

Genetic variability in European black grouse (*Tetrao tetrix*)

Jacob Höglund · Jobs Karl Larsson ·
Hugh A. H. Jansman · Gernot Segelbacher

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Abstract We studied microsatellite genetic variation in 14 different geographic populations of black grouse (*Tetrao tetrix*) across the European range. Populations were grouped in three different fragmentation categories: isolated, contiguous and continuous, respectively. Genetic diversity, measured as observed heterozygosity (H_O), expected heterozygosity (H_E) and allelic richness, were lower in isolated populations as compared to the other two categories that did not differ amongst one another. These results imply that lowered genetic variability in black grouse populations is negatively affected by population isolation. Our results suggest that the connectivity of small and isolated populations in Western Europe should be improved or else these face an increased risk of extinction due to genetic and demographic stochasticity.

Keywords Microsatellite · Genetic variation · Fragmentation · Isolation · Population size

J. Höglund (✉) · J. K. Larsson
Population Biology, Department of Ecology and Evolution,
Evolutionary Biology Centre, Uppsala University,
Norbyvägen 18D, SE-75236 Uppsala, Sweden

H. A. H. Jansman
Alterra, P.O. Box 47, 6700 AA Wageningen,
The Netherlands

G. Segelbacher
Max Planck Institute for Ornithology, Vogelwarte
Radolfzell, Germany

G. Segelbacher
Department of Wildlife Ecology and Management,
University Freiburg, Freiburg, Germany

Introduction

Population fragmentation and isolation may have detrimental effects both on the fitness and viability of extant populations, and also the evolutionary potential of species (see papers in Ferrière et al. 2004). Population genetic structure is predicted to affect population persistence and long-term survival where small and isolated populations face a higher risk of extinction (Frankham et al. 2002).

Black grouse are at present facing three rather different conditions in their European breeding range. First, in the north relatively continuous populations of black grouse are large and suitable habitat is abundant. Second, in some mountain areas, such as the Alps and the Scottish Highlands, populations are naturally fragmented but connected through dispersal. Black grouse populations in such areas are contiguous rather than continuous and may best be described as meta-populations. Third, in lowland areas of continental Western Europe and England/Wales a few black grouse populations are still present in highly isolated habitat fragments.

Population sizes of black grouse have declined dramatically over recent decades in a number of countries where populations now exist in isolated fragments (BirdLife International 2004). For example, in Britain population size was estimated in the range of 100 000 birds in the 1940:ies (Hancock et al. 1999). The present population size estimate is 13 000. In the Netherlands, population size was in the order of 10 000 birds before the Second World War and it is at present 35–50 (Jansman et al. 2004).

The aim of this study was to compare genetic diversity in continuous, contiguous and isolated black

grouse populations. We present reports of standard estimates of microsatellite genetic diversity in the three different types of population connectivity and discuss their implications for the conservation of black grouse in Europe.

Methods

We tested for differentiation between localities by computing pair-wise F_{ST} s according to Weir and Cockerham (1984) using Genetix 4.05.2 (Belkhir 2005). Designation of fragmentation category was based on the presence of suitable habitat and known occurrences of black grouse within the dispersal distance from a focal population (<100 km). We classified populations

into fragmentation categories as follows: populations from Scandinavia were classified as continuous. Seven populations from the Alps and one from the Scottish Highlands were classified as contiguous. Finally five populations were classified as isolated (Fig. 1).

We obtained tissue for DNA extraction from the different populations (see Table 1) between 1980 and 2003. Sources were feathers, egg remains (dried blood vessels), blood samples or muscle tissue (see Segelbacher 2002, Höglund et al. 1999). Genomic DNA was extracted from a circa 1 cm segment at the root end of feathers using the DNeasy Tissue Kit (Qiagen) as described by Segelbacher (2002). DNA from blood and tissue samples was extracted similarly or with a standard phenol/chloroform extraction. Individual samples were genotyped at 10

Fig. 1 Top: Map of Western Europe and the approximate location of the sampled populations. White dots- continuous populations, black dots- contiguous populations and grey dots- isolated populations. The map below indicates the breeding range



Table 1 Background data and genetic diversity of Black Grouse in Europe

Category	Population	Year sampled	<i>n</i>	AR	H_E	H_O	F_{IS}
<i>Continuous</i>	Jyväskylä	89–95	57	4.49	0.74	0.66	0.13
	Østfold	99	31	4.17	0.70	0.67	0.04
<i>Contiguous</i>	Abernethy	00	16	3.93	0.63	0.65	–0.05
	Allgäu	98–00	23	4.38	0.73	0.69	0.06
	Ammer	98–00	18	4.20	0.73	0.71	0.02
	Vorarlberg	98–00	24	4.07	0.70	0.66	0.06
	Haut Savoie	98–99	9	4.16	0.72	0.67	0.07
	Tauern	98–00	27	4.12	0.70	0.69	0.01
<i>Isolated</i>	Tessin	80–83	16	4.61	0.73	0.64	0.13
	Northern Pennines	00–03	21	2.85	0.57	0.48	0.15
	Salland	03	31	3.16	0.53	0.44	0.17
	Rhön	92, 95, 03	8	3.93	0.72	0.55	0.25
	Waldviertel	01–03	14	3.27	0.56	0.57	0.01
	Llandegla	04	8	2.81	0.52	0.53	0.00

n gives the number of individuals analysed, AR allelic richness (as per Goudet 2001) rarified to a constant sample size of 8, H_O the mean observed heterozygosity, H_E the expected heterozygosity, and F_{IS} (bold- estimate significantly different from zero)

tetranucleotide microsatellite loci (Tut1, Tut2, Tut3, BG 10, BG12, BG15, BG16, BG18, BG19, BG20). PCR amplifications and genotyping were conducted as described elsewhere (Segelbacher et al. 2000; Piertney & Höglund 2001). PCR-primers were labelled with fluorescent dye and products were resolved by electrophoresis on an ABI 377 genetic analyser. To detect whether contamination with exogenous DNA or PCR products had occurred, tubes without samples were included in the DNA extraction and PCR amplification procedure as negative controls. Amplification of the cloned locus aided in size determination and also served as a positive control. To avoid contamination, DNA extractions, pre PCR and post PCR pipetting were carried out in different rooms and aerosol-resistant filter pipette tips were used throughout. To obtain reliable genotypes, samples were typed at least three times to check for false homozygotes.

We tested for linkage disequilibrium within populations using GenePop on the web (Raymond and Rousset 1995). Relative genetic variation in each population was assessed using allele frequency data from which observed heterozygosity H_O , expected heterozygosity H_E (Nei 1987), allelic richness and F_{IS} (deviations from Hardy-Weinberg expectations) were calculated using Fstat (Goudet 2001). Two of the loci (BG12 and BG20) were accidentally not typed in two populations, calculations of allelic richness is thus based on 8 loci. Tests for differences among groups of populations in allelic richness, observed and expected heterozygosity and F_{IS} were performed using generalised linear models in R 2.1.0 (R Development Core Team 2004).

Results

We found significant deviations from linkage equilibrium among loci within populations in seven instances after correcting for multiple tests. However, no pair of loci appeared consistently among populations. There was thus no evidence of physical linkage among loci. All but one pair of populations, even within the Alpine region where populations are geographically close to one another, were significantly differentiated indicating low or no dispersal between localities (Table 2).

When comparing measures of genetic variation among fragmentation categories we found less observed heterozygotes H_O , lower levels of expected heterozygosity H_E and lower allelic richness in isolated populations compared to populations from contiguous and continuous settings (that were not significantly different from one another) (GLMs, H_O : $t_{13}=7.91$, $P < 0.0001$, H_E : $t_{13}=4.27$, $P = 0.0001$, allelic richness: $t_{13}=5.89$, $P < 0.0001$, Fig. 2). F_{IS} was not significantly different among isolation categories ($t_{13}=1.89$, $P = 0.09$, Fig. 2).

Discussion

Black grouse populations in Europe seem to fit a pattern where small and isolated populations are exhibiting lower genetic variation as compared to contiguous (metapopulation) and continuous ones. Our results are all likely consequences of the effects of mitigated migration and the increased importance of genetic drift in small and isolated populations

Table 2 Pair-wise F_{ST} s between sampling localities (above diagonal), significance (proportion out of 1000 bootstrap replicates when the simulated value exceeded the observed below the diagonal)

	Allgäu	Ammergebirge	HautSavoie	Tauern	Tessin	Vorarlberg	Jyväskylä	Østfold	Abermethy	Llandegla	N Pennines	Salland	Waldvier	Rhön
Allgäu	0.034	0.025	0.019	0.029	0.020	0.132	0.104	0.077	0.153	0.122	0.170	0.142	0.099	
Ammergebirge	0.001	0.028	0.042	0.039	0.029	0.134	0.115	0.070	0.154	0.130	0.189	0.173	0.107	
HautSavoie	0.02	0.03	0.051	0.047	0.034	0.135	0.128	0.052	0.154	0.108	0.184	0.143	0.073	
Tauern	0.002	0.001	0.005	0.023	0.041	0.145	0.094	0.081	0.153	0.137	0.211	0.162	0.101	
Tessin	0.003	0.001	0.005	0.12	0.012	0.142	0.105	0.068	0.105	0.109	0.226	0.180	0.111	
Vorarlberg	0.004	0.009	0	0	0	0.138	0.125	0.050	0.122	0.104	0.176	0.165	0.093	
Jyväskylä	0	0	0	0	0	0	0.079	0.182	0.230	0.202	0.233	0.226	0.164	
Østfold	0	0	0	0	0	0	0	0.168	0.222	0.175	0.211	0.192	0.148	
Abermethy	0	0.001	0	0	0	0	0	0	0.134	0.082	0.205	0.204	0.134	
Llandegla	0	0.001	0	0	0	0	0	0	0	0.147	0.315	0.316	0.168	
N Pennines	0	0	0	0	0	0	0	0	0.001	0	0.310	0.231	0.166	
Salland	0	0	0	0	0	0	0	0	0	0	0	0.288	0.234	
Waldviertel	0	0	0	0	0	0	0	0	0	0	0	0	0.136	
Rhön	0	0.006	0	0	0.001	0	0.001	0	0.001	0	0	0	0	

(Loeschcke et al. 1994, Frankham 1996, Frankham and Ralls 1998) and are in good congruence with results on capercaillie (*T. urogallus*, Segelbacher et al. 2003). Since migration between isolated populations is likely to be absent or very low and population size is small, the risk of consanguineous mating is increased, possibly leading to increased levels of inbreeding. Thus small and isolated black grouse populations may face increased risks of extinction due to detrimental genetic effects. We saw some evidence of this as isolated populations tended to display significant F_{IS} values more often than other populations (Table 1). However, we observed increased homozygosity also in continuous populations. The increased F_{IS} in the larger and connected populations may perhaps be explained by a Wahlund effect since larger populations display on several leks and tended to be sampled over several years. There may be undetected population structure in such settings (Wahlund 1928).

From a conservation perspective our results imply that isolated populations have lost genetic variation. In theory, loss of genetic variation occurs more rapidly in small populations as the strength of genetic drift is inversely related to effective population size. In the case of the black grouse, we have indications that the populations in the Netherlands, Rhön and Waldviertel were indeed small. Such populations may thus face an increased risk of extinction due to both genetic and demographic factors. Unless connectivity is artificially increased they are likely to become extinct in the near future. Indeed, documentations of small isolated populations of black grouse going extinct have been reported in Denmark (Holst-Jørgensen 2000) and mid-England (Hancock et al. 1999) in recent years. Until suitable stepping stone habitats can be created, managers may consider transplants to boost the genetic variation in the small and isolated populations in lowland Germany/Austria, the Netherlands and Britain. Transplantations are not without problems however, as this may lead to outbreeding depression and increased risk of spreading disease. Nevertheless, a balanced assessment of benefits and risks may reach the conclusion that transplantation is indeed a worthwhile conservation action (see e.g. Westemeier et al. 1998; Hogg et al. 2006).

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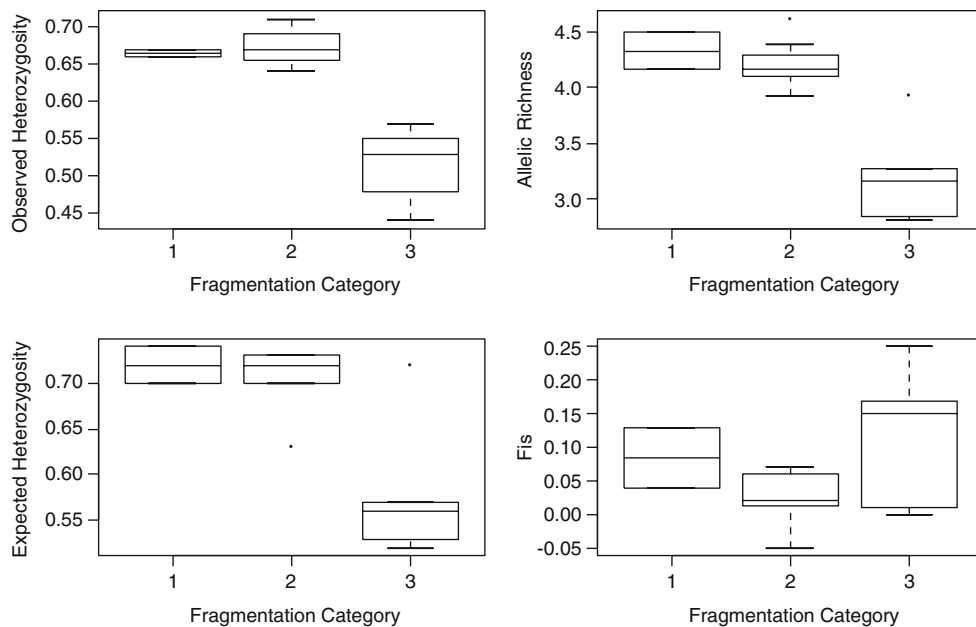


Fig. 2 Boxplots (median, first and third quartiles, range of 95% of variability and outliers as dots) of genetic diversity in three categories of West European black grouse populations. Fragmentation Categories: (1) Continuous, (2) Contiguous and (3) Isolated

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