

Phylogeography of the European capercaillie (*Tetrao urogallus*) and its implications for conservation

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Abstract The phylogeographic pattern of mitochondrial DNA variation in capercaillie (*Tetrao urogallus*) from 14 populations across the European range was examined using sequence variation in the central portion of the control region and the cytochrome B gene. Twenty-nine different haplotypes were resolved, which formed two distinct phylogenetic clades. The major division separated haplotypes found in the Pyrenees from those found in the rest of Europe. However, two Pyrenean birds were found within the central European clade. The Pyrenean population is likely to be derived from an Iberian glacial refugium, whereas the other European populations have been recolonized from a refugium in the East. Birds from the Eastern refugium spread west until they reached the Pyrenees, where they mixed with birds from the Iberian refugium. Connectivity between populations from central, eastern, and northern Europe and the Pyrenees was interrupted by the extinction of interconnecting populations, leading to the formation of a different subspecies (*Tetrao urogallus aquitanicus*). We argue that the Pyrenean birds should be

therefore regarded as a separate evolutionary significant unit, and we discuss the status of the other capercaillie populations in a conservation context.

Keywords *Tetrao urogallus* · Capercaillie · mtDNA · Control region · Cytochrome B

Introduction

The capercaillie (*Tetrao urogallus*, L. 1758) is the largest gamebird species of Western Palearctic boreal and montane forests, and is considered an important umbrella species in these ecosystems (Suter et al. 2002; Pakkala et al. 2003). It inhabits a continuous range in large parts of the Palearctic boreal forest, but is patchily distributed in temperate Europe (Storch 2001), primarily because of the scattered distribution of coniferous forest that remained after the last Ice Age, and due to human impact on landscapes. Population decline is an ongoing process across most of its geographic range, is most pronounced where forest fragmentation is highest, and already has led to the extinction of several populations in central Europe (Klaus et al. 1989).

Long-term efforts to conserve capercaillie in Europe require recourse to the causes and consequences of population fragmentation to ensure that management occurs at the appropriate geographic scale. Some capercaillie populations are already recognized to be subspecies, most notably those in the Pyrenees (Potapov and Flint 1989) and the Cantabrian mountains (Castroviejo 1975). A total of 12 different subspecies have been suggested to occur across the range (del Hoyo et al. 1994), though there is some uncertainty over whether the plumage and size characters used to define these are appropriate (Klaus et al. 1989;

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Storch 2001). Indeed, results from a Finnish study indicated that morphologically defined subspecies were not supported by genetic analyses (e.g., Liukkonen-Antilla et al. 2004).

Phylogeographic studies can provide useful information for delimitating evolutionary units and management units, thus enabling the design of efficient conservation strategies. There is a recognized need to accurately assess the phylogeographic structure of capercaillie in Europe to identify management and evolutionary significant units, and thus enhance future conservation effort. An earlier study based on microsatellite data (Segelbacher et al. 2003) has shown that most European capercaillie populations, which are geographically isolated, are genetically differentiated from each other. However, it is unclear if this pattern can be attributed to recent habitat fragmentation or is a result of long-term historical isolation. The quaternary glaciations in Europe explain patterns of phylogeographic structure in a wide range of species (Hewitt 2004). During periods of extensive glaciations, many species shifted their ranges to milder climate areas at low altitude (Taberlet et al. 1998), such as the Iberian Peninsula, which now represent important hot spots of genetic diversity at the edge of the distribution range (Hampe and Petit 2004).

In this study, we examine the patterns of mitochondrial DNA variation from 14 sampling locations that span the

complete European range of capercaillie. We not only identify how genetic variation is apportioned within and among populations, but also determine the evolutionary relationships among the populations studied. Sequence data can be utilized to separate the effects of contemporary processes such as recurrent gene flow and population isolation from historical population processes that also affect the spatial distribution of allelic variation.

Specifically, we focus on the following questions:

1. What are the evolutionary relationships among the studied populations?
2. How is the genetic variation apportioned within and among populations across the species' range across Europe?

This study will give a more detailed picture of the genetic diversity of the endangered populations in Europe and provide valuable information for conservation strategies.

Methods

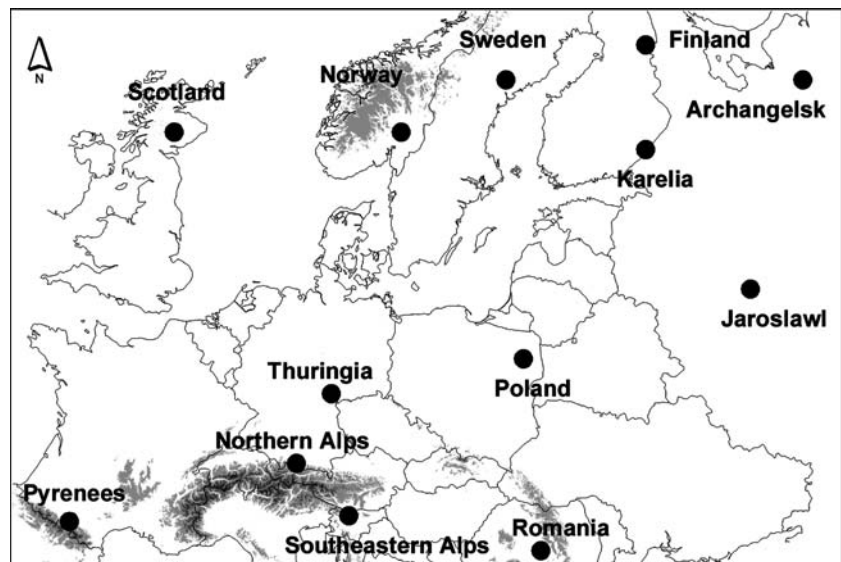
Samples were collected from 221 capercaillie from 14 sampling locations spanning the entire European distribution range (see Table 1 and Fig. 1). The majority of the

Table 1 Distribution of haplotypes across the different sampling locations. Codes at the lower end refer to Genbank accession numbers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Total	Different	Unique
Archangelsk					1	1																							2	2	1	
Jaroslavl						1				2																				3	2	0
Karelia				1						1																				2	2	0
Norway		1					1	1																						3	3	2
Sweden			1	1																										2	2	0
Northern Alps	1																1				1									3	3	0
Southern Alps	1	1									2	3	2	1	2	1	1				1									15	10	7
Black Forest				2						1							2	2												7	4	1
Pyrenees				1						1												1	2	1						6	5	3
Scotland			1														1									1	3	1	1	8	6	4
Thuringia																									1					1	1	1
Poland										1																				1	1	0
Romania									1																					1	1	1
Finland																			1											1	1	1

Combined dataset 809 bp, 55 sequences

Fig. 1 Map of the geographical locations of the sampled *Tetrao urogallus* populations



samples were molted feathers, with a few samples taken from museum skins. DNA was extracted as described previously (Segelbacher 2002).

A DNA sequence from all 221 individuals was obtained for the 5' hypervariable domain of the mtDNA control region. For a subset of these individuals ($n = 55$) a cytochrome *b* sequence was also obtained that was concatenated with the control region data to provide better resolution for phylogenetic analysis. The control region was PCR-amplified with the heavy primer (5'-GTG AGG TGG ACG ATC AAT AAA T-3') annealing in the control region and the light primer (5'-TTG TTC TCA ACT ACG GGA AC-3') annealing in the adjacent tRNA^{glu} region. The cytochrome *b* region was PCR-amplified using the primers F (5'-ATC CTC GCT GGC CTC CTA C-3') and R (5'-GGG TGA AAA TAG GGC TAA TGT G-3'). All reactions were performed in a total volume of 50 μ l in thin-walled 200 μ l tubes using an MJ Research (Waltham, MA, USA) PTC-100 thermal cycler. The reaction mix contained approximately 25 ng DNA, 2.5 mM MgCl₂, 75 mM Tris-HCl (pH 9.0), 20 mM (NH₄)₂SO₄, 0.01% (v/v) Tween-20, 0.2 mM of each nucleotide, 5 pmol of each primer and 1.0 unit of Bioline *Taq* polymerase. After an initial denaturation step of 3 min at 90 °C, 30 cycles of PCR were performed, each cycle consisting of 30 s denaturation at 90 °C, 30 s annealing at 55 °C, and 30 s extension at 72 °C. PCR products were purified using the Qiagen (Venlo, The Netherlands) PCR purification system, and sequenced using a PerkinElmer (Waltham, MA, USA) ABI 377 automated sequencer (cycle sequencing according to the manufacturer's instructions). Sequences have been deposited in GenBank (accession numbers DQ352072-DQ352131). Multiple sequence alignments were obtained

using BioEdit (Hall 1999), with subsequent confirmation by eye.

Within-population genetic diversity (haplotype and nucleotide diversity) was calculated using DnaSP version 4.0 (Rozas et al. 2003) and MEGA version 3 (Kumar et al. 2004). A phylogeny of the haplotypes was constructed using maximum likelihood (ML) within PAUP (version 4.0b8; Swoford 1993). The optimal model of sequence evolution used in the analysis was identified using Modeltest version 3.0 (Posada and Crandall, 1998), with selection based on the Akaike Information Criterion (AIC; Akaike 1974). It had the following parameters: base frequencies of 0.2625, 0.2691, 0.1832 and 0.2821 for A, C, G, and T nucleotides respectively; transition:transversion = 3.264; a proportion of invariable sites = 0.9436; and a gamma shape parameter = 0.1948. The ML topology was constructed using a heuristic search with a tree bisection and reconnection (TBR) algorithm, and was rooted through *Tetrao parvirostris*. The stability of internal nodes was assessed by bootstrap analysis (1,000 replicates).

Results

Mitochondrial DNA sequence variation

A total of 252 base pairs were unambiguously resolved for all 221 individuals from the control region. Among all European samples, overall species nucleotide diversity (π) was 0.0089 (SD = 0.0027), and haplotype diversity was 0.832 (SD = 0.016). Nucleotide diversity was highest in the Pyrenean birds. Birds from the southern part of the Alps display higher levels of diversity than in the Northern

Table 2 Genetic diversity in sampled populations where polymorphism was resolved

	<i>N</i>	<i>h</i>	π
Archangelsk	3	1.000 + 0.272	0.0053 + 0.0037
Jaroslavl	7	0.524 + 0.209	0.0023 + 0.0023
Karelia	2	1.000 + 0.500	0.0012 + 0.0006
Norway	8	0.821 + 0.101	0.0049 + 0.0026
Sweden	4	0.833 + 0.222	0.0046 + 0.0031
Northern Alps	20	0.811 + 0.052	0.0058 + 0.0025
Southern Alps	39	0.883 + 0.027	0.0078 + 0.0033
Black Forest	37	0.517 + 0.086	0.0041 + 0.0019
Pyrenees	22	0.662 + 0.071	0.0181 + 0.0036
Scotland	71	0.705 + 0.046	0.0045 + 0.0018
Thuringia	8	0.679 + 0.122	0.0033 + 0.0021

Control region, 252 bp; *N*, number of individuals sampled; *h*, haplotype diversity, π , nucleotide diversity, + standard deviation

Alps, and isolated populations like the Black Forest and Thuringia show decreased diversity levels (Table 2).

For 55 individuals we resolved a total of 809 bp across both the control region and the cytochrome *b* amplicons. Within these samples, 26 variable sites were resolved, which defined 29 haplotypes (Table 1). Of these, only six were found in more than one population.

The evolutionary relationships between all haplotypes are shown by an ML phylogeny in Fig. 2. The most striking feature of the topology was the well-supported separation of haplotypes 22–24 from all other haplotypes. Haplotypes 22–24 were found only in the Pyrenees. Two additional haplotypes (4 and 11) were resolved from the Pyrenean birds, though these formed a part of the other major clade, and were also found in other sampling locations. The diversity of the Pyrenean samples was higher than that of any other sampling location (Table 2).

Discussion

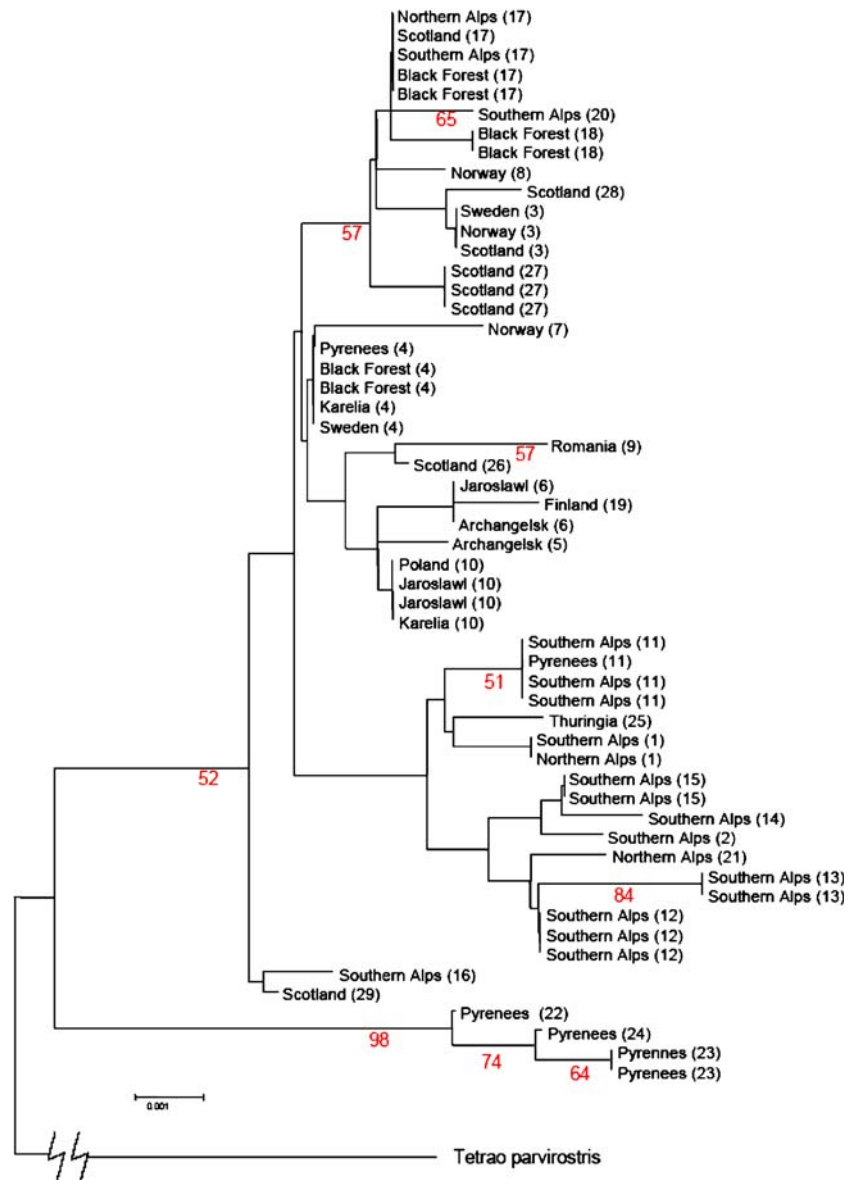
The patterns of phylogeographic structure indicate that extant European populations are derived from two primary glacial refugia, Iberia and Eastern Europe. After the ice retreated, the Iberian populations appeared to have remained restricted to Iberian populations (the Pyrenees and Cantabrian regions), whilst the remainder of Europe was likely recolonized from Eastern Europe. The prominent feature of the intraspecific phylogeny of capercaillie across Europe was a major division between three haplotypes found only in the Pyrenees and all other haplotypes. These clades were separated by several mutation steps, and such divergence is likely a consequence of isolation in separated glacial refugia. We also found the highest genetic diversity within the Pyrenean bird population. The idea that

marginal rather than central populations harbor the bulk of the species' genetic diversity is well supported by other studies (e.g., Petit et al. 2003; Hewitt 2004). Capercaillie reach their most westerly distribution in the Cantabrian mountains and the Pyrenees, and those birds could therefore be classified as rear edge populations according to Hampe and Petit (2005). As modern climate change is shifting species borders, the environmental response of species may be determined by populations at the range margins (Hampe and Petit 2005). These populations may act as a long term store of species' genetic diversity, and should therefore be of specific conservation concern. Moreover, capercaillie in the Pyrenees may have developed specific adaptations to their environment. Pyrenean birds differ from other European populations morphologically and ecologically, which might be an adaptation to the climate in south-western Europe. Based on our sequence data, a split indicates that the Pyrenean samples form an evolutionary significant unit, which is consistent with the subspecific status argued by Potapov and Flint (1989) and the subdivision detected with microsatellites (Segelbacher et al. 2003). This pattern is consistent when analysis is restricted to just the control region for all 221 individuals, and when other optimality criteria are used in phylogenetic analysis (data not shown).

The dominant European clade contains some Pyrenean birds. This suggests secondary contact between populations isolated after the ice age and indicates an admixture of different populations in the Pyrenean Mountains. Capercaillie habitat has been more widespread in the past, allowing gene flow across Europe. Whether such gene flow is still ongoing remains unclear. Current phylogeographic findings based on mtDNA reflect historic distribution patterns. Previously, a population existed in the Cevennes that likely formed a stepping stone for occasional migration between the Pyrenees and other populations such as the Alps. This population has become extinct, and as such it is likely that gene flow will have ceased, leading to the complete isolation of the Pyrenees.

Aside from the separation of Pyrenean samples, there is very little geographic structure within the main clade. Amongst all other clades we could not detect any further subdivision across European populations. Isolated populations in central Europe harbor less genetic variation than in the Alps, but the strong genetic differentiation detected with microsatellites indicates that fragmentation of these isolated populations occurred rather recently. The isolated Scottish population displays similar low levels of genetic diversity to other central European isolated populations. However, there appears to be no overall genetic divergence of Scottish samples, which reflects the fact that Scottish birds are descended from Scandinavian stock introduced to Scotland over the past few hundred years.

Fig. 2 Maximum likelihood derived phylogeny showing the relationships between all sample haplotypes, based upon concatenated mitochondrial control region and cytochrome *b* sequences. Bootstrap confidence indices (derived from 100 replicates) are provided on internal nodes. To facilitate the internal subdivision within *Tetrao urogallus*, the rooted branch to *Tetrao parvirostris* is truncated. Names of sampling locations and haplotype numbers in brackets are given



Ultimately, whilst our data do not indicate any long-term isolation of populations within the main clade, a population genetic analysis based on neutral markers would be most appropriate for an examination of the local genetic structure. Studies investigating levels of microsatellite diversity and divergence have highlighted that even populations isolated for only short time periods (i.e., less than 100 years) show high levels of population differentiation. These recently isolated populations already display lower genetic diversity and signs of genetic impoverishment (Segelbacher et al. 2003). Furthermore, a fine-scale genetic study based on microsatellites in the Black Forest highlighted that the genetic legacy of the frequent reintroductions of capercaillie over the last few decades could not be detected (Segelbacher, in review).

Our results suggest limited evidence for different subspecies of capercaillie within Europe. Certainly, there is no justification for the 12 subspecies argued by del Hoyo (1994). Such assertions have previously been criticized, as they were based on morphological divergence, which tends to be clinal, and may reflect local ecotypes as opposed to deeper evolutionary splits (Klaus et al. 1989; Storch 2001). The present study did not include samples from the Cantabrian mountains, which appear to be genetically differentiated from other European populations but are derived from the Iberian refugium (Duriez et al. 2007; Rodriguez-Munoz et al 2007). Our detected genetic pattern of recolonization would argue for two evolutionary significant capercaillie units within Europe: the Pyrenees and all other populations. More detailed sampling of capercaillie from locations not

represented in the current study, especially in the eastern mountains of south-eastern Europe, might identify further distinct clades indicative of further refugial populations.

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