Molecular Ecology (2003) **12**, 1773–1780 doi: 10.1046/j.1365-294X.2003.01873.x

From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe

G. SEGELBACHER,*† J. HÖGLUND‡ and I. STORCH†

*Max Planck Research Centre for Ornithology, Vogelwarte Radolfzell, Radolfzell, Germany, †Wildlife Research and Management Unit, Weihenstephan Centre of Life and Food Science, TU Munich, Freising, Germany, ‡Population Biology/EBC, Uppsala University, Uppsala, Sweden

Abstract

The capercaillie inhabits a continuous range in large parts of the Palearctic boreal forest, but is patchily distributed in temperate Europe. An ongoing population decline, largely related to human land use changes, has been most pronounced in central and western Europe, where some local populations have become extinct. In this study, we document the genetic differentiation of capercaillie populations at different stages along a gradient of spatial structuring from high connectivity (continuous range in the boreal forest) to a metapopulation systems (Alps) and recent (central Europe) and historic (Pyrenees) isolation. Four hundred and sixty individuals from 14 sample sites were genotyped at 10 polymorphic microsatellite loci to assess genetic structure and variation of capercaillie populations across its European range. As expected, differentiation was least pronounced within the continuous range in the boreal forest. Within the metapopulation system of the Alps, differentiation was less than among the isolated populations of central Europe (Black Forest, Fichtelgebirge, Thuringia, Vosges). In the long-isolated population of the Pyrenees, and the recently isolated populations of central Europe, genetic diversity was significantly reduced compared with the Alps and boreal forest. Our results agree with the concept of a gradual increase in genetic differentiation from connectivity to isolation, and from recent to historic isolation. Anthropogenic habitat deterioration and fragmentation thus not only leads to range contractions and extinctions, but may also have significant genetic and evolutionary consequences for surviving populations. To maintain high levels of genetic variation in species in fragmented habitats, conservation should aim at securing connectivity between spatially distinct populations.

Keywords: fragmentation, isolation, metapopulation, microsatellites, population structure, *Tetrao urogallus*

Received 27 November 2002; revision received 21 March 2003; accepted 21 March 2003

Introduction

The capercaillie *Tetrao urogallus* is closely associated with older stages of conifer-dominated forests and is considered an important umbrella species for boreal and montane forest biodiversity conservation (Suter *et al*. 2002). The species has a contiguous distribution in the boreal forest from Scandinavia to the Yenisey River in eastern Siberia. The distribution in western and central Europe, however, is scattered, due primarily to the patchy distribution of coniferous forest that remained after the last Ice Age, and isolation has led to the formation of subspecies, as in the Pyrenees (Potapov & Flint 1989) and the Cantabrian mountains (Castroviejo 1967, 1975).

During the 18th and 19th centuries, forest use practices such as the planting of conifers, extensive livestock grazing and litter collection improved habitat conditions for capercaillie, resulting in temporary range extensions in central Europe (Klaus *et al*. 1989; Storch 2001). During this time, the central European ranges of the capercaillie were probably well connected in a metapopulation (*sensu* Wiens 1996) pattern (Storch & Segelbacher 2000). More recently, since the second half of the 20th century, habitat deterioration related to changes in forestry and agriculture, excessive

Correspondence: G. Segelbacher. Max Planck Research for Ornithology. Fax: +49 7732 150169;
E-mail: segelbacher@vowa.ornithol.mpg.de

Fig. 1 Map of the geographical locations of the sampled populations.

human disturbance, increasing predator numbers and possibly climate change have led to an overall population decline and range contraction in western and central Europe (Storch 2000). As a result, the species has disappeared from most central European lowland forests (Storch 2001) and its remaining ranges have probably become isolated from one another as the former metapopulation system broke up (Storch & Segelbacher 2000).

Today, the capercaillie is red-listed throughout western and central Europe (Storch 2000). Habitat deterioration and small population size are considered the major threats (Storch 2000, 2001). Most of the capercaillies's central European ranges are limited in spatial extent to between a few hundred to a few thousand km^2, and remaining populations count < 100 (e.g. Fichtelgebirge) to maximally 1000 birds (Black Forest) (see Fig. 1, Table 1). The Alps are the central European stronghold of the capercaillie with an estimated total population size of > 30 000 birds extending over 150 000 km^2 (Storch 2000, 2001). In the Alps, forests are patchily distributed, and thus, capercaillie populations are spatially structured. Among local populations of the Alps, observational and genetic evidence indicates connectivity within a metapopulation system (Storch & Segelbacher 2000; Segelbacher & Storch 2002).

Spatial structuring of populations may have significant genetic consequences. Even among different sites within a contiguous range of a species, geographical distance will result in a certain degree of local differentiation (e.g. Grapputo *et al*. 1998). These differences become greater as connectivity through gene flow decreases. Thus, significant differentiation can be expected within metapopulation systems, and differences should be most pronounced among isolated populations. Given long enough, isolated populations may evolve into separate subspecies or species (Hewitt 1996, 2001). Many populations isolated by anthropogenic habitat fragmentation, however, will not persist for long (see, e.g. Hilton-Taylor 2000). Fragmentation is an ongoing process and its effects have to be ameliorated if we are to efficiently manage landscapes and species. In addition to demographic effects of habitat fragmentation, consequences for genetic population structure must be examined explicitly. Meaningful interpretation can be gained from the study of genetic structure over a range of levels of fragmentation.

Here, we document the genetic differentiation of capercaillie populations at different stages along a gradient of spatial structuring from high connectivity (contiguous range in the boreal forest) to metapopulation systems (Alps) and recent (central Europe) and historic (Pyrenees) isolation. We hypothesize that the genetic structuring among capercaillie populations will be greater in isolated ranges in central Europe than within the Alpine metapopulation system and expect the least pronounced structuring within the contiguous boreal parts of the range.

Materials and methods

Sampling

Between 1997 and 2000, feathers from 460 individuals were sampled as a source of genomic DNA at different locations over the entire European range of the capercaillie (Fig. 1) as described in Segelbacher (2002). Based

Table 1 Geographical distinct ranges of the capercaillie in Europe, estimated total population sizes per range (after Storch 2000, 2001; Baines & Andrew 2003; A Hurstel pers. commun.), study sites per range included in this study, and genetic variation estimated. *n*, number of individuals analysed, *A*, mean number of alleles per locus, *R*, allelic richness; H_O, mean observed heterozygosity; H_E, expected heterozygosity and F_{IS} per population

Range	Total population size	Study site	*n*	*A*	*R*	H_E	H_O	F_{IS}
		Norway	17	4.9	2.51	0.62	0.61	0.06
		Karelia	3	2.8	2.41	0.53	0.70	−0.11
		Archangelsk	44	5.3	2.39	0.59	0.60	0.10
Boreal forest	> 1 Million	Jaroslawl	18	4.5	2.39	0.58	0.56	0.07
		Alps north (Germany)	130	6.3	2.49	0.66	0.72	−0.08
		Alps south (Italy)	38	5.5	2.52	0.66	0.70	−0.05
		Alps southeast (Austria)	47	5.7	2.35	0.60	0.60	0.01
Alps	> 30 000	Slovenia	8	3.7	2.38	0.58	0.58	0.07
Pyrenees	> 5000	Pyrenees	16	3.0	1.99	0.45	0.44	0.07
Scotland	1000	Scotland	5	3.0	2.26	0.50	0.54	0.05
Black Forest	1000	Black Forest	62	5.1	2.22	0.54	0.53	0.02
Vosges	200	Vosges	52	4.6	2.15	0.53	0.56	−0.05
Fichtelgebirge	< 100	Fichtelgebirge	9	3.5	2.23	0.53	0.71	−0.26
Thuringia	< 100	Thuringia	11	4.2	2.39	0.57	0.57	0.05

on the known distribution of capercaillie, we classified sampling locations as part of the contiguous boreal range (Archangelsk, Jaroslawl, Karelia, Norway), large ranges (estimated population sizes > 5000 birds; Storch 2001) in central/western Europe (Alps, Pyrenees) and small isolated ranges in central/western Europe (populations < 2000 birds; Black Forest, Fichtelgebirge, Scotland, Thuringia, Vosges; Storch 2001). The Alpine sample comprised local populations within the Alps in Germany (Alps north), Italy (Alps south) and Austria (Alps east). All populations classified as small and isolated (see above) were separated from the nearest neighbouring population by at least 80 km. We included a few populations with low sample sizes in this study, as they fitted well into the overall picture. However, we consider data from sites with sample sizes < 10 individuals (see Table 1) as preliminary.

For the capercaillie, 12 subspecies are recognized based on morphological variation in various parts of the range (Potapov & Flint 1989). Their relevance, however, has not been examined genetically. According to Potapov & Flint (1989), the populations sampled in this study belong to the subspecies *Tetrao urogallus aquitanus* (Pyrenees), *major* (central Europe), *urogallus* (Scotland, Norway), *pleskei* (Jaroslawl) and *obsoletus* (Archanglsk); the Karelian site is located at the transition between *urogallus* and *pleski.* The capercaillie were reintroduced into Scotland in the 19th century by translocating birds from Sweden. In central Europe, largely unsuccessful restocking attempts with captive-bred birds of unclear origin are documented for the Black Forest and Thuringia (Klaus 1997; Storch 2001).

Genotyping

Genomic DNA was extracted from an ≈1 cm segment at the root end of feathers using the DNeasy Tissue Kit (Qiagen) as described by Segelbacher (2002). Individual samples were genotyped at 10 tetranucleotide microsatellite loci and polymerase chain reaction (PCR) amplifications and genotyping were conducted as described elsewhere (Segelbacher 2002). PCR fragments were separated by electrophoresis on 6% denaturing polyacrylamide gels and silver stained using routine approaches.

Data analysis

To investigate genetic structure within and between sample sites we used genotype and allele frequencies of the microsatellite loci. Departures from Hardy–Weinberg equilibrium (HWE) were tested for each of the 10 loci using GENEPOP Version 3.1d (Raymond & Rousset 1995b), which uses a Markov chain method following the algorithm of Guo & Thompson (1992). Relative amounts of genetic variation in each population were assessed using allele frequency data from which the mean number of alleles, allelic richness (Petit *et al*. 1998) and the unbiased expected heterozygosity (H_E; Nei & Roychoudhury 1974) were determined using FSTAT Version 2.93 (Goudet 2001). Allelic richness is a measure of the number of alleles independent of sample size, and hence allows comparison of this quantity between samples of different sizes. Tests for differences among groups of populations in allelic richness, observed heterozygosity (H_O), gene diversity and F_{IS} were calculated with the same program using 1000

permutations and a one-tailed test. We also tested population genetic structure by means of a hierarchical analysis of molecular variance (AMOVA) using ARLEQUIN Version 2.0 (Schneider *et al*. 2000) with three levels of population structure: total population, groups (boreal, Alpine and isolated populations) and individual populations.

Investigating the distribution of allele frequencies across populations can assess population differentiation. An exact probability test for departures from random allelic frequencies (Raymond & Rousset 1995a) was carried out using GENEPOP. Individual genotypes were ordinated in a multidimensional space by principal component analysis (PCA) based on PCAGEN (http://www.unil.ch/izea/softwares/pcagen.html).

Geographic distance was the straight-line distance between the centres of the different sampling areas. To examine isolation by distance we used a partial Mantel test implemented in FSTAT assuming that $F_{ST}/(1 - F_{ST})$ was linearly related to the distance between the populations. Pairwise F_{ST} estimates were obtained from GENEPOP 3.1d (Raymond & Rousset 1995b; as per Weir & Cockerham 1984) and significance tested by permuting genotypes among samples (1820 permutations, FSTAT). To reduce the likelihood of type I errors among multiple tests we applied a strict Bonferroni correction (Sokahl & Rohlf 1995).

Results

Genetic diversity within populations

We investigated genetic variation of all populations by analysing departures from Hardy–Weinberg distribution and linkage disequilibrium and by the levels of genetic diversity within populations. We did not find any evidence for linkage disequilibrium and only one population showed significant heterozygosity excess (Fichtelgebirge).

All microsatellite loci were polymorphic (4–10 alleles per locus) and observed heterozygosity was high (Table 1). The lowest degree of heterozygosity, allelic richness and mean number of alleles were detected in the Pyrenees. Alpine and boreal populations showed greater levels of genetic diversity. Within central Europe, isolated populations (Fichtelgebirge, Black Forest, Thuringia, Vosges) showed a significantly lower degree of allelic richness ($P = 0.03$), heterozygosity ($P = 0.05$) and genetic diversity ($P = 0.02$) than did the Alpine populations. Allelic richness was lower in isolated populations than in the boreal populations ($P = 0.04$).

Population differentiation

The exact tests revealed that the distribution of alleles was not identical across sample sites ($\chi^2 = \infty$, $P < 0.0001$). F_{ST} analysis across all populations and loci showed significant structuring, with $\theta = 0.102$ ($P < 0.001$). Pairwise F_{ST} values ranged from 0.019 to 0.237 (Table 2). The lowest F_{ST} values were found among the Alpine populations, suggesting high levels of gene flow between these populations. The highest pairwise F_{ST} estimates were found between the Pyrenees and the Vosges and with the boreal populations. These populations could be identified as the most genetically distinct relative to all other sampled.

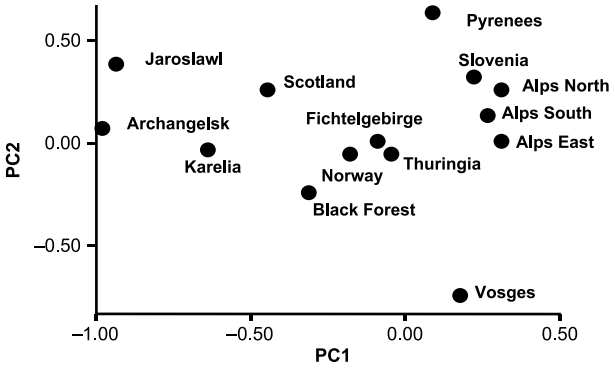

Fig. 2 Principal components analysis scores of European capercaillie microsatellite genotypes plotted on the two first axes (PC1, PC2) of a PCA performed using PCAGEN.

We investigated genetic variation between populations using an analysis of molecular variance (AMOVA). We found significant genetic differences between groups (boreal, Alpine and isolated populations) ($F_{CT} = 0.046$, $P < 0.0001$), among populations within groups ($F_{SC} = 0.060$, $P < 0.0001$) and among individuals within populations ($F_{ST} = 0.103$, $P < 0.0001$). Most of the variance (89%) was explained by the variation among birds within populations; whereas 5% of the variation was attributed to among groups and 5% to among populations.

The results of the PCA showed a similar pattern (Fig. 2). Scores of the local populations were plotted on two principal axes (PC1 and PC2), which cumulatively explained 52% of the total genetic diversity. This plotting showed a clear separation of the local populations of the boreal range, the Alpine populations and birds from the small populations.

Isolation by distance

A partial Mantel test revealed that F_{ST} pairwise comparisons were related to geographical distance. This analysis of isolation by distance detected a strong effect of geographical distance on genetic distance between all sample sites (partial Mantel test, $P = 0.004$) (Fig. 3). When only analysing the isolated populations in central Europe (Fichtelgebirge, Black Forest, Thuringia, Vosges) the effect of geographical distance on genetic distance was not significant ($P = 0.1$), whereas the remaining populations still showed a high correlation between geographical and genetic distance ($P = 0.0005$). Effects of isolation by

Table 2 Pairwise F_{ST} values between populations. Values given in bold are significant after Bonferroni correction

Populations	Alps north	Alps south	Alps east	Slovenia	Pyrenees	Vosges	Black Forest	Fichtelgebirge	Thuringia	Norway	Scotland	Karelia	Archangelsk	Jaroslawl
Alps north (Germany)	—													
Alps south (Italy)	**0.026**	—												
Alps east (Austria)	**0.036**	**0.019**	—											
Slovenia	0.046	0.027	0.022	—										
Pyrenees	**0.085**	**0.134**	**0.116**	0.116	—									
Vosges	**0.112**	**0.115**	**0.112**	**0.167**	**0.237**	—								
Black Forest	**0.092**	**0.087**	**0.091**	**0.132**	**0.178**	**0.139**	—							
Fichtelgebirge	**0.090**	0.102	**0.139**	0.189	0.247	0.188	**0.132**	—						
Thuringia	**0.065**	0.078	**0.088**	0.088	0.166	0.139	0.079	0.140	—					
Norway	**0.064**	**0.041**	**0.047**	0.047	0.154	**0.099**	**0.106**	0.096	0.110	—				
Scotland	0.099	0.092	0.096	0.096	0.199	0.207	0.103	0.176	0.129	0.069	—			
Karelia	0.102	0.083	0.056	0.056	0.199	0.155	0.113	0.188	0.104	0.036	0.088	—		
Archangelsk	**0.120**	**0.122**	**0.132**	**0.132**	**0.193**	**0.171**	**0.124**	**0.163**	**0.131**	**0.084**	0.111	0.021	—	
Jaroslawl	**0.120**	**0.111**	**0.132**	0.132	**0.164**	**0.216**	**0.141**	0.146	0.152	**0.074**	0.122	0.056	**0.027**	—

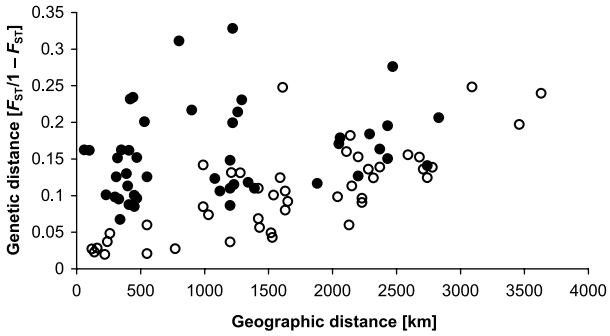

Fig. 3 Genetic distance ($F_{ST}/1 - F_{ST}$) was correlated to geographical distance. Filled circles represent the isolated central European populations (Black Forest, Vosges, Fichtelgebirge, Thuringia).

distance were still distinct when only analysing the populations in central and western Europe (data not shown).

Discussion

Population structure

This study elucidated the genetic structure of capercaillie populations across large parts of the species' European range. The genetic variation observed in these populations reflects different stages of an ongoing isolation process. Whereas the Pyrenees have been isolated for a long time, populations in central Europe probably became separated from the Alpine population and from each other during the 20th century, when many local populations disappeared (Storch 2001). Considering the relatively short natal dispersal distances of capercaillie (mean 1–2 km, median 5–10 km; review by Storch & Segelbacher 2000), it is likely that among these populations, dispersal, and thus gene flow, became restricted as neighbouring populations declined and became extinct (Storch 2000, 2001). Central European populations, except for those in the Alps, showed low genetic diversity, observed heterozygosity and allelic richness, suggesting that they were genetically isolated. Allelic richness was lowest in these isolated populations, indicating the importance of connectedness to other populations to maintain high levels of genetic diversity. The decreased levels of H_E and increased levels of genetic structure based on F_{ST} in the Pyrenean and central European populations are consistent with other studies of insular populations. In studies on brown bears (*Ursus arctos*) (Paetkau *et al*. 1998) and wolverines (*Gulo gulo*) (Kyle & Strobeck 2001), respectively, decreased genetic variability was attributed to population fragmentation from a previously larger contiguous population. Nucleotide diversity was lower in an isolated population of Siberian jay (*Perisoreus infaustus*) than in populations within its contiguous area of distribution, which suggests

that isolation by anthropogenic habitat fragmentation reduces gene flow (Uimaniemi *et al*. 2000) and is most distinct at the edge of the distribution range.

To explain the generally low diversity observed in the central European and Pyrenean populations of the capercaillie requires consideration of both natural (patchy post-Pleistocene distribution of coniferous forest) and anthropogenic (habitat deterioration and fragmentation) factors. We suggest that the observed level of genetic structuring in central and southern Europe reflects low effective population sizes, restricted gene flow and potentially the break-up of former metapopulation systems, whereas the level of genetic structure in the northern and Alpine regions was consistent with high levels of gene flow. Genetic diversity was lowest in the Pyrenean population, which is situated at the southwestern end of the capercaillie's distribution range, and is considered a distinct subspecies *Tetrao urogallus aquitanus*. This population is geographically isolated, and unlike in the central European populations, no other populations have existed within 300 km for centuries (Klaus *et al*. 1989). The reintroduced Scottish population, also geographically isolated, shows similarly low levels of genetic diversity, however, the sample size was quite small.

Although microsatellite markers may or may not fully reflect adaptive genetic variation in natural populations (Moss *et al*. 2003), small populations with low microsatellite variation have been shown to suffer from inbreeding depression, as documented in the greater prairie chicken *Tympanachus cupido pinnatus* (Westemeier *et al*. 1998). Thus, the reduced microsatellite variation we found in small and fragmented capercaillie populations may indicate reduced adaptive variation.

Population differentiation

Pairwise tests of F_{ST} showed significant differences between most of the isolated sample sites and the Alpine and boreal populations. Sample sizes below 10 individuals should however, be carefully interpreted as, e.g. in the Scottish and Karelian birds. Pairwise F_{ST} values were considerably lower between Alpine populations and gene flow was likely to be frequent among them. Although capercaillie numbers in the Alps may be at least locally declining (Storch 2001), populations seem still to be well connected. We therefore conclude that populations in the Alps show metapopulation structure, i.e. local populations are geographically distinct but connected by dispersal (Storch & Segelbacher 2000; Segelbacher & Storch 2002) and thus, genetic diversity across the Alps as a whole is maintained. Formerly connected populations as, e.g. Black Forest and Vosges, or Fichtelgebirge and Thuringia are now genetically isolated and therefore exhibit lower levels of genetic diversity. Although reduction in genetic diversity was significant for capercaillie populations in central Europe, it was still fairly high in comparison with studies of bottlenecked populations (Keller *et al*. 2001; Nichols *et al*. 2001). We found no evidence for a genetic bottleneck in any of the populations (data not shown), when testing using BOTTLENECK (Corunet & Luikart 1996) and the algorithm by Garza & Williamson (2001). Genetic differentiation was distinct, but fragmentation and isolation of the populations might be too recent a process to show severe genetic consequences. Interestingly, the populations of the Black Forest and the Vosges, which are separated by the Rhine valley, displayed very similar levels of genetic diversity, although restocking with captive birds was attempted (but might not have led to reproduction) in the Black Forest, but not the Vosges (Schroth 1991).

All currently described subspecies of the capercaillie have been classified according to their morphology and behaviour (Potapov & Flint 1989; see also Del Hoyo *et al*. 1994). Variation among the subspecies, however, is largely clinal. Allozyme data (Lindén & Teeri 1985) previously demonstrated that within the subspecies classified in Finland, there was distinct genetic differentiation, supporting the notion of genetic gradients even within a contiguous distribution. Our data showed that the Pyrenean birds are a genetically distinct unit, and thus agreed with the notion of a distinct subspecies. Genetically, the Scottish birds clustered closely with the boreal populations, because the recent population in Scotland descended from birds reintroduced from Sweden in the mid-1800s (Lever 1977; Starling 1991).

With the PCA of the genetic data, we found that Norwegian birds were quite distinct from Russian birds and more similar to those of central Europe, although geographical distances would have predicted the opposite. Because Fenno-Scandia was completely glaciated and later recolonized by two routes, one from the southwest and one from the northeast, there is a well known suture zone in various taxa across Sweden/Norway (Taberlet *et al*. 1998; Hewitt 2001). This has been demonstrated for rodents (Jaarola *et al*. 1999) and warblers (Bensch *et al*. 1999). Recently, Vernesi *et al*. (2002) found that Norwegian roe deer (*Capreolus capreolus*) were genetically closer to Alpine populations than to populations from northern Scandinavia. As our sample site is located in southern Norway this could likely explain why those birds were more related, albeit distantly, to those from central Europe than Russia.

From connectivity to isolation

Our genetic study of capercaillie across Europe confirms the notion of significant population declines and subsequent population fragmentation and isolation in parts of the range. These effects have clearly been most

pronounced in central Europe. Continued deterioration of habitats may lead to more populations becoming isolated remnants of a formerly contiguous or at least functionally interconnected European distribution. For capercaillie a demographic model based on Alpine populations suggested a minimum viable population size in an order of 500 birds (Grimm & Storch 2000). Our genetic data, however, show clear signs of reduced genetic variability already in ranges still counting up to 1000 birds. This indicates that a demographic minimal viable population of 500 birds may be too small to maintain high genetic variability.

We documented the genetic differentiation of capercaillie populations at different stages along a gradient of spatial structuring from high connectivity (contiguous range in the boreal forest) to metapopulation systems (Alps) and recent (central Europe) and historic (Pyrenees) isolation. Our results agree with the concept of a gradual increase in genetic differentiation from connectivity to isolation, and from recent to historic isolation. Our work confirms that both demographic (Grimm & Storch 2000) and genetic consequences (this study) of population fragmentation may contribute to the loss of biodiversity from man-dominated landscapes. Anthropogenic habitat deterioration and fragmentation not only leads to range contractions and extinctions, but may also have significant genetic, and thus evolutionary consequences for surviving populations. For the conservation of species inhabiting fragmented habitats, securing connectivity between local populations appears to be the major challenge.

Acknowledgements

Most feathers were collected by IS, GS and their students. We thank the many people who collected additional samples, and particularly Sepp Brunner, Jochen Fünfstück, Gerhard Gruber, Gerold Kilzer (Birdlife Vorarlberg), Siegfried Klaus, Juri Kurhinen, Peter Kuske, Emmanuel Menoni, Richard Mohr, Rainer Ploner, Francoise Preiss (GTV), Bernhard Scherer, Rudi Suchant (FVA), Peter Trontelj, Per Wegge, Hubert Zeiler and Albin Zeitler, who provided larger numbers of samples. Robert Paxton, Günther Steinbrück, Jürgen Tomiuk and two anonymous referees provided helpful comments on the manuscript. This research was funded by the DFG (STO 230/4). Funding from the Max-Planck Research Centre of Ornithology, Vogelwarte Radolfzell, to GS and IS allowed us to finalize the manuscript.

References

Baines D, Andrew M (2003) Marking of deer fences to reduce frequency of collisions by woodland grouse. *Biological Conservation*, **110**, 169–176.

Bensch S, Andersson T, Akesson S (1999) Morphological and molecular variation across a migratory divide in willow warblers, *Phylloscopus trochilus*. *Evolution*, **53**, 1925–1935.

Castroviejo J (1967) Eine neue Auerhuhnrasse von der Iberischen Halbinsel. *Journal für Ornithologie*, **108**, 220–221.

Castroviejo J (1975) *El Urogallo en espana*. Publicaciones del C.S.I.C. Monografias de la Estacion Biologica de Donana 3, Madrid.

Corunet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

Del Hoyo J, Elliott A, Sargatal J, eds. (1994) *Handbook of the Birds of the World*, 2. Lynx ediciones, Barcelona, Spain.

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, **10**, 305–318.

Goudet J (2001) FSTAT, *A Program to Estimate and Test Gene Diversities and Fixation Indices Version 2.9.3*. Available from http://www.unil.ch/izea/softwares/FSTat.html

Grapputo A, Pilastro A, Marin G (1998) Genetic variation and bill size dimorphism in a passerine bird, the reed bunting *Emberiza schoeniclus*. *Molecular Ecology*, **7**, 329–338.

Grimm V, Storch I (2000) Minimum viable population size of capercaillie *Tetrao urogallus*: results from a stochastic model. *Wildlife Biology*, **6**, 259–265.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics*, **48**, 351–372.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biology Journal of the Linnean Society*, **58**, 247–276.

Hewitt GM (2001) Speciation, hybrid zones and phylogeography — or seeing genes in space and time. *Molecular Ecology*, **10**, 537–549.

Hilton-Taylor C (2000) *2000 IUCN Red List of Threatened Species*. IUCN, Gland, Switzerland.

Jaarola M, Tegelström H, Fredga K (1999) Colonization history in Fennoscandian rodents. *Biology Journal of the Linnean Society*, **68**, 113–127.

Keller LF, Jeffery KJ, Arcese P *et al.* (2001) Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. *Proceedings of the Royal Society London B*, **268**, 1387–1394.

Klaus S (1997) Breeding and releasing projects for capercaillie in Germany. *Grouse News (Newsletter of the IUCN Grouse Specialist Group)*, **14**, 4–7.

Klaus S, Andreev AV, Bergmann H-H, Müller F, Porkert J, Wiesner J (1989) *Die Auerhühner*. *Neue Brehm Bücherei*. Ziemsen Verlag, Wittenberg Lutherstadt.

Kyle CJ, Strobeck C (2001) Genetic structure of North American wolverine (*Gulo gulo*) populations. *Molecular Ecology*, **10**, 337–347.

Lever C (1977) *The Naturalized Animals of the British Isles*. Hutchinson, London.

Lindén H, Teeri T (1985) Genetic differentiation in the capercaillie (*Tetra urogallus*), populations. *Hereditas*, **102**, 297–299.

Moss R, Piertney SB, Palmer SCF (2003) The use and abuse of microsatellite DNA markers in conservation biology. *Wildlife Biology*, **9**, in press.

Nei M, Roychoudhury AK (1974) Sampling variances of heterozygosity and genetic distance. *Genetics*, **76**, 379–390.

Nichols RA, Brufod MW, Groombridge JJ (2001) Sustaining genetic variation in a small population: evidence from the Mauritius kestrel. *Molecular Ecology*, **10**, 593–602.

Paetkau D, Waits LP, Clarkson PL *et al.* (1998) Variation in genetic diversity across the range of North American Brown Bears. *Conservation Biology*, **12**, 418–429.

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conservation Biology*, **12**, 844–855.

Potapov RL, Flint VE (1989) *Handbuch der Vögel der Sowjetunion. Band 4. Galliformes, Gruiformes*. Ziemsen Verlag, Wittenberg Lutherstadt, Germany.

Raymond M, Rousset F (1995a) An exact test for population differentiation. *Evolution*, **49**, 1280–1283.

Raymond M, Rousset F (1995b) GENEPOP Version 3.1d: population genetics software for exact tests and ecumenism. *Journal of Heredity*, **86**, 248–249.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN, Version 2.000: A Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Schroth K-E (1991) Survival, movements, and habitat selektion of released Capercaillie in the north-east Black Forest in 1984–89. *Ornis Scandinavica*, **22**, 249–254.

Segelbacher G (2002) Genetic structure of capercaillie populations: a non-invasive approach at multiple spatial scales. PhD Thesis, TU Munich.

Segelbacher G, Storch I (2002) Capercaillie in the Alps: genetic evidence of metapopulation structure and population decline. *Molecular Ecology*, **11**, 1669–1677.

Sokahl RR, Rohlf FJ (1995) *Biometry*. Freeman, New York.

Starling AE (1991) Workshop summary: captive breeding and release. *Ornis Scandinavica*, **22**, 255–257.

Storch I (2000) *Grouse Status Survey and Conservation Action Plan 2000–04*. WPA/BirdLife/SSC Grouse Specialist Group, IUCN, Gland, Switzerland/World Pheasant Association, Reading, UK.

Storch I (2001) *Capercaillie. BWP Update. The Journal of Birds of the Western Palearctic*. Oxford University Press, Oxford.

Storch I, Segelbacher G (2000) Genetic correlates of spatial population structure in central European capercaillie *Tetrao urogallus* and black grouse *T. tetrix*: a project in progress. *Wildlife Biology*, **6**, 305–310.

Suter W, Graf RF, Hess R (2002) Capercaillie (*Tetrao urogallus*) and avian biodiversity: testing the umbrella-species conept. *Conservation Biology*, **16**, 778–788.

Taberlet P, Fumagalli L, Wust-Saucy A-G, Coson J-F (1998) Comparative phyleogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.

Uimaniemi L, Orell M, Mönkkönen M, Huhta E, Jokimäki J, Lumme J (2000) Genetic diversity in the Siberian jay *Perisoreus infaustus* in fragmented old-growth forests of Fennoscandia. *Ecography*, **23**, 669–677.

Vernesi C, Pecchioli E, Caramelli D, Tiedemann R, Randi E, Bertorelle G (2002) The genetic structure of natural and reintroduced roe deer (*Capreolus capreolus*) populations in the Alps and central Italy, with reference to the mitochondrial DNA phylogeography of Europe. *Molecular Ecology*, **11**, 1285–1297.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Westemeier RL, Brawn JD, Simpson SA *et al.* (1998) Tracking the long-term decline and recovery of an isolated population. *Science*, **282**, 1695–1698.

Wiens JA (1996) Wildlife in patchy environments: metapopulations, mosaics, and management. In: *Metapopulations and Wildlife Conservation* (ed. McCullough DR). Island Press, Washington, DC.

This study formed part of the PhD of Gernot Segelbacher on population structure of capercaillie in Europe. He is now a research fellow at the Max Planck Research Centre of Ornithology and interested in effects of habitat fragmentation on bird populations and the application of genetics in conservation. Ilse Storch, who is associated with both the Technical Universität München and the Max Planck Research Centre of Ornithology, has been conducting research on grouse for > 15 years and chairs the IUCN Grouse Specialist Group. She initiated the project on spatial population structure of grouse of which this study was part. This work is part of the cooperation between GS and Jacob Höglund, Professor in Population Biology at the Evolutionary Biology Centre (EBC) at Uppsala University. He is currently working on genetic variation in natural populations.
