This is in contrast to other grouse species. For example, extensive population genetic analyses of Scandinavian willow grouse (*Lagopus lagopus*) populations were conducted in the 1980s and 1990s using allozymes (G y l l e n s t e n 1985, G y l l e n s t e n et al. 1985, R ø r v i k 1989, R ø r v i k & S t e e n 1989, R ø r v i k et al. 1990). These studies suggested that Scandinavian willow grouse harboured substantial genetic variation and that effective population sizes were large. Furthermore, within Scandinavia willow grouse showed rather little (if any) population differentiation.

The social organisation of grouse varies substantially (J o h n s g a a r d 1983, H ö g l u n d & A l a t a l o 1995), ranging from almost monogamous pair bonds or low

levels of polygamy, such as in willow grouse, to more extreme forms of polygamy in the lek mating system of capercaillie (*Tetrao urogallus*) and black grouse (Höglund & Alatalo 1995). The level of polygamy has effects on the population genetic effective population size, N_e, via the skew in the operational sex ratio potentially making N_e much smaller in lekking taxa. Furthermore, molecular genetic studies of grouse have revealed their phylogenetic relationships and suggest that willow grouse and black grouse have been separated for a relatively long period of time (in the order of a million years) (Drovetski 2002, Dimcheff et al. 2002, Lucchini et al. 2001). For these reasons the population genetic structure and levels of genetic diversity in black grouse may be expected to be different from willow grouse.

Population genetic structure and diversity may impact on population persistence. First, sub-structured populations with a patchy distribution may be more prone to both local and global extinction since low population size is a major determinant of extinction risk. Furthermore, the levels of genetic drift (the stochastic loss of genetic variation) and inbreeding are both processes known to increase in strength in small populations. Therefore, genetic drift and inbreeding may be predicted to be more severe threats of extinction in smaller populations.

In this review I address the following issues: (1) what is genetic variation and why is it relevant to study such in black grouse? (2) the difference between so-called adaptive genetic variation and neutral genetic variation, (3) the patterns of genetic variation published using neutral genetic markers and (4) variation in quantitative traits and in immune defence (MHC) loci.

The relevance of genetic variation studies

Population genetic structure is predicted to affect population persistence and long-term survival where small and isolated populations run a higher risk of extinction (Frankham et al. 2002). The stochastic process of genetic drift is inversely related to effective population size (N_e) and becomes a strong force when local population size drops to tens of individuals. In small populations, chance alone can make slightly deleterious alleles increase in frequency at the expense of well adapted alleles. Small and isolated populations are also subjected to increased levels of inbreeding. Increased levels of inbreeding often lead to inbreeding depression i.e. reduced survival and/or fecundity. Both drift and inbreeding thus negatively affect individual reproductive success, reducing populations even more and trapping them in a downward spiral towards extinction, a so-called "extinction vortex" (Loeschcke et al. 1994). Populations that have survived a bottleneck but lost genetic variation are furthermore less likely to be able to adapt to future changing selection pressures since adaptability is dependent on standing levels of variation and small populations may thus be more extinction prone (Soulé 1976, Lande 1988, Frankham 1996, Frankham & Kingsolver 2004). These effects may be long lasting since while populations may recover from population size bottlenecks in terms of numbers quickly, the regain of genetic variation takes longer, being directly dependent on the mutation rate (which is an exceedingly slow process) and gene flow between sub-populations (Frankel 1974, Lande & Shannon 1996, Frankham et al. 1999).

We have thus concluded: natural populations of higher diploid organisms are genetically variable, i.e. no two individuals are genetically alike (except for monozygotic twins). The smaller and more inbred the population, the more genetically similar are the individuals in

the populations. In order to track changes in the environment selection need genetic variants to choose from. If everyone is similar, the less the chance that any particular variant can meet a future challenge.

The only evolutionary mechanism that may create new genetic variation is mutation and recombination; the other forces either reduce (genetic drift and purifying selection) or maintain already existing variation (balancing selection and migration). It is believed that most mutations have a negative effect on individual fitness of the carrier and therefore are quickly eliminated from the population via purifying selection. However, mutations that do not affect fitness or which only slightly impedes it may persist. In the rare cases when mutations have a positive effect on fitness, they will increase in frequency. It follows that most of the genetic changes that can be tracked and observed in populations are either neutral, or nearly so, or positive with respect to fitness. Genetic changes that do not alter the phenotype of the individual, and therefore are inaccessible to selection (they are off course still affected by drift), are called neutral. The genetic changes that alter the phenotype and give rise to fitness differences are referred to as non-neutral.

In natural populations much of the standing genetic variation is neutral in the sense that the allelic variants in the population have almost identical fitness. This does not mean that any particular allele may always remain neutral. When circumstances change, a neutral allele may become favoured (or disfavoured). This is one reason that variable populations are believed to be more resilient to future changes. It is like keeping a box of various bolts and nuts in one's home. The more variable the assortment of bolts and nuts, the more future problems the house keeper is able to solve.

There are of course many ways to assay and measure genetic variation and partly the various measures depend on the kind of genetic marker used in the study. The most common measures with discrete allelic data are: various measures of heterozygosity (i.e. the proportion of bi-allelic individuals); and number of alleles (genetic variants) observed at a gene (locus). This last measure is often corrected for sample size differences among samples by rarefaction to the smallest sample size (Petit et al. 1998) and then called allelic richness. In the case that sequence data, i.e. the nucleic acid sequence at the stretch of DNA coding for an allele, is available, two more measures are commonly used. These are: nucleotide diversity π),which is the average number of nucleotide differences per site between any two randomly chosen sequences from a sample population (Nei & Li 1979, Nei 1987) and haplotype diversity which is defined as $1-\sum f_i^2$ where f_i is the frequency of the i:th haplotype. Haplotype diversity corresponds to the expected heterozygosity for a discrete codominant marker. Haplotypes are sets of closely linked nucleotides present on a chromosome that are inherited together and thus inherited as a single linkage group. With quantitative characters such as morphometric traits or life-history traits, the common statistical measures of the mean, variance, standard deviation, standard error and the coefficient of variation are available.

DNA fingerprinting and absence of multiple paternity

On of the first study to employ molecular genetic studies of black grouse was a study of paternity in black grouse clutches using DNA-fingerprinting (Alatalo et al. 1996), the most advanced method available at the time. Before molecular methods entered the fields of ecology and animal behaviour, researchers had to rely on field observations to score the reproductive success of individuals. Thus studies of the reproductive success on leks involved

individual markings and careful observations of the mating success of the males participating. From these observations it was suspected that female black grouse mated only once per year since they were rarely observed on the lek after having been seen copulating. This is peculiar since in lekking birds other than grouse, multiple mating and multiple paternity is common (Petrie et al. 1992, Höglund & Alatalo 1995, Lanctot et al. 1997).

By using DNA-fingerprinting, a technique involving digestion of the DNA with restriction enzymes and probing with a specific radioactive DNA probe, a specific band pattern of the fragments could be visualised. This band pattern is highly specific to an individual but each band on the gel, corresponding to a chunk of DNA of a specific length, is inherited from either the mother or the father. Thus related individuals share more bands than unrelated and the paternity of offspring of a known mother can be assigned to a male if he is within the sample of putative fathers.

Blood samples were taken from all males at the lek and from females visiting the lek. The females were also fitted with a radio transmitter so that later in the season when their clutches had just hatched, the female and the clutch could be located and the chicks could be blood sampled. The results showed that indeed among almost all sampled females, the father of the clutch was the male that she was observed copulating with at the lek. There was one case when the first copulation the female attempted was interrupted which was ambiguous and one female for which the observations were unreliable. All in all, however, the genetic analyses confirmed that in the vast majority of cases black grouse females mate only once per season and the mating takes place at the lek (Alatalo et al. 1996).

The above results have later been confirmed with microsatellite markers (see below) and a much larger data set (Lebigre et al. 2007). In the mating season of 2001–2005, 135 broods were sampled in a study area slightly larger than the previous one. In about 10% of the matings the female seemed to have mated with a male that was displaying solitarily. The other hens had mated on a lek. Like in the previous study, there was a strong consistency between the observations of which male a female had copulated with and true paternity, even when the copulation was disturbed by a neighbouring male. Multiple matings and multiple paternities were rare. These results imply that black grouse, and other grouse species (see e.g. Semple et al. 2001), are unique among birds in having evolved specific adaptations to ensure fertilisation with a single copulation. This is presumably an adaptation for females to control with whom she mates.

Allozyme and microsatellite variation within regions and leks

A population genetic study using allozyme variability at 38 loci detected slight but significant population structure among black grouse from 4 locations (Netherlands, Bavaria and two sites in Sweden, respectively) (Schreiber et al. 1998). However, polymorphisms could only be found for 3 of the loci and inferred levels of genetic variation were low (proportion of polymorphic loci was 0.08). It is unclear why the genetic variability was so low in this study but both biological reasons such a low N_e due to the mating system of the species and methodological reasons are possible.

Since allozyme studies yielded such low variability estimates, future studies have used more variable microsatellite loci. Microsatellites are short stretches of repetitive DNA-sequences scattered throughout genomes. These repetitive sequences (e.g. AT) may be repeated up to a hundred times but most commonly the length variation is in the order

of 10–20 allelic variants at a any locus. These DNA-sequences evolve via a peculiar evolutionary process. When the endogenous DNA-polymerase of the cell copies the microsatellite sequence during replication, the polymerase may sometimes make an error and either mis-incorporate or mistakenly remove a copy (so-called DNA-slippage). This is referred to as the step-wise mutation model. This mutational process is much more likely to happen than many other types of mutation (in the order of 10^{-3} instead of 10^{-6}/genome and generation). Therefore microsatellite loci often harbour a lot of genetic variation within populations. Microsatellites are often found upstream (i.e. before) protein coding genes and thus may have a regulatory function. However, most microsatellite loci used in population studies are neutral, meaning that the different alleles do not affect the fitness of the organism in any measurable way.

Microsatellites are found by cutting up the genomic DNA from the study species with restriction enzymes and ligating this DNA into bacteria use a phagemid vector. This so-called genomic library is then probed with a synthetic oligonucleotide mirroring a repeat sequence. The bacterial clones are allowed to grow and clones with positive inserts are sequenced. This will allow detection of not only the repeat sequence but also flanking regions around the repeat. The next step is to design so-called PCR-primers in the flanking regions close to the repeat sequence. With the aid of the primers, the target DNA can be manifold in a polymerase chain reaction (PCR) to screen allelic length variation in a large number of individuals.

So far two black grouse microsatellite libraries have been published (Caizergues et al. 2001, Piertney & Höglund 2001). Furthermore, a number of microsatellite loci cloned in the closely related capercaillie (*Tetrao urogallus*, Segelbacher et al. 2000) and the a bit more distantly related red grouse *(L. l. scoticus)* (Piertney & Dallas 1997) also work in black grouse. Additionally, some, but far from all, microsatellite loci originally developed in chicken (*Gallus gallus*) may yield amplification products in black grouse (Höglund et al. in prep.). Depending on quality needs and laboratory ease, the number of microsatellite loci working in black grouse is 10–30.

The first studies of microsatellite genetic variation in black grouse used microsatellites developed in red grouse. Of the ten primer pairs developed by Piertney & Dallas (1997) only two turned out to amplify reliable products in black grouse. It is well known that both amplification probability and variability at any given locus fall off in a negative exponential manner with phylogenetic distance when loci are cross amplified in a taxon in which it was not developed (Primmer et al. 2005). Thus the poor performance of the ten red grouse loci is not unexpected.

Nevertheless this initial study revealed that black grouse in an area approximately 50 x 50 km in central Finland showed signs of being geographically substructured (Höglund et al. 1999). Moreover, the effect was only evident in males whereas females did not show any signs of sub-structuring. This is not surprising since radio-telemetry studies of black grouse have shown that females disperse longer distances than males (Willebrandt 1988, Caizergues & Ellison 2002). More recent studies using a larger number of markers and also in other populations have corroborated the geographical sub-structuring of males (Segelbacher & Höglund 2000, C. Lebigre pers. comm.).

These results indicated that black grouse males were more related within than between leks (Höglund et al. 1999), a pattern also found in some (prairie chicken *Tympanuchus pallidicinctus*: Bouzat & Johnstone 2004, peafowl *Pavo cristatus*: Petrie et al.

1999, turkey *Meleagris gallopavo*: Krakauer 2005) but not all other galliforms (sage grouse *Centrocercus urophasianus*: Gibson et al. 2005). As such the pattern is consistent with but not firm evidence that kin-selection may operate on black grouse leks. The suggestion is that if larger leks attract more females, relatives may join a lek where a relative is the most successful breeder and gain via inclusive fitness even if such a joining male would not reproduce himself (Kokko & Lindström 1996). This hypothesis also predicts that settled males should allow settlement of kin but are aggressive to non-kin thus creating a pattern of clustered kin within the lek (see e.g. Shorey et al. 2000). However, this fine-scale pattern within leks seems to be absent in black grouse (C. Lebigre pers. comm.).

With respect to conservation biology this first study was not specifically designed to test issues in relation to population survival. However, the study did detect evidence of a metapopulation structure among black grouse within the study area in central Finland. The basic unit in this metapopulation is the winter flock. When the birds are not attending the lek both sexes aggregate in mixed sex flocks that roost and forage together. These social units occupy a home range and winter flocks are weakly albeit significantly genetically differentiated (Höglund et al. 1999). From a conservation perspective it may be relevant to consider the winter flock and the habitat in which it resides as a prime focus for targeting conservation efforts.

Genetic studies of the last remaining and isolated population in the Netherlands examined the impact of isolation and reduction in numbers on genetic diversity (Larsson et al. 2008). Genetic diversity in the last extant Dutch population was compared with museum samples from extinct Dutch populations and three extant black grouse populations from other parts of Europe. These extant populations were chosen as representatives of isolated (England) and non-isolated populations (Austria and Norway). Significantly lower genetic variation was found in the present Dutch population compared to the non-isolated populations in Austria and Norway and also when compared with the historical Dutch population. These results stress the strong impact of genetic drift imposed by the reduction in numbers observed in the Netherlands. The numbers of displaying male black grouse used to be in the order of 9 000 around the time of the 2nd World War, when the number of sites (populations) was in the order of 200. In the 21st century one site remains and the number of displaying males is presently around 20–30.

Impacts on genetic diversity of a population size reduction can be estimated by examining the difference between the expected heterozygosity under Hardy-Weinberg equilibrium (H_e) and the heterozygosity expected at mutation-drift equilibrium (H_{eq}) (Cornuet & Luikart 1996, Piry et al. 1999). In populations that have not been reduced in numbers and that are near mutation-drift equilibrium, H_{eq} will equal H_e (Luikart & Cornuet 1998). Since alleles are lost more rapidly than heterozygosity during a population decline, the effect will be a heterozygosity excess (higher H_e) in reduced populations (Piry et al. 1999). Threatened species are rarely assayed continuously during population size reductions for genetic diversity. Thus the method outlined above has appeal since it allows detection of loss of genetic variation from only a single 'snap-shot' point estimate. Despite that strong genetic drift was evident in the present Dutch population in comparison to the reference populations we could not detect any heterozygosity excess. Using this test on each population, signs of heterozygosity excess were only found in the also isolated English population.

Simulating the effect of a population reduction on the Dutch population from 1948 onwards using census data and with the Dutch museum samples as a model for the genetic diversity in the initial population, revealed that the loss in number of alleles and observed heterozygosity was according to genetic drift expectations and within the standard error range of the present Dutch population (Fig. 1). Thus, the effect of the strong decline in the number of grouse on genetic diversity was only detectable when using a reference from the past. The lack of evidence for a population reduction in the present Dutch population by using the rationale outlined above may be attributed to a rapidly established new equilibrium owing to a very small effective population size.

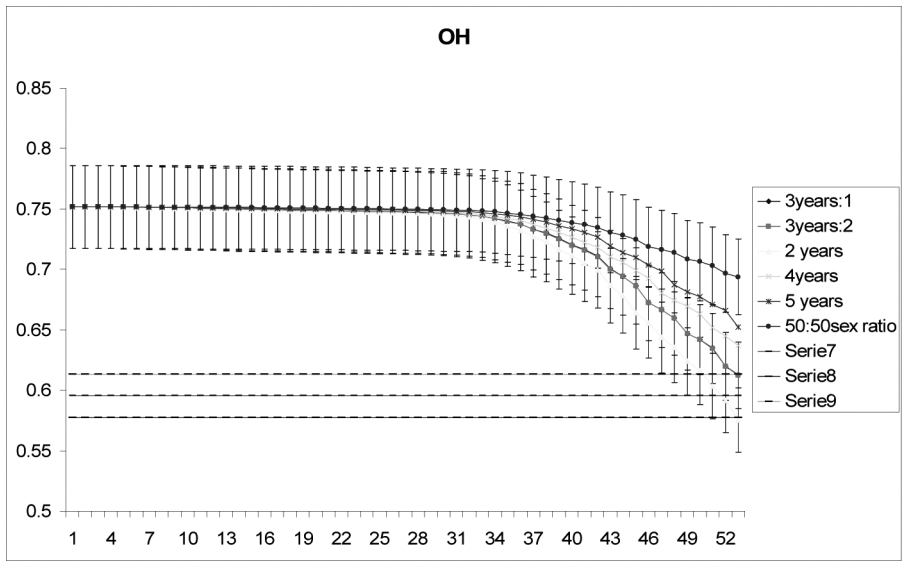

Fig. 1. The simulated observed heterozygosity loss in black grouse over 52 years in the Netherlands. The simulations start at the heterozygosity determined from museum samples stored before 1940. The observed heterozygosity was simulated assuming the censused number of males had a possibility to breed each year. The line around 0.6 is the mean of observed heterozygosity in the present Dutch population with 95% confidence intervals. The different simulations are varied according to generation time and sex ratio assumptions (adapted from L a r s s o n et al. 2008). With more realistic assumptions of mating skew, i.e. about a tenth of all males reproduce each year – the 9th time series, the simulations fall within observed levels.

M i c r o s a t e l l i t e v a r i a t i o n a m o n g p o p u l a t i o n s

As outlined above, one of the most important parameters in understanding the conservation status of a species is the extent of fragmentation and population structure. Subdivided populations in which subunits are disconnected are much more prone to local, and in the long run global, extinction.

The first study to investigate an association of habitat fragmentation with genetic structure of black grouse took advantage of the differences in connectivity among populations in Finland and in the Alps (C a i z e r g u e s et al. 2003). In this study the genetic differentiation of males among nine localities in continuous lowland habitats in Finland was compared to the genetic differentiation among 14 localities in fragmented habitats in the Alps (France, Switzerland and Italy). Significant genetic differentiation was found in both of these regions. However, the average differentiation was more than three

times higher in the Alps than in Finland. This shows that Alpine subpopulations are less connected than the Finnish, in other words gene flow is higher among Finnish populations.

The greater differentiation found in the Alps was attributed to the presence of mountain ridges rising above natural habitats of the species, which form barriers to gene flow, and to a higher influence of genetic drift resulting from lower effective sizes in the more fragmented habitats in the Alps. Similar results with more differentiation in the fragmented parts of the Alps have also been found in capercaillie (Segelbacher & Storch 2002, Segelbacher et al. 2003).

In a study using microsatellite genetic variation in 14 different geographic populations of black grouse across the west European range (Höglund et al. 2007), we found an additional result to the comparison of Finnish and Alpine black grouse: that genetic diversity tended to be lower in the more fragmented habitats. In this later study, populations were grouped in three different fragmentation categories: isolated, contiguous and continuous, respectively. Genetic diversity was lower in isolated populations than in the other two categories, which had equal diversity. The contiguous category involved Alpine populations and the continuous category consisted of populations from Fenno-Scandia. Thus this study, while corroborating the general proposition of lower diversity in more fragmented habitats, could not repeat the result when comparing the Alps (contiguous) and Fenno-Scandia (continuous). However, the numbers of sampled populations were lower and the absence of a difference between contiguous and continuous could well be attributed to lower statistical power.

In the western part of their range, black grouse were formerly found in both Britain and Ireland. The Irish population is now extinct and the range of British black grouse has contracted within historic times (Holloway 1996). At present British black grouse are found in three regions: Wales, northern England/south Scotland and the Scottish highlands. We used microsatellites to study genetic differentiation among British black grouse (Larsson et al. submitted). Samples from each of the Scottish highlands, England and Wales were significantly differentiated, suggesting little or no gene flow between these localities. Genetic distance analysis revealed that all the populations are genetically distinct. The population genetic variation in England and Wales showed signatures compatible with inbreeding being a major determinant of the present low genetic diversity whereas no such signature could be detected in the Scottish sample. This suggests that English and Welsh populations have suffered more severely from the negative effects of population decline and isolation than the Scottish. The Scottish population is presently also the largest.

The results of all these studies imply that lowered genetic variability in black grouse populations may be explained by population isolation and more intense genetic drift in small populations. The results also suggest that the connectivity of small and isolated populations in Western Europe should be improved or else face an increased risk of extinction due to genetic and demographic stochasticity (Höglund et al. 2003).

Heterozygosity fitness correlations

As mentioned above, inbreeding may severely hamper individual survival and performance and thus contribute to population declines and eventually local extinction. Much focus in conservation biology has therefore been directed towards detecting and measuring the negative effects of inbreeding in endangered populations (reviewed in Hedrick & Kalinowski 2000). Ideally inbreeding should be estimated with the aid of pedigree information (Wright 1922), a method commonly employed to minimise inbreeding within

zoo populations (Kalinowski & Hedrick 1998). However, pedigree information is not easily obtained in wild free ranging populations and especially not in lekking species where males and females to a large extent live separate lives. Researchers have therefore tried various methods to estimate the negative effects of inbreeding indirectly. Most of the methods are based on the fact that inbreeding reduces heterozygosity and therefore less heterozygous individuals should be more inbred (Coulson et al. 1998, Coltman et al. 1998, Pemberton et al. 1999). This approach has lately been severely criticised on both empirical and theoretical grounds and now methods to infer pedigrees via molecular data is strongly advised (Pemberton 2008). Nevertheless, many studies have indeed found statistical associations between various measures of individual heterozygosity and measures of individual performance (e.g. Slate et al. 2000, Rossiter et al. 2001).

We did one such study on data from a large sample of males whose performance had been monitored in the field and which had been genotyped at 15 microsatellite loci. We studied male lifetime lekking performance, and related this to indirect measures of inbreeding in a wild black grouse population in central Finland between 1989 and 1995 (Höglund et al. 2002). Inbreeding was estimated with two estimates of heterozygosity (the lower the heterozygosity the more inbred). We found a significantly positive relationship between one of the measures and lifetime copulation success (LCS), while the relationship with the other and LCS was close to being significant. We also found that males that never obtained a lek territory had lower mean heterozygosity than males that were observed on a territory at least during one mating season in their life. Furthermore, among males that were successful in obtaining a lek territory, LCS and heterozygosity were highest for those males that held central territories. These data imply that inbred males have a disadvantage (or outbred males have an advantage) in the competition for territories that may explain the relationships with LCS and inbreeding.

This study was one of the first attempts to link measures of inbreeding and lifetime fitness in a non-isolated population. This is important in establishing that the relationships found in previous studies of closed island and captive populations are not artefacts of low gene flow created by limited dispersal but a general feature of wild vertebrate populations. Furthermore, that signs of inbreeding depression could be found in the large, connected and numerous Finnish population suggest that inbreeding effects should not be ignored in conservation studies of black grouse. If anything the negative effects of inbreeding are expected to be even stronger in threatened and isolated black grouse populations in central and western Europe.

Immune defence genes and quantitative trait variation

In all the examples above the patterns of genetic variation have been inferred using neutral genetic markers. This means that we can exclude natural selection as a force acting on the allele frequency changes observed in the markers used among and within populations. In fact, this is a desired feature because neutrality allows us to infer important properties of the populations such as the level of genetic differentiation among the populations and thus the level of gene flow between localities. Furthermore, the neutrality of the markers allows us to make inferences about the level of inbreeding both at the scale of subpopulations and at the individual level.

When it comes to comparisons of the level of genetic variation among populations and any judgements about whether any given population has been subjected to elevated levels

of loss of genetic variation, the neutral markers are also informative given that the markers are a random selection of loci representative of the whole genome of the organism under study. It is questionable, but not completely unrealistic, to assume that a sufficient number of markers have been used in most studies of black grouse to allow the investigator to make inferences about whole genome processes. We do indeed think that Dutch and Welsh black grouse at present harbour less genetic variation than Scandinavian, not only at the studied marker loci but throughout their entire genomes.

Most studies of endangered species typically employ a suite of microsatellite markers in the order of 10–20 independently segregating markers. Of course it does not matter for a population that genetic variation has been lost at any particular neutral microsatellite locus. This is a consequence stemming from the fact that the marker is neutral. However, the very fact that neutral markers are neutral makes further inferences hard to make in a conservation perspective. How much microsatellite variation can be lost before the population becomes so impoverished that it needs to be restored genetically? How much can two populations drift apart before the populations become separate conservation units? Which reference population should be used to restock an endangered population? Should we take a genetically similar population which is low in genetic variation or a genetically distant one which harbours more allelic variants? Does microsatellite genetic variation say anything about adaptation to local conditions? These are all well founded questions which may not be easy to answer. Especially when it comes to the issue of local adaptation, researchers have turned to "ecologically" more relevant genetic variation. This is variation which for other reasons can be inferred to be ecologically relevant. One such suite of genes are those involved in coding for proteins involved in the immune defence system of vertebrates.

The major histocompatibility complex (MHC) is a set of closely linked genes, all to some extent involved in coding for proteins that signal the presence of foreign pathogens in the body. Mhc class II genes code for cell-surface proteins that signal pathogens to T-lymphocytes, thus triggering the adaptive immune response. In other vertebrates, MHC-genes are among the most variable genes known. In humans, more than 400 allelic variants have been discovered at the homologous loci.

We have developed a protocol and markers for studying Mhc class II loci in black grouse (S t r a n d et al. 2007). We found that black grouse have a low number of Mhc class II B (BLB) and Y (YLB) genes with variable diversity and expression. This is similar to what has been described in the avian model, the chicken. We were thus the first to show that another bird species shares several features of the simple Mhc organisation of the chicken. The black grouse BLB genes showed the same level of polymorphism that has been reported in chicken, and we also found indications of balancing selection in the peptide-binding regions. Balancing selection is a form of selection which is frequency dependent. For example it may favour certain allelic variants when rare and select against them when common. This form of selection is thus a mechanism maintaining genetic variation at the locus under selection.

The YLB genes were less variable than the BLB genes, also in accordance with earlier studies in the chicken, although their functional significance still remains obscure. We hypothesized that the YLB genes could have been under purifying selection. Future studies are under way using these markers to determine if isolated populations have lost more MHC variation than neutral variation and to study the fitness of consequences of MHC variation at the individual level.

Another approach to infer whether selection has been involved in shaping and maintaining population differences is to compare the level of differentiation observed in

neutral genetic markers with what can be observed in quantitative traits such morphology or life-history variables (Merilä & Crnokrak 2001, McKay & Latta 2002). The differentiation among population in neutral loci is calculated as

$$F_{ST}=\sigma^2_a/(\sigma^2_a+\sigma^2_b+\sigma^2_w)$$

where σ^2_a is the among sample genetic variance component, σ^2_b is the between individual within sample component and σ^2_w is the within individual component (Weir & Cockerham 1984).

The quantitative differentiation is calculated as

$$Q_{ST}=\sigma^2_{gb}/(\sigma^2_{gb}+2\sigma^2_{gw})$$

where σ^2_{gb} is the additive genetic variance for the quantitative trait among populations and σ^2_{gw} is the additive genetic variance within populations (Wright 1951, Lande 1992, Spitze 1993).

When population differentiation in neutral and selected genetic variation is compared, Q_{ST} being larger than F_{ST} implies local adaptation, i.e. that natural selection on the quantitative character has caused more divergence in this trait than what would be expected from drift alone, which is inferred from F_{ST}. If Q_{ST} equals F_{ST} genetic drift is inferred to be the only necessary force acting on the quantitative trait. Finally, if Q_{ST} is less than F_{ST}, this implies uniform selection in all the subpopulations acting against the forces of genetic drift in the subpopulations.

It is often a formidable task to estimate the additive genetic variation of quantitative characters in natural populations. A first step is usually to grow offspring from the various populations under identical conditions to rule out any differences among populations as being environmental rather than genetic in origin, a so-called common garden experiment. Fortunately such studies have been performed in the Netherlands in the past. Hazebroek (1986) studied the length of the tarsi in black grouse of different origins and compared birds from captive stocks with wild birds of both sexes from the Netherlands and Sweden. The results showed that birds from the Netherlands were larger than Scandinavian black grouse when controlling for sexual dimorphism. More importantly, when comparing birds from both countries raised in captivity, and thus under presumably benign and common conditions, the differences remained. This strongly suggests that the size differences among Dutch and Swedish black grouse reflect genetic rather than environmental differences.

We have begun studies of quantitative differentiation by comparing skins stored in museums in the Netherlands and Sweden. We have measured wing length, tarsus length and beak width on 60 skins from Sweden and 27 from the Netherlands which have been collected during the last 150 years. In all but one measurement in both sexes (male beak width which did not differ) the Dutch birds were significantly larger.

As the additive genetic component to these traits is unknown, we adopted the approach outlined by Seather et al. (2007) to infer Q_{ST}. The results on female tarsus length are shown in Fig. 2. Since the F_{ST} calculated from microsatellite divergence among Dutch and Scandinavian black grouse is 0.21+0.07 SD and significant at $P<0.0001$, the additive genetic component needs to be quite high (both the assumed proportion of variance among populations due to additive effects and the heritability need to be higher than 0.5) if Q_{ST} is indeed larger than F_{ST}. Morphological traits in natural populations often show heritabilities in

the order 0.6 (B o a g & G r a n t 1978) and thus it is not unrealistic to infer that different natural selection regimes in the Netherlands and Sweden are responsible for the observed size differences. The Dutch population is presently at such low numbers that genetic drift is a very potent force and thus the selection on size needs to be very large to play any role in the future evolution of this population. Clearly N_e needs to be larger if adaptations to local conditions in Dutch black grouse shall prevail.

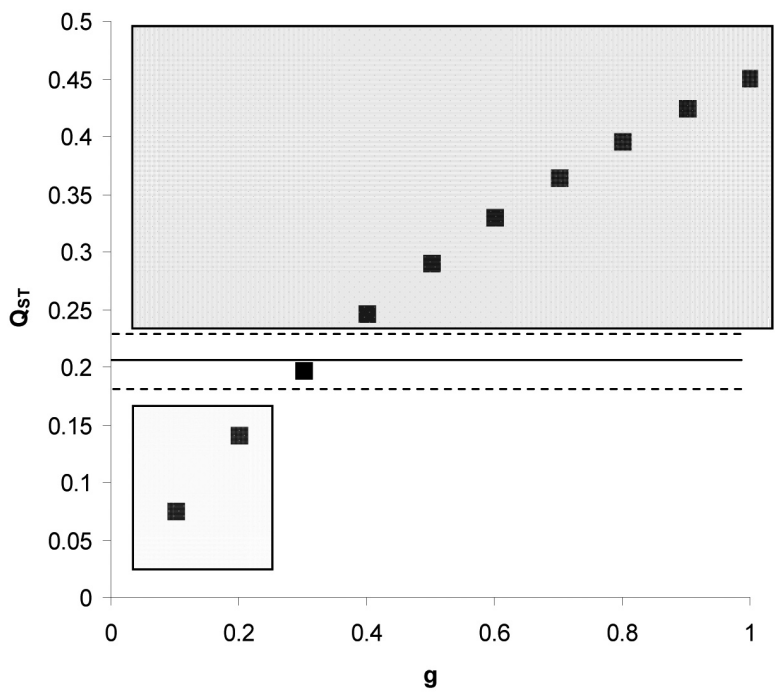

Fig. 2. Quantitative trait differentiation for tarsus length in female black grouse comparing the Swedish and Dutch populations in relation to varying assumptions about the assumed proportion of variance among populations due to additive genetic effects. If Q_{ST} lies in the blue area, the difference is due to varying selection pressures in the two regions. The line through the graph is the observed value of F_{ST} as determined from microsatellite loci with 95 percent confidence limits. Values of Q_{ST} within the limits could be ascribed to genetic drift. Values of Q_{ST} within the yellow area would be ascribed to uniform selection in both regions.

Conclusions

Population genetic data from black grouse have accumulated over the last decades. The results have been used to increase our knowledge in such different areas as reproductive biology, mating systems and, more recently, conservation biology. The results clearly show that in the western part of the range, populations are genetically differentiated and that small and isolated populations have lost genetic variation because of increased levels of inbreeding and genetic drift. It is still a contentious issue what to do with small and isolated populations. On the one hand if left alone, small remnant populations will without doubt sooner or later go extinct (K l a u s 1994). To maintain them in the long run, at least some need to be genetically restored and ideally population connectivity should be increased. On the other hand, genetic restorations need to be done with care. If not carefully planned there is a large risk that unique local adaptations may be ruined.

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