Genetic correlates of spatial population structure in central European capercaillie Tetrao urogallus and black grouse T. tetrix: a project in progress

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Genetic correlates of spatial population structure in central European capercaillie *Tetrao urogallus* and black grouse *T. tetrix*: a project in progress

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Capercaillie *Tetrao urogallus* and black grouse *T. tetrix* are threatened species in central Europe. Their habitats are fragmented both at a continental and a regional scale, and spatial connectivity may play an important role for their (meta-)population dynamics and persistence. In order to identify conservation priorities, it is important to know if and to what extent exchange between local populations occurs. In this paper, we present the rationale and techniques of an ongoing project into the spatial structure of capercaillie and black grouse populations in central Europe using non-invasive genetic methods. In this project, we assess the genetic differentiation of spatially distinct populations using microsatellite analysis based on DNA extracted from feathers. This approach will allow us to identify critical geographic distances beyond which demographic connectivity between populations is not assured. We expect to find a correlation between geographic and genetic distance.

Key words: black grouse, capercaillie, conservation genetics, genetic markers, metapopulation, microsatellites, Tetrao tetrix, Tetrao urogallus

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Capercaillie *Tetrao urogallus* and black grouse *T. tetrix* are listed in the Red Data Books in most western and central European countries (Storch 2000). Because their distribution ranges are highly fragmented, and many populations are small and spatially separated (see Bergmann & Klaus 1994, Klaus & Bergmann 1994), dispersal may play an important role for the dynamics and persistence of populations and metapopulations. Information on dispersal patterns, however, is lacking. In this paper, we present the rationale and techniques of an ongoing project into the spatial structure of capercaillie and black grouse populations in central Europe using non-invasive genetic methods. The study will provide data which allow us to determine the degree of exchange between populations, to identify critical distances for population connectivity, and to detect sex-specific and species-specific differences in dispersal patterns.

The metapopulation hypothesis

The landscape of central Europe is dominated by farmland. Forests are distributed as isolated fragments within a matrix of open land; small woodlots no more than a few hectares in size prevail. Contiguous forests covering several hundred square kilometres are restricted to mountainous regions. These are the areas where most of the remaining capercaillie and black grouse populations are found. Capercaillie are coniferous forest obligates. They prefer extensive areas of forest with moderate canopy cover and rich ground vegetation dominated by ericaceous shrubs such as bilberry *Vaccinium myrtillus*. The black grouse is a species with broader requirements. It inhabits forest edges and early stages of forest succession. Moorland, heaths, treeline habitats and alpine pastures are typical habitats. In central Europe, both species have their strongholds in the Alps (see Klaus, Andreev, Bergmann, Müller, Porkert & Wiesner 1989, Klaus, Bergmann, Marti, Müller, Vitovic & Wiesner 1990, Storch 2000).

In their boreal distribution range, capercaillie and black grouse live in contiguous forest landscapes. In central Europe, however, grouse habitats, and thus populations, are spatially structured at two hierarchical levels of scale: forest-dominated uplands versus farmland-dominated lowlands at the continental scale (i.e. central Europe), and forested mountain slopes versus open habitats at the regional scale (e.g. in the Alps). As a consequence, distribution ranges of grouse are separated by up to 100 and more kilometres at the continental scale (see Bergmann & Klaus 1994, Klaus & Bergmann 1994), and local populations by up to 10 and more kilometres at the regional scale (I. Storch, unpubl. data). From this distribution pattern, metapopulation structure has been hypothesised for central European capercaillie and black grouse populations (see Rolstad 1991, Storch 1993, 1995, 1997a,b). The distribution of capercaillie and black grouse has always been patchy in central Europe due to the naturally patchy distribution of suitable habitats. However, the degree of fragmentation and the properties of both habitat patches and the surrounding matrix have been altered by human land use practices. Accordingly, in the course of the 20th century grouse populations may have been shifting towards decreasing connectivity along the gradient from spatially structured to metapopulation to isolated populations.

Dispersal distances in capercaillie and black grouse are roughly known from marked birds. Summarising published results, average seasonal movements of 1-2 km may be expected for adults and median dispersal distances of less than 10 km may be expected for juvenile birds (Table 1). The longest dispersal distances recorded are 34 km for black grouse (Marja-

Table 1. Juvenile dispersal distances and adult seasonal movement distances of black grouse (Bg) and capercaillie (Ca). All data come from radio-marked or wing-tagged birds. Not all studies distinguished sexes and some reported mean, other median distances. Maximum distances recorded (Max) were rounded to full kilometres.

Species	Sex	Mean	SE	Median	N	Max	Location	Author
Juvenile dispersal, Bg				<2	68	26	Scandinavia	Myrberget 1978
		6.3			4	8	Sweden	Willebrand 1988
				9.5	28	34	Finland	Marjakangas et al. 1991
		6.2	1.6	2.0	29	26	Fennoscandia	Swenson 1991
	♀	8.0		7.5	16	29	French Alps	A. Caizergues, pers. comm.
	♂	1.1		0.8	11	8	French Alps	A. Caizergues, pers. comm.
Juvenile dispersal, Ca	♀				?	24	Fennoscandia	Koivisto 1963*
	♂				?	4	Fennoscandia	Koivisto 1963*
				<2	39	75	Scandinavia	Myrberget 1978
		6.7	1.1	3.0	55	38	Fennoscandia	Swenson 1991
	♀	>5.0			1		German Alps	Storch 1993
	♂	0.6			1		German Alps	Storch 1993
	♀	5.2			18		Northern Ural	Beshkarev et al. 1995
	♂	1.2			6		Northern Ural	Beshkarev et al. 1995
	♀					30	Scotland	R. Moss, pers. comm.
Adult movements, Bg				<2	12	3-5	Scandinavia	Myrberget 1978
				<1	38	9	Sweden	Willebrand 1988
				1.1	12	9	French Alps	Ellison et al. 1989
Adult movements, Ca				3-5	8	11-20	Scandinavia	Myrberget 1978
	♀	1.9			15	8	Norway	Rolstad et al. 1988
	♂	1.5			44	10	Norway	Rolstad et al. 1988
				<1	11	8	Pyrenees	Ménoni 1991
	♀	0.8	0.3		7	7	German Alps	Storch 1995
	♂	1.4	0.4		19	9	German Alps	Storch 1995
	♀					18	Scotland	R. Summers & R. Procter, pers. comm.

* Cited in Klaus et al. 1989

kangas, Aspegren & Kyllönen 1991) and 75 km for capercaillie (Myrberget 1978). Comparing the spatial distribution of habitats and recorded dispersal distances in capercaillie and black grouse, genetic and demographic isolation at a continental scale may be assumed. At a regional scale, it is likely that juveniles disperse between local populations so that metapopulation structure can be assumed (Storch 1993, 1995, 1997a). Furthermore, due to their supposedly better colonisation abilities (see Klaus et al. 1990), black grouse may show a different pattern in the differentiation of subpopulations than capercaillie.

DNA from feathers: techniques and experience

In the past, dispersal studies on birds had to rely on classical methods such as radio-tracking or banding. However, these methods require a lot of work, provide limited data sets and cause a relatively high level of disturbance to the birds, which is particularly problematic when dealing with endangered species. To our knowledge, there is no published example of a study on grouse in central and western Europe that achieved more than anecdotal data on dispersal rates and distances. Advances in molecular biology now allow us to address the questions of dispersal and gene-flow more effectively. Genetic information on individuals and populations can be obtained by analysing DNA from feathers or faeces (see Taberlet, Waits & Luikart 1999). These techniques may provide large data sets with relatively little effort and disturbance to the study species.

We collected feather samples from capercaillie and black grouse on a continental scale (distribution areas; e.g. Black Forest, Alps, Vosges) in central Europe and on a regional scale (mountain ranges) in the Alps. For both capercaillie and black grouse, we attempt to analyse feathers from 10-20 areas at either scale. Each sample should comprise feathers from at least 10 birds. Individuals will be separated genetically during the analyses. Most samples obtained are moulted feathers collected during the summer months; others came from carcasses and birds killed by hunters. Feathers were stored in a dry and dark place and frozen at minus 20° C as soon as possible after collection. DNA was extracted using a silica column method (Qiagen) following the protocol of the manufacturer. PCR-amplifications were then performed in 10µl reactions in an Eppendorf Gradient thermal cycler. PCR fragments were resolved by electrophoresis on 6% denaturing polyacrylamide gels and afterwards stained with silver.

Microsatellites, short repetitive tandem sequences of DNA, are genetic markers that allow us to measure gene flow and to quantify genetic variability (Queller & Strassmann 1993, Schlötterer & Pemberton 1994). They also allow us to reconstruct kin relationships (Primmer, Møller & Ellegren 1995, Blouin, Parsons, Lacaille & Lotz 1996). Microsatellites have been used successfully to estimate exchange rates between vertebrate populations (Waser & Strobeck 1998). Due to the large variability of microsatellite loci, the populations from which dispersing individuals have originated can be identified with high reliability. Examples of microsatellite-based dispersal studies exist for fish (Nielsen, Hansen & Loeschcke 1997), birds (Haig, Gratto-Trevor, Mullins & Colwell 1997), and mammals (Favre, Balloux, Goudet & Perrin 1997). These studies imply that there is a close correlation between the distribution of genotypes and the frequency of dispersal between populations (Favre et al. 1997). Thus, genetic studies are suitable both to add to our understanding of dispersal in grouse and to test hypotheses concerning the exchange between populations (Favre et al. 1997, Waser & Strobeck 1998) and within metapopulation systems (Hastings & Harrison 1994, Harrison & Hastings 1996).

When we began our study, techniques to extract DNA from feathers had been tested successfully for several grouse species, including the black grouse (e.g. Piertney & Dallas 1997). Comparing different extraction methods we found that a silica based column method proved to give the best yield of DNA and the most reliable results in PCR compared to the Chelex method or classical phenol-chloroform extraction. We also tested DNA extraction from faeces, but results were less promising compared to feathers. In particular, we had lower success with correctly genotyping individuals with DNA from faeces. Possibly, DNA from grouse faeces is more degraded than that from feathers (see Taberlet et al. 1999).

Primmer, Møller & Ellegren (1996) found that microsatellites could be cross-species amplified in a wide range of bird species. However, against expectation domestic chicken *Gallus gallus* primers proved not to be suitable in turkey *Meleagris gallopavo* (Liu, Crooijmans, van der Poel & Groenen 1996) and sage grouse *Centrocercus urophasianus* (Oyler-McCance, Kahn, Burnham, Braun & Quinn 1999). Even primers developed in red grouse *Lagopus lagopus scoticus* are of limited use in sage grouse and black grouse (Oyler-McCance et al. 1999, Höglund, Alatalo, Lundberg, Rintamäki & Lindell 1999). Our own experience agrees with these results. We tested more than 30 microsatellite primer pairs developed for the domestic chicken (Cheng,

Levin, Vallejo, Khatib, Dodgson, Crittenden & Hillel 1995, Hanotte, Pugh, Maucher, Dawson & Burke 1997) and 16 primer pairs developed for the red grouse (Piertney & Dallas 1997, Piertney, MacColl, Bacon & Dallas 1998) for their use in capercaillie and black grouse. We found only one chicken primer pair (Lei0319) and five red grouse primer pairs (LLST1, LLSD2, LLSD3, LLSD4, LLSD8) showing clear scorable bands, of which only three (Lei0319, LLST1, LLSD3) amplified loci with ≥3 alleles in both species. Apparently, microsatellite primers developed in related species of the order Galliformes are only of little use in tetraonids, especially if hypervariable loci are necessary for analysis.

A set of hypervariable microsatellites is available for black grouse from other studies (S. Piertney, pers. comm.). For capercaillie, we developed specific microsatellite primers (Segelbacher, Paxton, Steinbrück, Trontelj & Storch 2000). As the frequency of repeat sequences generally seems to be very low in birds (Primmer, Raudsepp, Chowdhary, Møller & Ellegren 1997) we used an enrichment protocol (Piertney et al. 1998) to isolate highly variable microsatellites. The loci showed high polymorphism which proved their suitability for kinship analyses and population studies (Segelbacher et al. 2000).

In spring 2000, we have begun to analyse the first batches out of about 1,500 feather samples from capercaillie and black grouse from the Alps and different parts of Europe. We expect to finish the laboratory work in the course of 2001, and to be able to present the results in 2002.

Perspectives

Based on capercaillie and black grouse feather samples from different parts of Europe and from different mountain ranges across the Alps, we expect to gain insight into the effects of geographic distance and habitat fragmentation on genetic differentiation, and on species-specific and sex-specific (see Mossmann & Waser) differences in dispersal patterns. Specific statistical methods (see review by Luikart & England 1999) are available for the analysis of microsatellite data which allow us to estimate dispersal rates from the relative distributions of genotypes within and between populations (Favre et al. 1997). In order to validate our results, however, it will also be important to relate genetic to behavioural data whenever possible. Therefore, we intend to compare our genetic results with those from radiotracking studies on dispersing birds going on elsewhere (e.g. A. Caizergues, pers. comm., P. Wegge, pers. comm.). To assess the relative importance of geographic distance and habitat fragmentation for the genetic differentiation of grouse populations, we plan to compare results from the Alps, i.e. a patchy distribution range, with results from a contiguous range in the boreal forest. Furthermore, we may be able to analyse existing feather and tissue samples from small, isolated populations collected during the process of extinction. This will allow us to assess potential changes in genetic variation during a population decline until extinction.

Genetic studies such as the one introduced here can be powerful tools to improve our understanding of dispersal patterns in grouse. They may help us estimate the frequency of exchange between populations (Favre et al. 1997, Waser & Strobeck 1998) and to assess the spatial structure of populations and metapopulations (Hastings & Harrison 1994, Harrison & Hastings 1996). Thus, we expect that our study will make a contribution to identify critical distances of population connectivity, to develop spatial models of (meta-)population dynamics, and last but not least, to improve capercaillie and black grouse conservation and management approaches in central Europe.

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