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How many of them? Genetic diversity, survival and effective population size of the capercaillie population from the Gorce Mountains

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Abstract: Population size and effective population size are important factors affecting probability of extinction of small, isolated population. Hence, from conservation perspective, it is recommended to monitor changes in population size of endangered species. Genetic methods, based on genetic profiling of non-invasive samples of biological material, despite some limitations, were proved to be efficient method in tracking individuals in the field and estimate populations' parameters. We used this strategy to investigate isolated population of the capercaillie (*Tetrao urogallus*) in the Gorce Mountains. In two study periods (2012–2013 and 2017–2018) almost 400 faeces and feathers were collected. Microsatellite genotyping was performed to identify individuals and estimate genetic diversity. We found that population is stable in terms of size and genetic indices, although allelic richness has significantly increased between 2012–2013 and 2017–2018. In the overall study period (2012–2018) there were 52 individuals identified. However, only 10 birds were found in both study periods. This suggests low survival in the population. Moreover, genetic data indicated low effective population size of the capercaillie in the Gorce Mts. Thus, we suggest that monitoring, either genetic and based on field-surveys, should be implemented in the management and protection of this population.

Key words: *Tetrao urogallus*, the Carpathians, non-invasive sampling, microsatellites, genetic tagging

INTRODUCTION

The populations of endangered species are usually small and isolated. As an after-effect, they are prone to stochastic demographic events and/or genetic processes, that increase probability of extinction (Frankham 2014). Hence, it is important for conservation efforts to monitor these isolated populations, both in terms of changes in number of individuals and the level of genetic diversity (Schwartz et al. 2007; Frankham et al. 2015).

The capercaillie (*Tetrao urogallus* Linnaeus, 1758) is one of the most endangered woodland bird in Central and Western Europe. In this region the species occurs mainly in montane coniferous forests, while majority of lowland populations have disappeared or is threatened with extinction (Storch 2007). Among montane populations, the Carpathians constitute major stronghold of the capercaillie in Central Europe. For example, the overall size of population in Poland has been recently estimated at 530–630 birds, and among them Carpathian stronghold is believed to support about 50% of individuals and 57–60% of leks (Zawadzka et al. 2019). However, recent genetic studies has suggested that Carpathian population is fragmented, and birds in some areas exhibit evidently decreased level of genetic diversity, probably due to persisting isolation (Rutkowski et al. 2017a; Klinga et al. 2019). The stronghold in the Gorce Mts in one of the examples. This region is separated from other Carpathian populations by an extensive non-forested area and the large urbanized area of Nowy Targ (Fig. 1). It was shown that the type of environment, especially presence of human settlements, can effectively reduce

gene flow between strongholds of the capercaillie in the Carpathians (Klinga et al. 2019). As suggested by survey-based studies, in the 1970s and 1980s there were less than 20 birds in the Gorce Mts (Zawadzka 2014). Towards 20th century the population has increased in size, and was probably demographically stable between 1990 and 2000, with the estimated size of 20–30 individuals (Żurek & Armatys 2011). However, observational data suggested that in the second decade of 21st century the number of birds has increased to 40 individuals (Zawadzka et al. 2019). Indeed, the genetic analysis of non-invasive samples collected throughout the Gorce Mts between 2009 and 2013 indicated population size of at least 44 individuals (Rutkowski et al. 2017a). These results suggest that the capercaillie from the Gorce Mts could have experienced long term demographic bottleneck effect in the second half of 20th century, and recently has undergone increase in population size. Indeed, genetic data suggested decline and recovery of this population over different time scales, both recent and historic (Rutkowski et al. 2017a). While historic bottleneck effect should be interlinked with colonization of the Carpathians after the last glacial maximum (Rutkowski et al. 2016), the recent signals might be connected with fluctuation in population size during 20th century.

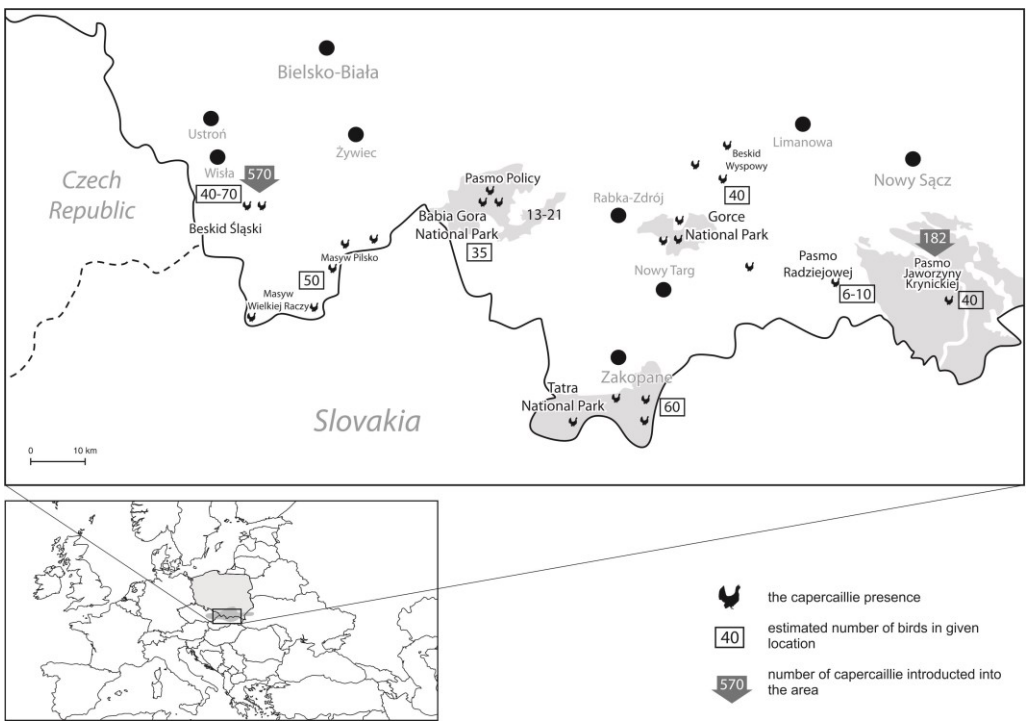

Fig. 1. Distribution of the capercaillie in the Polish part of the Carpathians, including stronghold in the Gorce National Park, where majority of samples for this study were collected. Black dots indicated main cities in the region. The estimated number of birds is given after Zawadzka et al. (2019).

Genetic methods, based on molecular markers and non-invasive sampling enable to study populations of endangered species at various levels, from tracking individuals to estimate gene flow (Poggenburg et al. 2018). Thus, to verify population trends in the capercaillie from the Gorce Mts we compared genetic data from two field surveys: in the beginning and at the end of the second decade of the 21st century (years 2012–2013 and 2017–2018). In these periods

non-invasive samples were intensively collected throughout the Gorce Mts. They were genotyped with microsatellite markers to identify individual birds and estimate genetic diversity of the population. We also used obtained genetic data to estimate effective population size, because number of individuals participating in the gene pool can differ substantially from census population size, especially in lekking species, such as the capercaillie.

MATERIAL AND METHODS

Sample collection

Non-invasive samples – faeces and feathers of the capercaillie – were collected in the Gorce Mts, especially Gorce National Park (Fig. 1), during field surveys in two periods: 2012–2013 and 2017–2018. Surroundings of the known leks were surveyed, as well as regions, where the capercaillie has been frequently observed. Searching for faeces samples were conducted between February and May, during snow retention. Additionally, some feather samples were collected between June and September. After being collected in the field, the faeces were covered with silica gel to dehydrate the sample, then froze and stored in a freezer at -72°C until extraction. The feathers were stored in paper envelopes or plastic vials, while after delivery to the laboratory, they were kept in a freezer at -4°C. In total, 240 samples were collected between 2012 and 2013, and 156 between 2017 and 2018. They were mainly faeces (96% of all samples collected).

Laboratory analysis

DNA from faeces was extracted using the NucleoSpin Soil Kits (MACHEREY-NAGEL, distributed in Poland by AQUA LAB), as described previously (Rutkowski et al. 2017a). DNA extractions from feathers were performed using a NucleoSpin Tissue Kits (MACHEREY-NAGEL) in line with the standard protocol. As the material constituted non-invasive samples, several measures were taken (as described in Rutkowski et al. [2017a]) in association with the DNA isolation process in order to minimise problems of contamination.

All the extractions were made subject to microsatellite genotyping, using multiplex PCR. We amplified 8 microsatellite loci, i.e. TuT1, TuT2, TuT3, TuT4, TTT1, Bg12, Bg16 and Bg18 (tetranucleotide repeats) (Segelbacher et al. 2000; Caizergues et al. 2001; Piertney & Höglund 2001). Microsatellites were amplified in two multiplex reactions, using reaction mixtures and conditions as described previously (Rutkowski et al. 2017a). The genotyping analyses were performed using a CEQ 8000 sequencer (BECKMAN COULTER, distributed in Poland by Comesa-Polska).

To obtain reliable genetic data, several measures were taken to avoid genotyping errors, as described in Rutkowski et al. (2017a). Briefly, all PCR reactions were repeated at least twice. All extracts lacking the PCR product in both replicates were excluded from further analysis. Equally, extracts with two identical genotypes in both independent PCRs were classified as successfully genotyped. All extracts showing signs of contamination (more than two microsatellite alleles at particular loci) were excluded from further analysis. Two additional PCRs were performed in the case of differences between genotypes obtained in the two first PCRs, which could be explained by typical technical problems observed frequently during the microsatellite genotyping of non-invasive samples (i.e. 'allelic drop-out' or 'false alleles'). Consensus genotypes were then created on the basis of the genotypes obtained in all four reactions. The extracts showing evidently different genotypes in successive PCR reactions were excluded from further analysis.

Statistical analysis

We assumed that the presence of identical microsatellite genotypes in two or more independent samples attested to the samples belonging to the same individual. Comparisons of genotypes were performed using GenAlEx v. 6.501 (Peakall & Smouse, 2012).

Based on unique genotypes, basic genetic measures were estimated: (i) for each period (2012–2013 and 2017–2018) the deviation from the Hardy-Weinberg Equilibrium was assessed using Fisher's exact test in Genepop v.4 (Raymond & Rousset, 1995; Rousset 2008), with the following settings: 10,000 dememorisation, 1000 batches and 10,000 iterations; (ii) mean values for basic genetic indices, i.e. allelic richness – the number of alleles corrected for sample size using the rarefaction method (R, [Petit et al. 1998]); observed (H_O) and expected heterozygosity (H_E, [Nei 1978]) and inbreeding coefficient (F_{IS}). These analyses were performed using GenAlEx and FSTAT version 2.9.3.2 (Goudet 2001). The allelic richness coefficient (R), observed heterozygosity (H_O) and expected heterozygosity (H_E) were compared between populations in ANOVA models and a post hoc Tukey HSD test was applied to all pairwise comparisons. Basic measures of genetic diversity were also estimated for the unique genotypes, identified in both periods of the study. Similarly, for each locus, as well as for a combination of 8 loci, Probability of Identity (the average probability that two unrelated individuals, randomly sampled from a population, will have the same genotype, $P_{(ID)}$) was calculated using GenAlEx v. 6.501. Additionally, we also calculated Probability of Identity with account taken of genetic similarity among siblings ($P_{(ID\text{-}Sibs)}$).

Because 'null alleles' could significantly affect microsatellite data, we also analysed identified genotypes in the PopGenReport V. 3.0.0 package in the R environment (R Core Team 2017) to identify possible problems, interlinked with 'nulls'. We used a method after Brookfield (1996), as this performs better when all genotyped individuals have at least one allele detected (i.e. there are no missing data).

Potential changes in allele frequencies between two study periods were evaluated using F_{ST} (Weir & Cockerham 1984), as based on the Infinite Allele Model of mutation. Pairwise F_{ST} value and its significances were calculated in FSTAT.

Effective population size (Ne) of the capercaillie population in the Gorce Mts was estimated using NeEstimator 1.3 (Peel et al. 2004). We used Single Sample Method based on Linkage Disequilibrium (Bartley et al. 1992), estimating Ne for each study period, as well as Moment Based Temporal Approach (Waples 1989, 1991), treating 2012–2013 sample as generation '0', and 2017– 2018 as generation '5'.

Results

We successfully genotyped 228 samples (≈57% of all samples collected): 149 from 2012–2013 (≈62%), and 79 from 2017–2018 (≈50%). Among them 52 unique genotypes were found. In 2012–2013 we found 34 unique genotypes, while in 2017–2018 28 genotypes. However, among genotypes found in 2017–2018, 10 were previously found in 2012–2013. Hence, we can state that at least 52 individuals have lived in the Gorce Mts between 2012–2018, although only 10 of them were identified in both study periods.

The method of Brookfield (1996) indicated the possibility of there being null alleles for locus TuT1, although with a low frequency (<2.5%).

Per locus Probability of Identity ($P_{(ID)}$) ranged from 0.14 (locus TuT4) to 0.48 (TuT1). For the combination of 8 loci, both $P_{(ID)}$ and $P_{(ID\text{-}Sibs)}$ were 0.001 and 0.008, respectively (Table 1). Hence, according to our data, the expected number of different individuals with the same genotype was very low.

Table 1. Microsatellite polymorphisms in the investigated population of the capercaillie from Gorce National Park. Only unique genotypes, identified in both periods, were used for calculations (N = 52). A — number of alleles per locus; H_O — heterozygosity observed; H_E — heterozygosity expected; *HWE* — P-values for *HWE* exact test for heterozygote deficiency/excess (* — P<0.05; ns — non-significant (P>0.05); F_{IS} — fixation index (* — significant after Bonferroni correction); $P_{(ID)}$ by locus — probability of identity for each locus; All $P_{(ID)}/P_{(ID\text{-}Sibs)}$ — probability of identity for combination of 8 loci/probability of identity for combination of 8 loci, taking into account the genetic similarity among siblings.

Locus	A	H_O	H_E	*HWE*	F_{IS}	$P_{(ID)}$ by locus
Bg16	4	0.577	0.592	ns	0.036	0.252
TTT1	4	0.519	0.516	ns	0.004	0.274
TuT2	6	0.712	0.608	*	-0.161	0.231
Bg12	6	0.462	0.631	*	0.278*	0.194
TuT1	2	0.250	0.448	*	0.442	0.484
TuT4	6	0.731	0.698	ns	-0.037	0.144
TuT3	5	0.615	0.531	ns	-0.150	0.266
Bg18	3	0.654	0.617	ns	-0.051	0.227
Mean/Overall	4.86	0.610	0.599	*	-0.008	
$P_{(ID)}/P_{(ID\text{-}Sibs)}$						<0.001/0.008

Population from the Gorce Mts was in Hardy-Weinberg Equilibrium in both study periods (Fisher's Exact test, P>0.05), and F_{IS} was low (0.001 and 0.03 for 2012–2013 and 2017–2018, respectively) and statistically non-significant after Bonferroni correction (280 randomisations, adjusted P-value = 0.0035). Calculations performed based on 52 unique genotypes, identified in both study periods, indicated overall significant heterozygote excess, although F_{IS} value was low and not significantly different from zero (Table 1). Allelic richness was higher in 2017–2018 (R = 4.57) then in 2012–2013 (R = 3.58) and the difference was statistically significant (Tukey HSD test: P = 0.048). Observed heterozygosity (H_O) and expected heterozygosity (H_E) were similar in both study periods (H_O = 0.604 and 0.592; H_E = 0.597 and 0.598 for 2012–2013 and 2017–2018, respectively) and did not differ significantly.

There were no differences in allele frequencies between study periods, as indicated by low and non-significant F_{ST} (0.0032; P > 0.05).

Both, Single Sample Method and Moment Based Temporal Approach indicated low effective population size of the capercaillie population from the Gorce Mts. For 2012–2013 Single Sample Method suggested Ne = 10.2 (95% CI 8–13.5 individuals); for 2017–2018 slightly higher: Ne = 15.6 (95% CI 11.4–22.3 individuals). Temporal Approach, assuming that interval between study periods correspond to 5 generations of the capercaillie, indicated Ne = 13.4 within a range of 6.6–23.8 individuals.

DISCUSSION

Three main issues emerged from our study. Firstly, the population size seems to be stable, although there was a low number of genotypes (thus low number of individuals) found in both study periods; second: genetic diversity, estimated as a number of microsatellite alleles, has increased during the study period; third: the effective population size in the Gorce Mountains seems to be very low.

Genetic data, obtained during two study periods, indicated that investigated population of the capercaillie in the Gorce Mts is demographically stable. In both periods similar number of unique genotypes was identified, despite difference in number of collected and successfully genotyped samples. We can stated that in particular study period about 30 individuals lived in the area. The combining data set (a period of six years) suggested that at least 52 birds have inhabited the Gorce Mts. Although simple count of unique genotypes may only approximately reflect actual population size, for example due to bias towards low values (Mills et al. 2010), it was shown

that genetic methods could be more appropriate to estimate population size than field surveys. In general, field surveys, for example counting birds on leks, usually underestimates abundance of the capercaillie (Jacob et al. 2010). On the other hand, in many cases estimation of population size using genetic data corresponds very well to observational data (Rutkowski et al. 2017b; Santorek et al. 2018).

In this study we found that majority of the genotypes (individuals) were identified in a single season — we found only 10 individuals in both study periods. Low number of individuals found in temporal surveys of the capercaillie is not exceptional. Field survey and genetic analysis of non-invasive samples in Switzerland Prealps in two time periods (2003 and 2008) allowed to identify 86 individuals, however no genotype was found in both years (Kormann et al. 2012). Such low 'genetic recapture' could be caused be a few factors. Firstly, despite a quite intensive collection of biological remains, some individuals could be hard to sample, the most probably females. For example direct observations of females are rare during field surveys, which cause that their biological traces (feathers, faeces) could be easily overlooked, especially in difficult mountain terrain (Jacob et al. 2010). Secondly, genotyping errors could occur when using DNA from non-invasive samples as a template for PCR amplification of microsatellite loci (Gageneux et al. 1997; Taberlet et al. 1996; Bradley & Vigilant 2002). As a result, 'ghost genotypes' are identified in population, instead of increasing recapture rate. Being aware of the risk, we applied methodological improvements to reduce genotyping errors. Indeed, such a strategy was shown to effectively reduce problems interlinked with allelic dropout or amplification of non-target DNA (Rutkowski et al. 2017a). However, we cannot completely excluded that some errors influenced our results. Thirdly, low number of individuals found in both study periods could reflect low survivability of the capercaillie in the Gorce Mts. Although the capercaillie is believed to be long-lived species, its annual survival is low, even in large, stable populations (Wegge et al. 1987; Moss et al. 2008; Åhlen et al. 2013). Predation is the main cause of mortality in the capercaillie (Wegge & Kastdalen 2007). In small, isolated population genetic processes, such as inbred reducing viability, constitute additional factor, which could potentially decrease survival rate (Saccheri et al. 1998). Indeed, genetic data suggest that genetic diversity, including heterozygosity is low in the capercaillie from the Gorce Mts (Rutkowski et al., 2017a; this study). In this context, finding only 10 individuals, which survived time period of 4–6 years cannot be seen as a something of a surprise. Nonetheless, considering elusiveness of the species, mountain terrain hard to penetrate and possible genotyping errors, the issue of survival rate of the capercaillie in the Gorce Mts requires further studies.

Apart from low number of individuals found in both study periods, suggesting substantial mortality, the genetic pool of the capercaillie in the Gorce Mts seems to be very stable. There was no genetic differentiation between genetic pools of the two study periods. However, there were new alleles found in the population, as allelic richness was significantly higher in 2017–2018 than 2012–2013. There are two possible explanations of an appearance of additional microsatellite alleles, assuming that mutation are negligible in such short period: some portion of genetic pool was not identified in the first period or new allelic forms have appeared in population with migrating individuals. The first issue was already discussed above and it is obvious that overlooking of same individuals, thus some microsatellite alleles, cannot be excluded. However, it should be noted that in the first period of the study (2012–2013) clearly more samples were collected and successfully genotyped. Hence, there was greater chance to identify more alleles in the first study period. The other explanation is interlinked with the possibility of a gene flow. The Gorce Mts are located nearby areas inhabited by the capercaillie, e.g the Tatra National Park or Jaworzyna Krynicka (Fig. 1). Genetic data suggested that population form the Gorce has recently been isolated from other Carpathian strongholds (Rutkowski et al. 2017a). Although this population seems to be spatially isolated

from large population in Tatras in the south, the gene flow can possibly occur from the Beskid Sądecki on the east (throughout Radziejowa Range) and Babia Góra on the west (throughout Polica Range). Recently, the population from Beskid Sądecki has been reinforced by introduction of more than 550 individuals (Zawadzka et al. 2019). Hence, it is possible that some of them dispersed after introduction, enriching genetic pool of other Carpathian strongholds. To verify existence of gene flow between the Gorce Mts further studies are needed, including genetic data from other Carpathian strongholds and potential corridors, connecting them.

Genetic data suggested low effective population size of the capercaillie in the Gorce Mts. The minimum effective size for a population to be viable in the short term should be 50, while maintaining of a long term genetic potential requires minimum effective population size of 500–5000 individuals (Frankham et al. 2010). Hence, in the light of our research population of the capercaillie in the Gorce Mts should be considered as endangered with extinction. We indicated, that in single study period only 10–15 (max. 15–20) individuals contribute to genetic diversity of the subsequent generation. However, these low values, clearly lower than number of individuals in the population, estimated based on unique genotypes, could be explained by breeding system of the capercaillie or the imperfection of the method used to access Ne. In birds species with lek breeding behaviour, such the capercaillie, effective population size of males can be significantly reduced (Jonhson et al. 2004). Considering recent filed surveys, the number of active leks in the Gorce Mts does not exceed five. Hence, assuming presence of single dominant male on each lek, the effective population size of 15–20 individuals seems to be realistic. However, to estimate Ne we used method based on the Linkage Disequilibrium (LD) (Bartley et al. 1992; Waples & Do 2008). The principle of the method is that, as Ne decreases, genetic drift with few parents generates non-random associations among alleles at different loci, i.e. LD (Waples & Do 2010; Waples & England 2011). The method is susceptible to be down-biased by processes other than small Ne (Luikart et al. 2010; England et al. 2010; Waples & England 2011). Thus, we can expect, that low effective size of population from the Gorce Mts reflect population subdivision, immigration or admixture. The effect of such processes on effective population size in the Gorce Mts should be further investigated.

On the other hand, we can also suggest that low effective size properly reflects genetic and demographic conditions of small, isolated population. Assuming that number of identified unique genotypes identified in our study is more or less equal to census population size, the ratio Ne/Nc in the Gorce Mts would be about 0.29 for 2012–2013, and 0.53 for 2017–2018. These values correspond well to Ne/Nc ratio in other, even clearly larger, populations of the capercaillie (Vázquez et al. 2013). Hence, it is possible that our estimates correspond well to the low number of individuals contributing to genetic pool in population of a small size. Because our estimates of survival and effective population size are rather low, we suggest that monitoring, either genetic and field-survey, should be implemented in the management and protection of the population of the capercaillie form the Gorce Mts.

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REFERENCES

ÅHLEN P.-A., WILLEBRAND T., SJÖBERG K. & HÖRNELL-WILLEBRAND M. 2014. Survival of female capercaillie *Tetrao urogallus* in northern Sweden. Wildlife Biology 1 9(4): 368–373. DOI: https://doi.org/10.2981/13-025

BARTLEY D., BAGLEY M., GALL G. & BENTLEY B. 1992. Use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations. Conservation Biology 6 (3): 365–375. DOI: https://doi.org/10.1046/j.1523-1739.1992.06030365.x

BRADLEY B. J. & VIGILANT L. 2002. False alleles derived from microbial DNA pose a potential source of error in microsatellite genotyping of DNA from faeces. Molecular Ecology Notes 2 (4): 602–605. DOI: https://doi.org/10.1046/j.1471-8286.2002.00302.x

BROOKFIELD J. F. Y. 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. Molecular Ecology 5(3): 453–455. DOI: https://doi.org/10.1046/j.1365-294X.1996.00098.x

CAIZERGUES A., DUBOIS S., MONDOR G. & RASPLUS J.-F. 2001. Isolation and characterisation of microsatellite loci in black grouse (*Tetrao tetrix*). Molecular Ecology Notes 1 (1-2): 36–38. DOI: https://doi.org/10.1046/j.1471-8278.2000.00015.x

ENGLAND P., LUIKART G., WAPLES R. 2010. Early detection of population fragmentation using linkage disequilibrium estimation of effective population size. Conservation Genetics 11 (6): 2425–2430. DOI: https://doi.org/10.1007/s10592-010-0112-x

FRANKHAM R. 2010. Inbreeding in the wild really does matter. Heredity 104 (2): 124.

FRANKHAM R., BRADSHAW C. J. A. & BROOK B. W. 2014. Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation 170: 56–63. DOI: https://doi.org/10.1016/j.biocon.2013.12.036

FRANKHAM R. 2015. Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Molecular Ecology 24 (11): 2610–2618. DOI: https://doi.org/10.1111/mec.13139

GAGNEUX P., BOESCH C. & WOODRUFF D. S. 1997. Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. Molecular Ecology, 6 (9): 861–868. DOI: https://doi.org/10.1111/j.1365-294X.1997.tb00140.x

GOUDET J. 2001. FSTAT V2.9.3, a program to estimate and test gene diversities and fixation indices.

JACOB G., DEBRUNNER R., GUGERLI F., SCHMID B. & BOLLMANN K. 2010. Field surveys of capercaillie (*Tetrao urogallus*) in the Swiss Alps underestimated local abundance of the species as revealed by genetic analyses of non-invasive samples. Conservation Genetics 11 (1): 33–44. DOI: https://doi.org/10.1007/s10592-008-9794-8

JOHNSON J. A., BELLINGER M. R., TOEPFER J. E. & DUNN P. 2004. Temporal changes in allele frequencies and low effective population size in greater prairie-chickens. Molecular Ecology, 13 (9): 2617–2630. DOI: https://doi.org/10.1111/j.1365-294X.2004.02264.x

KLINGA P., MIKOLÁŠ M., SMOLKO P., TEJKAL M., HÖGLUND J. & LADISLAV P. 2019. Considering landscape connectivity and gene flow in the Anthropocene using complementary landscape genetics and habitat model ling approaches. Landscape Ecology 34(3): 521–536. DOI: https://doi.org/10.1007/s10980-019-00789-9

KORMANN U., GUGERLI F., RAY N., EXCOFFIER L. & BOLLMANN K. 2012. Parsimony-based pedigree analysis and individual-based landscape genetics suggest topography to restrict dispersal and connectivity in the endangered capercaillie. Biological Conservation 152: 241–252. DOI: https://doi.org/10.1016/j.biocon.2012.04.011

LUIKART G., RYMAN N., TALLMON D., SCHWARTZ M. & ALLENDORF F. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. Conservation Genetics 11 (2): 355–373. DOI: https://doi.org/10.1007/s10592-010-0050-7

MILLS L. S., CITTA J. J., LAIR K. P., SCHWARTZ M. K. & TALLMON D. A. 2000. Estimating animal abundance using noninvasive DNA sampling: Promise and pitfalls. Ecological Applications, 10 (1): 283–294. DOI: https://doi.org/10.1890/1051-0761(2000)010[0283:EAAUND]2.0.CO;2

MOSS R., PICOZZI N., SUMMERS R. W. & BAINES D. 2008. Capercaillie *Tetrao urogallus* in Scotland - demography of a declining population. Ibis 142 (2): 259–267. DOI: https://doi.org/10.1111/j.1474-919X.2000.tb04865.x

NEI M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89 (3): 583–589.

PEAKALL R. & SMOUSE P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics Applications Note 28 (19): 2537–2539. DOI: https://doi.org/10.1093/bioinformatics/bts460

PEEL D., OVENDEN J. R. & PEEL S. L. 2004. NeEstimator: software for estimating effective population size, Version 1.3. Queensland Government, Department of Primary Industries and Fisheries.

PETIT R. J., EL MOUSADIK A. & PONS O. 1998. Identifying populations for conservation on the basis of genetic markers. Conservation Biology 12 (4): 844–855. DOI: https://doi.org/10.1111/j.1523-1739.1998.96489.x

PIERTNEY S. B. & HÖGLUND J. 2001. Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). Molecular Ecology Notes 1 (4): 303–304. DOI: https://doi.org/10.1046/j.1471-8278.2001.00118.x

POGGENBURG C., NOPP-MAYR U., COPPES J. & SACHSER F. 2018. Shit happens … and persists: decay dynamics of capercaillie (*Tetrao urogallus* L.) droppings under natural and artificial conditions. European Journal of Wildlife Research 64: 29. DOI: https://doi.org/10.1007/s10344-018-1187-9

R CORE TEAM. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available: https://www.R-project.org/

RAYMOND M. & ROUSSET F. 1995. GENEPOP (version 1.2): Population Genetics Software For Exact Tests And Ecumenicism. Heredity 86 (3): 248–249. DOI: https://doi.org/10.1093/oxfordjournals.jhered.a111573

ROUSSET F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources 8: 103–106. DOI: https://doi.org/10.1111/j.1471-8286.2007.01931.x

RUTKOWSKI R., JAGOŁKOWSKA P., ZAWADZKA D. & BOGDANOWICZ W. 2016. Impacts of forest fragmentation and post-glacial colonization on the distribution of genetic diversity in the Polish population of the hazel grouse *Terastes bonasia*. European Journal of Wildlife Research 62 (3): 293–306. DOI: https://doi.org/10.1007/s10344-016-1002-4

RUTKOWSKI R., ZAWADZKA D., SUCHECKA E. & MERTA D. 2017a. Conservation genetics of the Capercaillie in Poland – delineation of Conservation Units. PLoS One 12 (4): e0174901. DOI: https://doi.org/10.1371/journal.pone.0174901

RUTKOWSKI R., DULISZ B., SZCZEPAŃSKI S., NOWAKOWSKI J. J., ZWIJACZ-KOZICA T. & KRZAN P. 2017b. Conservation genetics of the capercaillie in Poland — estimating the size of the Tatra National Park population by the genotyping of non-invasive samples. Fragmenta Faunistica 60 (2): 119–128.

SACCHERI I., KUUSSAARI M., KANKARE M., VIKMAN P., FORTELIUS W. & HANSKI I. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392 (6675): 491–494.

SANTOREK A., KULIGOWSKA B., SZCZEPAŃSKI S., DULISZ B. & RUTKOWSKI R. 2018. Ocena liczebności głuszca (*Tetrao urogallus*) w Babiogórskim Parku Narodowym na podstawie analiz genetycznych prób nieinwazyjnych. Studia i Materiały CEPL w Rogowie 54 (4): 125–133.

SEGELBACHER G., PAXTON R. J., STEINBRUCK G., TRONTELJ P. & STORCH I. 2000. Characterization of microsatellites in capercaillie *Tetrao urogallus* (Aves). Molecular Ecology 9(11): 1934–1935. DOI: https://doi.org/10.1046/j.1365-294x.2000.0090111934.x

SCHWARTZ M. K., LUIKART G. & WAPLES R. S. 2007. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution 22(1): 25–33. DOI: https://doi.org/10.1016/j.tree.2006.08.009

STORCH I. 2007. Conservation Status of Grouse Worldwide: an update. Wildlife Biology 13 (SP1): 5–12. DOI: https://doi.org/10.2981/0909-6396(2007)13[5:CSOGWA]2.0.CO;2

TABERLET P., LUIKART G. & WAITS L. P. 1999. Noninvasive genetic sampling: Look before you leap. Trends in Ecology and Evolution 14 (8): 293–332. DOI: https://doi.org/10.1016/S0169-5347(99)01637-7

VÁZQUEZ J. F., PÉREZ T., ALBORNOZ J. & DOMÍNGUEZ A. 2013. Census and effective population size of the endangered Cantabrian capercaillie (*Tetrao urogallus*) estimated from non - invasive samples. Grouse News 46: 12–26.

WAPLES R. S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics 121 (2): 379–391.

WAPLES R. S. 1991. Genetic methods for estimating the effective size of Cetacean populations. Report of the International Whaling Commission Special Issue 13: 279–300.

WAPLES R. S. & DO C. 2008. ldne: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8 (4): 753–756. DOI: https://doi.org/10.1111/j.1755-0998.2007.02061.x.

WAPLES R. S. & DO C. 2010. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evolutionary Applications 3 (3): 244–262. DOI: https://doi.org/10.1111/j.1752-4571.2009.00104.x

WAPLES R. S. & ENGLAND P. R. 2011. Estimating contemporary effective population size on the basis of linkage disequilibrium in the face of migration. Genetics 189 (2): 633–644. DOI: https://doi.org/10.1534/genetics.111.132233

WEGGE P., LARSEN B. B., GJERDE I., KASTDALEN L., ROLSTAD J. & STORAAS T. 1987. Natural mortality and predation of adult capercaillie in southeast Norway. In: Lovel T. & Hudson P. (Eds.). Proceedings of the 4th International Symposium on Grouse. Lam. Germany: 49–56.

WEGGE P. & KASTDALEN L. 2007. Pattern and causes of natural mortality of capercaille, *Tetrao urogallus*, chicks in a fragmented boreal forest. Annales Zoologici Fennici 44 (2): 141–151.

WEIR B. S. & COCKERHAM C. C. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38(6): 1358–1370.

ZAWADZKA D. 2014. Podręcznik najlepszych praktych ochrony głuszca i cietrzewia [The handbook of the best practices of the Capercaillie and the black grouse protection]. Centrum Koordynacji Projektów Środowiskowych, Warszawa. [In Polish]

ZAWADZKA D., ŻUREK Z., ARMATYS P., STACHYRA R., SZEWCZYK P., KORGA M., MERTA D., KOBIELSKI J., KMIEĆ M., PREGLER B., KRZAN P., RZOŃCA Z., ZAWADZKI G., ZAWADZKI J., SOŁTYS B., BIELAŃSKI J., CZAJA J., FLIS-MARTYNIUK E., WEDIUK A., RUTKOWSKI R. & KRZYWIŃSKI A. 2019. Liczebność i rozmieszczenie głuszca w Polsce w XXI w. Sylwan 163 (9):773–783. DOI: https://doi.org/10.26202/sylwan.2019029

ŻUREK Z. & ARMATYS P. 2011. Występowanie głuszca *Tetrao urogallus* w polskich Karpatach Zachodnich — wnioski z monitoringu w latach 2005-2010 oraz końcowa ocena liczebności karpackich subpopulacji głuszca i cietrzewia. [The occurence of Capercillie in Polish Western Carpathians — conclusions from the monitoring in the years 2005-2010 and the final assessment of the quantity of Carpathians subpopulations of Capercaillie and Black Grouse]. Stud. i Mat. CEPL, Rogów 13: 229-240. [In Polish]

STRESZCZENIE

[**How many of them? Genetic diversity, survival and effective population size of the capercaillie population from the Gorce Mountains**]

Wielkość populacji, a szczególnie efektywna wielkość populacji są ważnymi czynnikami, decydującymi o możliwości przetrwania małych, izolowanych populacji. Z punktu widzenia ochrony gatunków zagrożonych, wiedza o tych parametrach jest kluczowa dla podejmowania właściwych działań i decyzji konserwatorskich. Metody genetyczne, oparte na profilowaniu genetycznym prób nieinwazyjnych są efektywną metodą monitorowania populacji gatunków rzadkich bądź trudnych do bezpośredniej obserwacji. Taką strategię wykorzystano w badaniach izolowanej populacji głuszca (*Tetrao urogallus*) w Gorcach. W dwóch okresach (lata 2012–2013 i 2017–2018) zebrano na tym terenie blisko 400 prób nieinwazyjnych (głównie odchodów). Identyfikację osobniczą i szacowanie parametrów populacyjnych przeprowadzono z wykorzystaniem mikrosatelitarnych markerów genetycznych. Stwierdziliśmy, że populacja głuszca w Gorcach jest stabilna pod względem genetycznym i demograficznym, chociaż zmienność genetyczna zwiększyła się istotnie między dwoma okresami badań. Łącznie, w latach 2012–2018, na terenie Gorców żyły co najmniej 52 głuszce, ale tylko 10 osobników występowało na terenie badanej populacji w obydwu okresach badań. To może sugerować niską przeżywalność ptaków. Ponadto, dane genetyczne wskazują, że efektywna wielkość populacji gorczańskiej jest bardzo niska. Uzyskane wyniki sugerują potrzebę monitorowania populacji głuszca w Gorcach, zarówno w oparciu o metody obserwacyjne, jak i genetyczne.

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