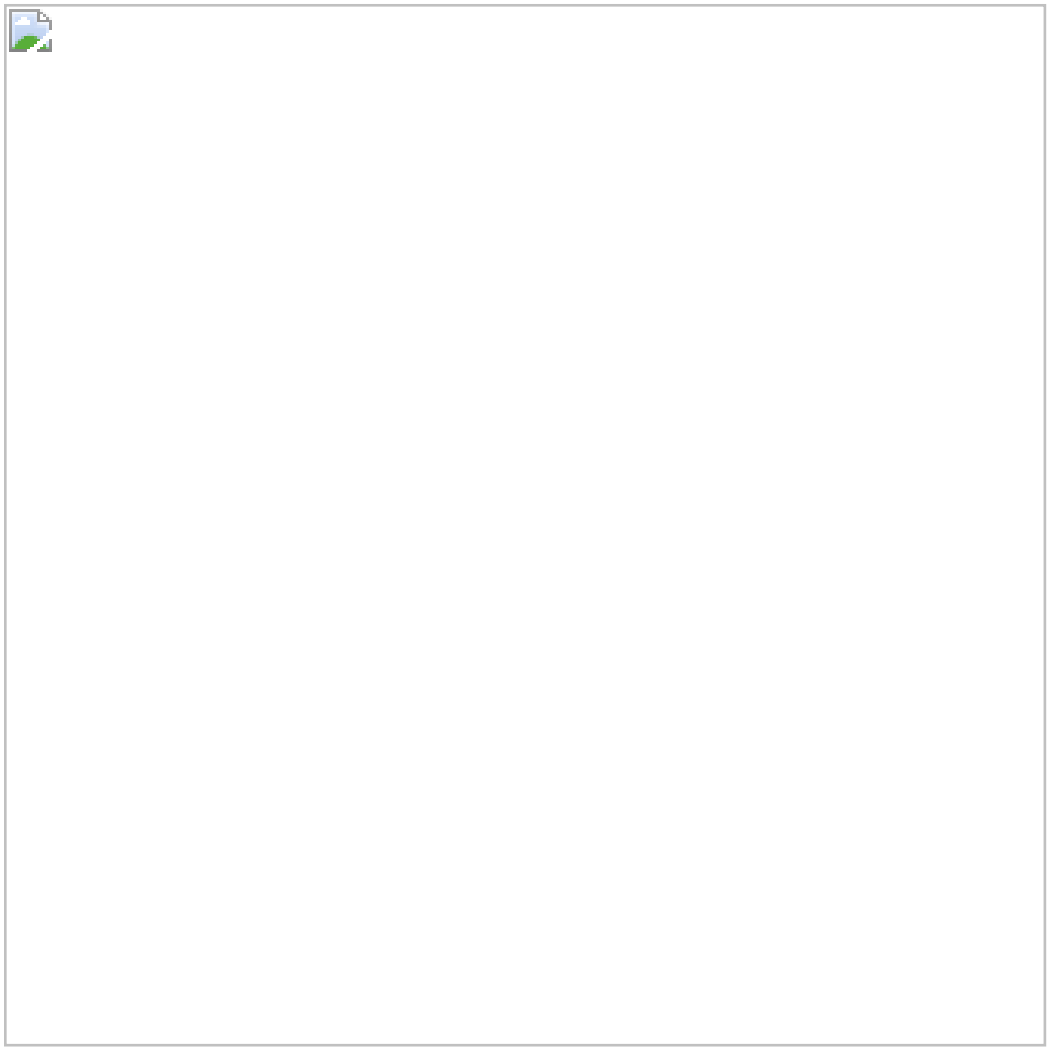

Genetic Variability of Polish Population of the Capercaillie Tetrao urogallus

Authors: Rutkowski, Robert, Niewęgłowski, Hubert, Dziedzic, Roman, Kmieć, Marek, and Goździewski, Jan

Source: Acta Ornithologica, 40(1) : 27-34

Published By: Museum and Institute of Zoology, Polish Academy of Sciences

URL: https://doi.org/10.3161/068.040.0108

ACTA ORNITHOLOGICA
Vol. 40 (2005) No. 1

Genetic variability of Polish population of the Capercaillie *Tetrao urogallus*

Robert RUTKOWSKI[1], Hubert NIEWĘGŁOWSKI[2], Roman DZIEDZIC[3], Marek KMIEĆ[4] & Jan GOŹDZIEWSKI[5]

[1]Museum and Institute of Zoology Polish Academy of Sciences, Wilcza 64, 00–679 Warsaw, POLAND, e-mail: robertrut@miiz.waw.pl
[2]Institute of Animal Reproduction and Food Research PAS, Tuwima 10, 10–747 Olsztyn, POLAND, e-mail: hubert@pan.olsztyn.pl
[3]Agricultural University of Lublin, Akademicka 13, 20–950 Lublin, POLAND, e-mail: Dzied@ursus.ar.lublin.pl
[4]Forest District Ruszów SF NFH, Zgorzelecka 1, 59–930 Ruszów, POLAND, e-mail: keram@rubikon.pl
[5]Polish Hunting Association, 1 Maja 25, 16–400 Suwałki, POLAND

Rutkowski R., Niewęgłowski H., Dziedzic R., Kmieć M., Goździewski J. 2005. Genetic variability of Polish population of the Capercaillie *Tetrao urogallus*. Acta Ornithol. 40: 27–34.

Abstract. The Capercaillie is one of the most seriously endangered bird species in Poland. It currently numbers around 650 individuals that live in four isolated populations (Lower Silesian Forest, Janów Lubelski Forest, Carpathians, Augustów Forest). This study investigated genetic variability based on the polymorphism of six microsatellite loci in the surviving Polish populations of the Capercaillie and compares the results with the analogous variability in two large, contiguous populations in Russia. The following parameters were estimated: mean number of alleles per locus, allelic richness, mean effective number of alleles per locus, heterozygosity in each of the populations investigated. Differentiation between pairs of populations was assessed using F_{ST}. The results show that despite some inevitable reduction in genetic variability, most of the Polish populations retained a substantial level of microsatellite polymorphism. Only in the population from Janów Lubelski Forest was there a significant reduction in variability, probably due to long isolation and the recent decline. That this population has long been isolated was also confirmed by the pronounced genetic differentiation from the other Polish populations. The Carpathian population of the Capercaillie was found to be genetically structured, and in the Lower Silesian Forest population heterozygosity was low, possibly as a result of the lek mating system and also the dramatic reduction in numbers.

Key words: Capercaillie, *Tetrao urogallus*, genetic variability, population structure, microsatellites

Received — Sept. 2004, accepted — March 2005

INTRODUCTION

The Capercaillie prefers old, mainly coniferous forests with damp soil, diversified with bogs, areas of peat or glades, with dense undergrowth of ericaceous plants (Cramp & Simmons 1980). The species still occupies most of its original range and occurs throughout the boreal forests from eastern Siberia to Scandinavia. However, in some countries of Central and Western Europe Capercaillie becomes extinct. The most numerous European population occurs in Scandinavia, where the number of individuals is estimated at 600 000 (Storch 2000). The Alps are the central European stronghold of the Capercaillie with an estimated total population size above 30 000 birds (Storch 2001). The size of Russian population of the species is estimated at 1.4 mln individuals (Storch 2000).

Formerly widespread breeding bird in Poland, the Capercaillie has disappeared from central and north-western parts of the country in 18th and 19th centuries. In 20th century, the decline of the species was still fast and severe, especially before 1994, when ban of hunting on leks was introduced. Now, the Capercaillie distribution in Poland is restricted to four isolated regions: Augustów Forest (50–70 males), Janów Lubelski and Solska Forest (60–80 males), Carpathians (120–150 males) and Lower Silesian Forest (40–45 males) (Tomiałojć & Stawarczyk 2003, Fig. 1). However, recent information from forest

rangers indicate, that the number of birds living in Lower Silesian Forest does not exceed 30 individuals (M. Kmieć, pers. com.). In Białowieża Forest single birds are occasionally observed, probably moving from the Belarusian part of the Forest. The total population size (males and females) in Poland can be estimated at 550–750 individuals (Tomiałojć & Stawarczyk 2003).

Among the main threats for Polish populations of the Capercaillie, habitat degradation and fragmentation are considered as the most important. Moreover, fragmentation of habitat is known to affect genetic population structure, and it may also lead to the loss of genetic variation and increased inbreeding, especially in case of population of limited size (Soulé 1987, Frankham 1995, Frankham et al. 2002). Although the importance of inbreeding in natural population has been questioned for a long time, the new evidence has appeared, which suggests that inbreeding and loss of genetic variation are important factors, increasing the probability of extinction (Westemeier et al. 1998, Keller & Waller 2002). Genetic changes in population structure magnify the extrinsic sources of jeopardy, making populations susceptible to diseases, unfavorable enviromental changes and random demographic events (Soulé & Mills 1998). Thus, the knowledge of genetic variation in endangered species is considered as more and more crucial factor in planning conservation strategies (Hedrick 2001).

Microsatellites are one of the most popular genetic markers, frequently used in estimation of genetic variability. They are defined as tandem repeats of short (two-, three-nucleotide etc.) DNA motif. Microsatellites are found in large numbers and are relatively evenly distributed throughout the genome (Edwards et al. 1991, Stallings et al. 1991). High level of polymorphism and possibility of analysis using fast and effective technique of PCR cause that microsatellites are useful genetic markers for genetic investigation at the population level. They have been proved to be useful in the analysis of levels of variation within populations and among them (Girman et al. 2001, Lee et al. 2001, Roeder et al. 2001).

Genetic variability assessed by the analysis of microsatellite polymorphism and genetic structure of Central and West-European populations of the Capercaillie has been carefully studied (Segelbacher & Storch 2002, Segelbacher et al. 2003). The studies presented greater level of genetic diversity in large, contiguous boreal populations and large Alpine populations than in small and isolated populations from Central and Western Europe. A high level of genetic diversity was also found to be maintained in local, spatially isolated Alpine populations through existence of gene flow (Segelbacher & Storch 2002). In small, isolated populations from Central Europe an increased level of genetic structure was found, and the authors suggested that this structuring and decrease in genetic variability are connected with the low effective population size, restricted gene flow and disturbance in functioning of a former metapopulation system (Segelbacher et al. 2003).

Until now, genetic investigations of Polish populations of the Capercaillie have not been carried out. As the existence of the species in Poland is highly threatened (Tomiałojć & Stawarczyk 2003), information about genetic variability within remaining populations and genetic differentiation among them has become important. The aim of the study was to estimate genetic variability based on microsatellite typing in Polish populations of the Capercaillie and compare the results with variability of the numerous, continuous population inhabiting Russia. Polish populations of the Capercaillie are isolated and small, thus vulnerable to random factors reducing genetic variability. We hypothesise, that because of fragmentation, isolation and in some cases severe reduction in number of individuals, Polish populations should exhibit lower level of genetic variability compared to the Capercaillies from Russia. We also hypothesise that isolation and low population size should induce pronounced genetic differentiation among Polish populations of the species.

STUDY AREA

Samples for genetic analysis were collected from four Polish populations and from two populations from Russia (Fig. 1). Polish samples originated from Lower Silesian Forest (LSF, Table 1), Carpathians (Car), Janów Lubelski Forest (JLF) and Augustów Forest (AF). All samples from LSF and JLF were collected in 2003; samples from Car originated from years: 2002 (2 samples) and 2003 (7 samples), and samples from AF from years: 2003 (4 samples) and 2004 (7 samples). Russian samples originated

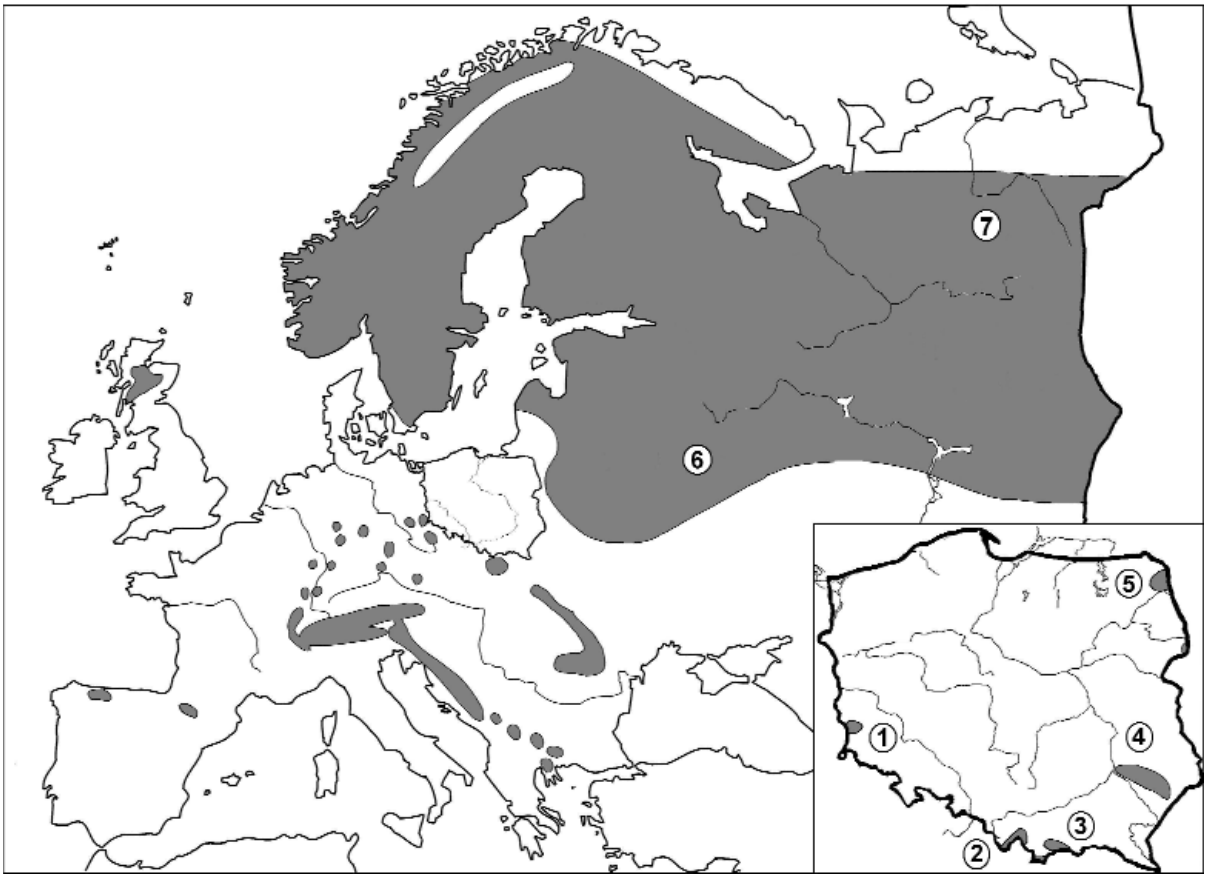

Fig. 1. Present distribution of the Capercaille in Poland and Europe (in grey, Tomiałojć 2000, Tomiałojć & Stawarczyk 2003) and study sites: 1 — Lower Silesian Forest, 2 and 3 — Carpathians (2 — Moravian-Silesian Beskid Mountains, 3 — Gorce Mountains), 4 — Janów Lubelski Forest, 5 — Augustowska Forest, 6 — Kirovskaya Oblast', 7 — Ukhtinskiy Rayon.

form Ukhtinskiy Rayon (R-U) and Kirovskaya Oblast' (R-K) and were collected in 2002 and 2003 (Table 1). Samples consisted of different sexes, but in many cases there was a lack of information about sex of sampled individual.

Table 1. Composition of a sample types in investigated populations. LSF — Lower Silesian Forest, Car — Carpathians, JLF — Janów Lubelski Forest, AF — Augustowska Forest, R-U — Ukhtinskiy Rayon, R-K — Kirovskaya Oblast', Total — total number of samples, N — sample size.

Population	N	Tissue	Feathers	Feaces	Egg membrane
Poland					
LSF	11	1	8	1	1
Car	9	1	2	6	
JLF	14		14		
AF	11		11		
Russia					
R-U	13	13			
R-K	8	7	1		
Total	66	22	36	7	1

Collected samples were: non-invasively collected feathers, faeces and egg membrane and tissue samples from dead individuals. Composition of a type of samples in each investigated population is presented in Table 1.

METHODS

DNA isolation

DNA from feathers was extracted using QIAamp DNA Mini Kit (QIAGEN). Only root end of feathers, approximately 0.5 cm long, were used for extraction. They were crushed, placed in 180 µl of ATL buffer and incubated at 56°C over night with 20 µl of proteinase K (20 µg/ml). Then, extraction followed standard protocol. DNA was eluted in 100 µl of elution buffer. DNA from feaces was isolated using DNA Stool Mini Kit (QIAGEN), according to the method described by Segelbacher & Steinbrück (2001). In the case of non-invasively

collected feathers and feaces all reagents, tubes and pipettes were exposed to ultraviolet light for 15 min. prior to extraction process. DNA was isolated from small sets of samples (5–10) and blank control sample (all reagents without DNA) was always included in each group. Isolation of DNA from tissue was carried out using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma), according to standard protocol.

The concentration of DNA was estimated using spectrophotometer Helios Beta (Spectronic Unicam).

Amplification of microsatellite loci

Six microsatellite loci, described for the Capercaillie (Segelbacher et al. 2000) were analyzed: TUT1, TUT2, TUT3, TUT4 (tetranucelotide repeat motif), and TUD4 and TUD5 (dinucleotide repeat motif). Microsatellite markers were amplified using PCR method. Each reaction was performed in 25 µl of reaction mix, which contained: 10 pmol of each primer, 10–50 ng of template DNA, 12.5 µl of REDTaq PCR ReadyMix (Sigma) and 7.5 µl of PCR grade water (Sigma). Prior to PCR all reagents, tubes and pipettes were exposed to ultraviolet light for 15 minutes. The following PCR profiles were used in a Techne Touchgene thermocycler: initial denaturation — 3 min in 94°C; 35 cycles of 30 s in 94°C, 45 s in 55°C, 45 s in 72°C; one cycle of 30 s in 94°C, 1 min in 55°C, 5 min in 72°C. Forward primers were fluorescently labelled on their 5′ ends with Fam 5.

In the case of DNA samples extracted from feathers and feaces, following adjustments were applied to reduce probability of allelic drop-out and false alleles scoring: each amplification was performed twice, independently at three different DNA dilutions (1:1, 1:3 and 1:6), samples from extraction of blank controls were included for each set of reaction at similar dilutions, and negative PCR control was always included for each set of reaction. No amplification product was found in any blank and negative controls after electrophoresis in agarose gels and analysis in automatic sequencer. All samples showing a different number of alleles or amplification of alleles of different sizes at three dilutions were excluded from further analysis. Additionally, all homozygotes, rare genotypes and samples with poor DNA quality and quantity were reamplified and analyzed independently at least twice.

Microsatellite typing

The length of amplified fragments was estimated using ABI Prism 3700 automated sequencer. Data were analyzed using GENESCAN 3.1.2 (PE Biosystems).

Analysis of genetic variability

For each population, relative amounts of genetic variability were assessed using allele frequency data. GenalEx version 5.04 (Peakall & Smouse 2001) was used to estimate mean number of alleles per locus (A) and effective number of alleles (A_{ef}) in each population. Effective number of alleles enables more meaningful comparison of allelic diversity to be performed across loci with diverse allele frequency distribution (Frankham et al. 2002).

Allelic richness (R) (Petit et al. 1998), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E) (Nei & Roychoudhury 1974) and fixation index (F_{is}) were calculated using FSTAT version 2.9.3 (Goudet 2001). Allelic richness is a measure of the number of alleles independent of sample size and allows comparison of allelic diversity between samples of different sizes (Goudet 2001). Fixation index is a measure of deviation from heterozygosity expected under Hardy-Weinberg equilibrium. Magnitudes close to zero are expected under random mating, while positive values indicate homozygosity excess. The significance of F_{is} was tested with 720 randomisations. Significance threshold was adjusted in order to take into account multiple comparisons (Bonferoni correction, Sokal & Rolf 2003). Differences among Polish and Russian populations in mean number of alleles per locus (A), allelic richness (R) and observed heterozygosity (H_O) were tested using Wilcoxon signed rank test. Differences between two groups of populations (Poland and Russia) in allelic richness and observed heterozygosity were calculated using FSTAT version 2.9.3 and significance was tested using 2000 permutation and one-tailed tests.

Genetic differentiation between investigated populations was examined applying F_{ST} measure. Pairwise F_{ST} values were obtained and their significance was tested with FSTAT version 2.9.3.

RESULTS

The six microsatellite loci assayed were polymorphic with allele numbers ranging from 5 to 14 (Table 2). Loci TUD4 and TUD5 were

Table 2. Polymorphism of analyzed microsatellite loci. Populations — see Table 1, Total — total number of alleles in locus.

Population	Number of alleles in locus					
	TUT1	TUT2	TUT3	TUT4	TUD4	TUD5
LSF	5	5	3	4	5	8
Car	3	3	6	5	5	7
JLF	2	4	4	3	4	3
AF	3	4	6	4	5	6
R-U	8	4	3	4	6	8
R-K	4	5	6	3	6	8
Total	8	5	12	7	13	14

the most polymorphic, with 13 and 14 alleles respectively. The lowest number of alleles was found in locus TUT2.

Mean number of alleles per locus (A) was higher in sample coming from Russia than in each of Polish populations, however a significant difference was found only for comparison JLF—R-K (Wilcoxon signed rank test, p = 0.039). Among Polish samples, the highest mean number of alleles was observed in LSF. Allelic richness (R) and effective number of alleles (A_{ef}), which provide more robust comparison of the samples that differ in size and alleles frequency distribution, reached the lowest magnitude in JLF. Both indicators were significantly lower in JLF than in R-K (Wilcoxon signed rank test, p = 0.028) and R-U (Wilcoxon signed rank test, p = 0.046). Although allelic richness (R) and effective number of alleles (A_{ef}) were also lower in other Polish populations in comparison to samples from Russia, the differences were not statistically significant (Table 3).

Comparison among populations from Poland indicated significantly lower allelic richness (R) and effective number of alleles (A_{ef}) in JLF than in Car and AF (p = 0.028, Table 3).

Table 3. Genetic variability in analyzed populations, expressed in terms of microsatellites polymorphism. Populations — see Table 1, A — mean number of allels per locus, A_{ef} — effective number of alleles, R — allelic richness, H_O — observed heterozygosity, H_E — expected heterozygosity, F_{is} — fixation index; * — p = 0.05, ns — not significant.

Population	Parameters of genetic variability					
	A	R	A_{ef}	H_O	H_E	F_{is}
LSF	5.0	4.5	2.8	0.35	0.59	0.45*
Car	4.8	4.6	3.1	0.44	0.65	0.36*
JLF	3.3	3.0	1.8	0.38	0.42	0.14 ns
AF	4.7	4.2	3.1	0.67	0.68	0.02 ns
R-U	5.5	4.9	3.7	0.69	0.70	0.05 ns
R-K	5.3	5.3	4.0	0.60	0.71	0.23 ns

Observed heterozygosity (H_O) was high in two populations from Russia and in AF coming from Poland. LSF presented lower level of observed heterozygosity than R-U and R-K (p = 0.027 and p = 0.044 respectively) and Car lower level than R-U (p = 0.027). Differences for JLF and Russian populations were not significant. Fixation index (F_{is}) values were significant only in the case of LSF and Car (Table 3).

Comparison between two groups of populations: Russia and Poland, indicated higher allelic richness (R) and observed heterozygosity (H_O) in samples from Russia (R = 5.112, H_O = 0.659) than in group of Polish populations (R = 4.097, H_O = 0.459) but neither differences in allelic richness (R) and heterozygosity (H_O) were significant (p = 0.06 and p = 0.13 for R and H_O respectively). The AF population represents a considerably higher level of heterozygosity than other Polish populations and its exclusion from the Polish group made differences in heterozygosity between Poland (H_O = 0.387) and Russia (H_O = 0.659) statistically significant (p = 0.03).

Pairwise F_{ST} values ranged from 0.041 to 0.225, indicating moderate and great genetic differentiation among investigated populations (Table 4). The lowest F_{ST} and small differentiation were observed between R-K and other populations, except JLF. The highest differentiation was found between AF and JLF populations.

Table 4. Pairwise F_{ST} values for each population pair. Underline values are significant after Bonferroni correction (p < 0.05). Populations — see Table 1.

	Car	JLF	AF	R-U	R-K
LSF	0.056	0.104	0.148	0.087	0.043
Car		0.188	0.121	0.131	0.041
JLF			0.225	0.159	0.121
AF				0.117	0.056
R-U					0.047

DISCUSSION

This study is a first attempt to elucidate the level of genetic variability of Polish populations of the Capercaillie. Bearing in mind that sample sizes are small, especially in case of Car populations from Poland and R-K from Russia, the results should be treated as preliminary. As we hypothesized, small population size and isolation of local populations resulted in lower genetic variability in Polish populations in

comparison with large and continuous populations from Russia. Analyses of genetic differentiation confirmed that Polish populations are isolated and gene flow among them is highly reduced.

Samples R-U and R-K presented level of genetic variability, expressed in terms of allelic diversity and heterozygosity, generally higher than in each of Polish populations and comparable with results obtained for Russian Capercaillies in other studies (Segelbacher et al. 2003). However, it seems likely that most Polish populations retained quite large portion of variability in microsatellite loci. In the case of LSF, Car and AF differences with Russian populations in allelic diversity were not significant. Moreover, mean numbers of alleles per locus in these Polish populations (magnitudes from 4.7 to 5.0) were higher than the estimate reported by Segelbacher et al. (2003) for the Capercaillie population from Slovenia (A = 3.7), where the number of individuals is estimated at more than 30 000, and very similar to the estimate for Black Forest (Germany) (A = 5.1), where the total population size is estimated at 1000 individuals. However, our study applied different sets of microsatellite markers, thus comparisons can be questionable.

JLF population presented lower genetic variability in comparison to both, Russian and other Polish Capercaillies. Indicators of genetic variability in JLF were similar to these found by Segelbacher et al. (2003) in populations coming from Pyrenees and Scotland. The total size of these populations is estimated at more than 5000 and 1000 individuals, respectively (Storch 2001). Both of them are geographically isolated from other European populations. Birds from Pyrenees have been isolated for a long time (Tomiałojć 2000) and the analysis of microsatellite polymorphism indicates that they form a genetically distinct unit (Segelbacher et al. 2003). Populations from Scotland descended from birds reintroduced from Sweden in the mid-1800s (Lever 1977) and its number has severely declined since the 1970s (Baines et al. 2004). Similarly, JLF population is considered as rather dense but it has been isolated for a few hundred years and presently declining (Głowaciński & Profus 2001). Moreover, pairwise F_{ST} values (Table 4), showing great differentiation, confirmed this long isolation from other Polish populations and analysis of microsatellite polymorphism indicated that these processes have already resulted in reduced genetic variability in JLF.

The level of observed heterozygosity in two Polish populations: LSF and Car is significantly lower than in populations coming from Russia (this study) and in other populations from central Europe, even small and isolated (Segelbacher et al. 2003). We also found significant heterozygote deficiency in these populations. The most probable explanation is existence of population structure due to habitat fragmentation. Homozygote excess in population divided into smaller units is well recognized phenomenon (Hartl & Clark 1997). Especially Car population is spatially subdivided and gene flow among local populations in this region seems to be strongly limited, resulting in lower observed heterozygosity. The environment occupied by LSF population is continuous, thus in this case we cannot predict spatial fragmentation. An explanation for significant homozygote excess in LSF may be the Capercaillie mating system. The bird species with the lek mating system and low level of dispersal may be particularly prone to show spatial genetic structuring, even in continuous habitats, as only few males tend to contribute to reproduction (Bouzat & Johnson 2004). Methodological difficulties, such as a presence of null alleles or bias due to small sample size could not also be excluded.

The least pronounced genetic differentiation (F_{ST} = 0.056) (Table 4) among populations from Poland was observed between Car and LSF. These populations were probably connected in the past through Sudety Mountains — Tomiałojć & Stawarczyk (2003) reported presence of isolated breeding grounds of the Capercaillies in Sudety, e.g. in Śnieżnik Massif, even in 1970s. Thus, it is possible that genetic isolation of LSF and Car populations is more recent process, comparing to isolation between other Polish populations. Except JLF also AF seems to be genetically isolated from other Polish populations for a long period of time. However, in contrast to JLF, AF populations does not exhibit any substantial reduction in genetic variability. Both of them are of similar sizes (Tomiałojć & Stawarczyk 2003), but AF lies in occurrence range of large and numerous populations of the species from the territory of Belarus and Lithuania. It is possible that gene flow between them and Polish AF populations was presented not long time ago or it may constantly exist.

CONCLUSIONS

We presented the results of a preliminary analysis of microsatellite polymorphism in four Polish Capercaillie population. The investigation confirmed that that the small population size and isolation decrease genetic variability. JLF population, limited in size and isolated for a long time lost some genetic variability. Populations from AF, LSF and Car seem to retain substantial level of variability despite their drastic reduction in size. Moreover, populations Car and LSF presented low level of heterozygosity, probably, due to fragmentation (the former) and extremely low population size (the later). Predicting the future of Polish Capercaillies, we assume that they are susceptible for factors reducing genetic variability. To stop this process, from genetics point of view, conservation efforts should concentrate not only on "passive" protection of local populations but also on "active" conservation, conducted in two main directions. First, the protection of local genetic pool through controlled and steered captive breeding programmes should be assured. This should stop or at least slow down decreasing trends in genetic variability of native Polish populations. Simultaneously, intensive efforts should be taken in habitat improvement and reconstruction of gene flow among isolated, local populations.

ACKNOWLEDGEMENTS

The study was supported by Regional Direction of National Forests in Wrocław in 2003 and Forests Inspectorate in Wisła. Authors thank Alain Caizergues and two anonymous referees for helpful comments of the manuscript. Many thanks to Mrs Grażyna Góral — without her help this work would be impossible to complete. We also thank many people who collected samples, especially dr Dorota Zawadzka, who delivered samples form Augustowska Forest. Patrycja Jagołkowska-Tkaczuk was very helpful with laboratory analysis.

REFERENCES

Baines D., Moss R., Dugan D. 2004. Capercaillie breeding success in relation to forest habitat and predator abundance. J. Appl. Ecol. 41: 59–71.

Bouzat J. L., Johnson K. 2004. Genetic structure among closely spaced leks in a peripheral population of lesser prairie-chickens. Mol. Ecol. 13: 499–505.

Cramp S., Simmons K. E. L. (eds). 1980. The birds of Western Palearctic. Vol. II. Oxford Univ. Press.

Edwards A., Civitello A., Hammond H. A., Caskey C. T. 1991. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am. J. Human Gen. 49: 746–756.

Frankham R. 1995. Inbreeding and extinction: a threshold effects. Conserv. Biol. 9: 792–799.

Frankham R., Ballou J. D., Briscoe D. A. 2002. Introduction to Conservation Genetics. Cambridge Univ. Press.

Girman D. J., Vila C., Geffen E., Creel S., Mills M. G. L., McNutt J. W., Ginsberg J., Kat P. W., Mamiya K. H., Wayne R. K. 2001. Patterns of population subdivision, gene flow and genetic variability in the African wild dog (*Lycaon pictus*). Mol. Ecol. 10: 1703–1723.

Głowaciński Z., Profus P. 2001. [Capercaillie]. In: [Polish Red Book Data. Vertebrates]. Państwowe Wydawnictwo Rolnicze i Leśne. Warszawa, pp. 173–177.

Goudet J. 2001. FSTAT V2.9.3, a program to estimate and test gene diversities and fixation indices. (available: http://www.unil.ch/izea/softwares/fstat.htlm).

Hartl D. L., Clark A. G. 1997. Principles of Population Genetics. 3rd ed. Sinauer Associates, Inc, Sunderland, MA.

Hedrick P. W. 2001. Conservation genetics: where are we now? TREE 16: 629–636.

Keller L. F., Waller D. M. 2002. Inbreeding effects in wild populations. TREE 17: 230–241.

Lee P. L. M., Bradbury R. B., Wilson J. D., Flanagan N. S., Richardson L., Perkins A. J., Krebs J. R. 2001. Microsatellite variation in the yellowhammer *Emberiza citrinella*: population structure of declining farmland bird. Mol. Ecol. 10: 1633–1644.

Lever C. 1977. The Naturalised Animals of British Isles. Hutchinson, London.

Nei M., Roychoudhury A. K. 1974. Sampling variances of heterozygosity and genetic distance. Genetics 76: 379–390.

Paekal R., Smouse P. E. 2001. GenAlEx V5: Genetic Analysis in Excel. Population genetic software for teaching and research. (available: http://www.anu.ed.au/BoZo/GenAlEx/).

Petit R. J., el Mousadik A., Pons O. 1998. Identifying populations for conservation on the basis of genetic markers. Conserv. Biol. 12: 844–855.

Roeder A. D., Marshall R. K., Mitchelson A. J., Visagathilagar T., Ritchie P. A., Love D. R., Pakai T. J., McPartlan H. C., Murray N. D., Robinson N. A., Kerry K. R., Lambert D. M. 2001. Gene flow on the ice: genetic differentiation among Adélie penguin colonies around Antarctica. Mol. Ecol. 10: 1645–1656.

Segelbacher G., Steinbrück G. 2001. Bird faeces for sex identification and microsatellite analysis. Vogelwarte 41: 139–142.

Segelbacher G., Storch I. 2002. Capercaillie in the Alps: genetic evidence of metapopulation structure and population decline. Mol. Ecol. 11:1669–1677.

Segelbacher G., Hoglund J., Storch I. 2003. From connectivity to isolation: genetic consequences of population fragmentation in across Europe. Mol. Ecol. 12: 1773–1780.

Segelbacher G., Paxton R. J., Steinbruck G., Trontelj P., Storch I. 2000. Characterization of microsatellites in capercaillie *Tetrao urogallus* (AVES). Mol. Ecol. 9: 1934–1935.

Sokal R. R., Rolph F. J. 2003. Biometry. Freeman, New York.

Soulé M. E. 1987. Viable Populations for Conservation. Cambridge Univ. Press, New York.

Soulé M. E., Mills S. L. 1998. No need to isolate genetics. Science 282: 1658–1659.

Stallings R. L., Ford A. F., Nelson D., Torney D. C., Hilderbrand C. E., Moyzis R. K. 1991. Evolution and distribution of (GT)n repetitive sequences in mammalian genomes. Genomics 10: 807–815.

Storch I. 2000. Grouse Status Survey and Conservation Action Plan 2000-4. WPA/Bird Life/SSC Grouse Specialist Group, IUCN, Gland, Switzerland/World Pheasant Association, Reading, UK.

Storch I. 2001. *Tetrao urogallus*. Capercaillie. BWP Update. The Journal of Birds of the Western Palearctic. Oxford Univ. Press.

Tomiałojć L. 2000. An East-West gradient in the breeding distribution and species richness of the European woodland avifauna. Acta Ornithol 35: 3–17.

Tomiałojć L., Stawarczyk T. 2003. [The avifauna of Poland. Distribution, numbers and trends]. PTPP "pro Natura", Wrocław.

Westemeier R. L., Brawn J. D., Simpson S. A., Esker T. L., Jansen R. W., Walk J. W., Kershner E.L., Bouzat J. L., Paige K. N. 1998. Tracking the long-term decline and recovery of an isolated population. Science 282: 1695–1698.

STRESZCZENIE

[Zmienność genetyczna polskich populacji głuszca]

Celem pracy było oszacowanie zmienności genetycznej na podstawie analizy sześciu markerów mikrosatelitarnych w czterech polskich populacjach głuszca (Bory Dolnośląskie — LSF, Karpaty — Car, Lasy Janowskie — LJF i Puszcza Augustowska — AF) i dwóch populacjach z terenu Rosji (rejon Uchty — R-U i rejon Kirova — T-K, Tab. 1). Dla każdej badanej populacji oszacowano następujące wskaźniki zmienności genetycznej: średnią liczbę alleli w badanych loci (A), średnią liczbę alleli efektywnych, zasobność alleliczną oraz heterozygotyczność oczekiwaną (H_O) i obserwowaną (H_E). Dla poszczególnych par populacji wynaczono wartość F_{ST} w celu oszacowania zróżnicowania genetycznego. Wyniki wykazały, że w małych, izolowanych populacjach głuszca z terenu Polski doszło do ograniczenia zmienności genetycznej. Wskaźniki zmienności, opierające się na polimorfizmie sekwencji mikrosatelitarnych były wyższe w przypadku populacji rosyjskich niż w populacjach krajowych (Tab. 2). Najsilniej proces zmniejszania się zmienności genetycznej jest widoczny w populacji z Lasów Janowskich. Wynika to najprawdopodobniej z tego, że populacja ta jest izolowana od innych populacji głuszca od kilkuset lat. Zróżnicowanie genetyczne między głuszcami z Lasów Janowskich i pozostałymi badanymi populacjami (Tab. 4) potwierdza długotrwałą izolację. Pozostałe polskie populacje, mimo drastycznego spadku liczebności w ostatnich dziesięcioleciach i istniejącej obecnie izolacji, zachowały znaczną część zmienności genetycznej (Tab. 3). Jednak w przypadku Borów Dolnośląskich i Karpat zaobserwowano istotny niedobór heterozygot (Tab. 4). Zjawisko to może być spowodowane izolacją poszczególnych siedlisk w obrębie populacji karpackiej oraz drastycznym spadkiem liczebności w populacji z Borów Dolnośląskich. Prawdopodobnie na różnice w heterozygotyczności oczekiwanej i obserwowanej ma także wpływ system rozrodczy głuszca, szczególnie w małych populacjach.

Badania genetyczne potwierdziły konieczność czynnej ochrony gatunku na terenie Polski. Zabiegi ochroniarskie powinny się skupiać nie tylko na ochronie siedlisk, ale także na ochronie puli genowej polskich głuszców i odtworzeniu przepływu genów między izolowanymi populacjami.