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Genetic structure and diversity of the capercaillie (*Tetrao urogallus*) population in Belarus in the context of delineation of two subspecies: *major* and *pleskei*

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abstract

In the present article, an analysis of the genetic diversity and differentiation of the Belarusian capercaillie subpopulations from the range of two subspecies —*Tetrao urogallus major* and *Tetrao urogallus pleskei* — distinguished on the basis of morphological and behavioural (mating vocalization) criteria was carried out. The microsatellites were chosen as genetic markers. A total of 53 specimens were used for genetic analysis (23 specimens from the range of *T. u. major* and 30 specimens from the range of *T. u. pleskei*). In this study, we aimed at resolving the following questions: (1) Does the capercaillie subpopulation from the range of *T. u. major* in Belarus exhibit genetic isolation from the rest of the population in the country? (2) Should we consider the western subpopulation of the capercaillie in Belarus a management unit? Our data allows concluding that the genetic diversity of the studied capercaillie subpopulations is sufficiently high. The eastern subpopulation of the capercaillie is characterised by slightly higher values of all estimates of genetic diversity. A total of 35 unique alleles were detected in the studied capercaillie population. Of them, 10 alleles (29%) were discovered among the specimens sampled from the western subpopulation of the capercaillie. Genetic analysis for the presence of bottleneck events did not reveal any evidence of those in the demographic history of the studied Belarusian capercaillie subpopulations. Bayesian analysis of genetic structure has indicated the presence of two clusters, corresponding to the eastern and western capercaillie subpopulations in Belarus. The obtained genetic structure of the capercaillie population is also supported by the results of the factorial correspondence analysis. The results of genetic structure and diversity analysis indicate that the capercaillie population in Belarus possesses a degree of genetic differentiation on subpopulation level and a lack of clear isolation between the studied subpopulations. As recommendations for the conservation of the western subpopulation of the capercaillie in Belarus we propose to conduct genetic monitoring of the newly created population, as well as genetic analysis of the specimens used for breeding.

Генетична структурованість та різноманітність популяції глушця (*Tetrao urogallus*) у Білорусі в контексті виділених двох підвидів: *major* та *pleskei*

Костянтин Гомель, Тетяна Павлющик, Михаіл Никифоров, Арсеній Волністий

Резюме. У цій роботі проведено аналіз генетичної різноманітності та генетичної диференціації білоруських субпопуляцій глушця з територій проживання двох підвидів — *Tetrao urogallus major* і *Tetrao urogallus pleskei* — що виділяються на підставі морфологічних та поведінкових (звук пробки, що відкривається, в пісні *T. u. major*) критеріїв. Як генетичні маркери використовувалися мікросателіти. Сумарний обсяг вибірки склав 53 особи (23 особи з території проживання підвиду *T. u. major* та 30 особин з території проживання підвиду *T. u. pleskei*). Метою статті було отримання відповідей на два питання: 1) чи спостерігається чи відсутня генетична ізоляція субпопуляції глушця з території проживання західного підвиду *T. u. major* у Білорусі? і 2) чи слід західну субпопуляцію глушця розглядати як самостійну одиницю управління? Отримані дані дозволяють зробити висновок про досить високу генетичну різноманітність досліджених субпопуляцій глушця. Східна субпопуляція глушця характеризується дещо вищими значеннями всіх показників генетичної різноманітності. Усього для досліджуваної популяції глушця встановлено 35 унікальних алелей. З них 10 алелів (29%) відзначено у західній субпопуляції глушця. Аналіз наявності у минулому у досліджуваних субпопуляціях глушця різкого скорочення чисельності не показав присутності цієї події у їхній демографічної історії. Байєсовський аналіз генетичної структурованості показав наявність двох кластерів, що відповідають східній та західній субпопуляціям глушця у Білорусі. Виявлена генетична структурованість популяції глушця в Білорусі підтверджується факторним аналізом відповідності (FCA). Таким чином, результати аналізу генетичної структурованості та генетичної різноманітності дають підстави припускати, що всередині білоруської популяції глушця існує поділ генетичної різноманітності на субпопуляційному рівні, при цьому немає чітко вираженої генетичної диференціації та ознак ізоляції окремих субпопуляцій. Як рекомендації щодо збереження західної субпопуляції глушця в Білорусі пропонується проведення генетичного моніторингу стану новоствореної популяції, а також здійснення генетичного аналізу особин, що використовуються для цілей рятівного розведення.

Ключові слова: *Tetrao urogallus major*, *Tetrao urogallus pleskei*, глушець, генетична різноманітність, генетична диференціація, самостійна одиниця управління, Білорусь.

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Introduction

The western capercaillie (*Tetrao urogallus* Linnaeus, 1758) is a nesting, sedentary, and non-abundant species in Belarus, with the springtime population number estimated at 8 to 9 thousand individuals across the country [Pavlushchick 2018]. It is one of the more valuable game bird species nesting in the woodlands of Belarus for a very long time and having high numbers in the past.

The state of the capercaillie population in Belarus is highly dependent on the state of woodland areas. Earlier analysis of capercaillie distribution across woodland biotopes has revealed a direct connection between the abundance of this species in the area and the prevalence of mature and maturing pine forests, which act as the main preferred habitat of the capercaillie [Dolbik 1974]. However, the woodland areas of Belarus had undergone tremendous changes from the middle of the 16th century until the 1990s due to anthropogenic factors. The period of WWII alone had accounted for a loss of nearly 1.5 billion ha of Belarusian forests. Many forested areas were heavily damaged in combat action. The wooded portion of the country's territory declined to the lowest point in the preceding history of the state, being at 19.7% by 1944. The total area of mature forests fell by 37% from 1940 to 1945 [Baginskii & Esimchik 1996]. In the post-war period, deforestation continued with Belarus becoming a timber-deficit region by the 1960s, and reaching the lowest portion of mature forests among the post-Soviet states by 1988–1993 [Baginskii & Esimchik 1996; Pugachevsky 1999].

Most authors agree that the decline of capercaillie in Belarus in the 1960s–1970s (from 1.5 birds/1000 ha in 1956–1957 to 0.6 birds/1000 ha in 1977–1978) was specifically caused by the loss of mature and maturing pine forests [Dolbik 1974; Ivanyutenko & Semashko 1989].

According to the 1988 survey, capercaillie numbers in Belarus have stabilized at 5.5–6.0 thousand birds and even displayed limited recovery by that point [Ivanyutenko *et al.* 1992]. The authors explained this with the plantings from reforestations of the early post-war period finally reaching the stage optimal for capercaillie habitation, which substantially increased the area of their potential habitats.

Two subspecies of capercaillie are specified in Belarus: *Tetrao urogallus major* C. L. Brehm, 1831 (=*Tetrao urogallus crassirostris*) and *Tetrao urogallus pleskei* Stegmann, 1926, based on some differences in their size, plumage colouration, and mating vocalisation [Tukallo 1927; Fedyushin 1928; Domaniewski & Rydzewski 1937]. *T. u. major* is characterised by higher body mass and size compared to *T. u. pleskei.* However, only body mass and beak measurements have shown statistically significant differences between the two subspecies. The ratio between beak lengths from tip to nostril and from tip to the edge of rhamphotheca is considered less than 0.73 for *T. u. major* and more than 0.74 for *T. u. pleskei* [Pavluschik & Chercas 1999].

Contemporary official statistical survey data indicates the growth of capercaillie abundance in Belarus compared to the last quarter of the 20th century. Nevertheless, when considered separately, the two subspecies —*T. u. major* and *T. u. pleskei* — show different tendencies.

The Belarusian population of the *T. u. major* currently acts as the core of the last viable isolated group of this subspecies, which inhabits western Belarus, eastern Poland, and Lithuania. At present, the Belarusian range of *T. u. major* is limited to Brest, Grodno, and partly Minsk (Volozhinskii and Stolbtsovskii administrative districts) regions. The second half of the 20th century was marked by a population decline of *T. u. major* in Belarus by nearly a factor of 10 [Nikiforov *et al.* 1996; Pavluschik et al. 1999]. The current springtime population estimates of *T. u. major* in Brest and Grodno regions are set at about 460–495 individuals, whereas *T. u. pleskei* has shown population growth over the last 20 years—from 7700 individuals in 2001–2005 to 8200 in 2020.

To estimate the viability and stability of the capercaillie population in Belarus, especially concerning the subpopulation of *T. u. major*, the understanding of the genetic characteristics of the population is of great importance. The latter is in line with the fact that the most active scientific work on the conservation of capercaillie populations is conducted with applying data from molecular genetics.

The existing data on genetic diversity and structure of capercaillie populations indicate that the population of boreal forests area is in a generally good state [Segelbacher *et al.* 2003]. While studies of populations from Central [Segelbacher & Storch 2002; Segelbacher *et al.* 2003a; Segelbacher *et al.* 2003b; Segelbacher *et al.* 2008; Rutkowski *et al.* 2017b], Western [Fameli *et al.* 2017], and Southern Europe [Klinga *et al.* 2015; Klinga *et al.* 2020] show how necessary is the timely consideration of genetic characteristics for a conservation strategy under the conditions of continuously increasing anthropogenic pressure expressed in continuous transformation of the species' natural habitat. The consequential fragmentation of habitats presents a significant source of genetic diversity loss due to disruption of gene flow, decline of population groups, and shrinkage the population of leks [Cayuela *et al.* 2021]. These effects could cause further disruption of populations' adaptive potential and viability, and eventually lead to extinction [Segelbacher *et al.* 2003b].

It is also crucial to remember the necessity of practicing locally adapted approaches to identify the adverse factors affecting the studied species, as generalised and simplified approaches tend to produce contradictory explanations concerning stability of the capercaillie populations [Wegge & Rolstad 2011; Mikoláš *et al.* 2015]. Erroneous estimations of adverse factors can lead to false conclusions and, eventually, to ineffective recommendations for conservation efforts.

Effective and successful development of methods for maintaining and conservation of stable capercaillie populations require a combined consideration of demographic as well as genetic data for analysis [Cayuela *et al.* 2021]. Among the genetic methods, the effective population size monitoring,

gene flow determination, genetic diversity and structure analysis are all fitting solutions [Vázquez *et al.* 2013]. Regarding the samples for conducting genetic studies of capercaillie populations, non-invasive are preferable from conservation point of view. As examples of successful studies following such guidelines, we can mention Segelbacher *et al.* 2003b, Vázquez *et al.* 2013, Morán-Luis *et al.* 2014, Rösner *et al.* 2014, and Rutkowski *et al.* 2017a. This general approach should necessarily be maintained in relation to captive breeding groups used for re-introduction of capercaillie in new habitats, as well as wild populations.

In our earlier molecular genetic studies concerning the genetics of capercaillie population in Belarus, only the mitochondrial DNA control region as a genetic marker was used [Homel *et al.* 2019]. According to our knowledge, no detailed studies of the genetic structure of the Belarusian capercaillie population based on a wider analysis using microsatellites or similar genetic markers have been carried out before, despite it being a priority task for estimating the species' viability and determining its conservation status in the country. Additionally, the issue of two subspecies requires a more detailed investigation due to works concerning the species' phylogeography [Duriez *et al.* 2007; Bajc *et al.* 2011; Homel *et al.* 2019], as well as the potential question of delineating independent management units for efficient conservation efforts.

In this work, we aimed at resolving the following questions: (1) Does the capercaillie subpopulation from the range of *T. u. major* in Belarus exhibit genetic isolation from the rest of the population in the country? and (2) Should we consider the western capercaillie subpopulation in Belarus a management unit?

Materials and Methods

A total of 53 specimens (feathers, muscle tissue) were collected for the genetic diversity and structure analysis of the capercaillie population in Belarus (Supplement 1).

The spatial distribution of capercaillie specimens included in the study of the genetic structure of the species in Belarus is shown in Fig. 1.

To study the genetic structure of the species in Belarus, the eastern subpopulation was further divided into a number of smaller local population groups according to the geography of the specimen collection: northern (n = 12), central (n = 14), and central-southern (n = 2) (Fig. 1; Supplement 2). Two more individuals of capercaillie from the eastern subpopulation were not included into the population groups-level analysis of genetic structure of the species as it was impossible to assign them to a particular group.

DNA extraction. DNA extraction from capercaillie muscle tissues was carried out with Animal and Fungi DNA Preparation Kit (Jena Bioscience). For feather samples, a specialised protocol for DNA extraction was used [De Volo *et al.* 2008].

Microsatellite panel and PCR. For the genotyping of capercaillie samples, 14 pairs of microsatellite markers were selected: BG10, BG12, BG15, BG16, BG18, TUD1, TUT1, TUT2, TUT3, TUT4, TUD4, TUD5, TTT1, and BG14 [Rösner *et al.* 2014; Strzała *et al.* 2015; Rutkowski *et al.* 2017b] (Supplement 3). For BG10–BG18, TUD4, TUD5, and TTT1 loci, the Cy5 fluorescent dye (PRIMETECH ALC) at the 5' end of the forward primer was used, for the rest of loci—the Cy5.5 fluorescent dye (PRIMETECH ALC) at the 5' end of the forward primer was used.

A low level of errors associated with the quality of reading of fragment analysis data as well as with the amplification are noted for the used microsatellite loci [Strzała *et al.* 2015; Rutkowski *et al.* 2017b; Fameli *et al.* 2017]. Microsatellite loci BG10 and BG12 were not used in the current work on the genotyping of capercaillie population due to the linkage with sex chromosomes [Strzała *et al.* 2015].

The reaction mixture contained 2.5 µl Taq-Buffer with $(NH_4)_2SO_4$ 10x (Thermo Scientific), 2.5 µl dNTPs Mix (10x, 2 mM each, Thermo Scientific), 3 µl $MgCl_2$ (25 mM, Thermo Scientific), 2 µl for forward and reverse primers (5 pmol/µl, PRIMETECH ALC), 0.1 µl Taq-polymerase (500 U, Thermo Scientific), 2 or 4 µl of DNA extract depending on the source of the DNA. Thus, 2 µl of DNA was used

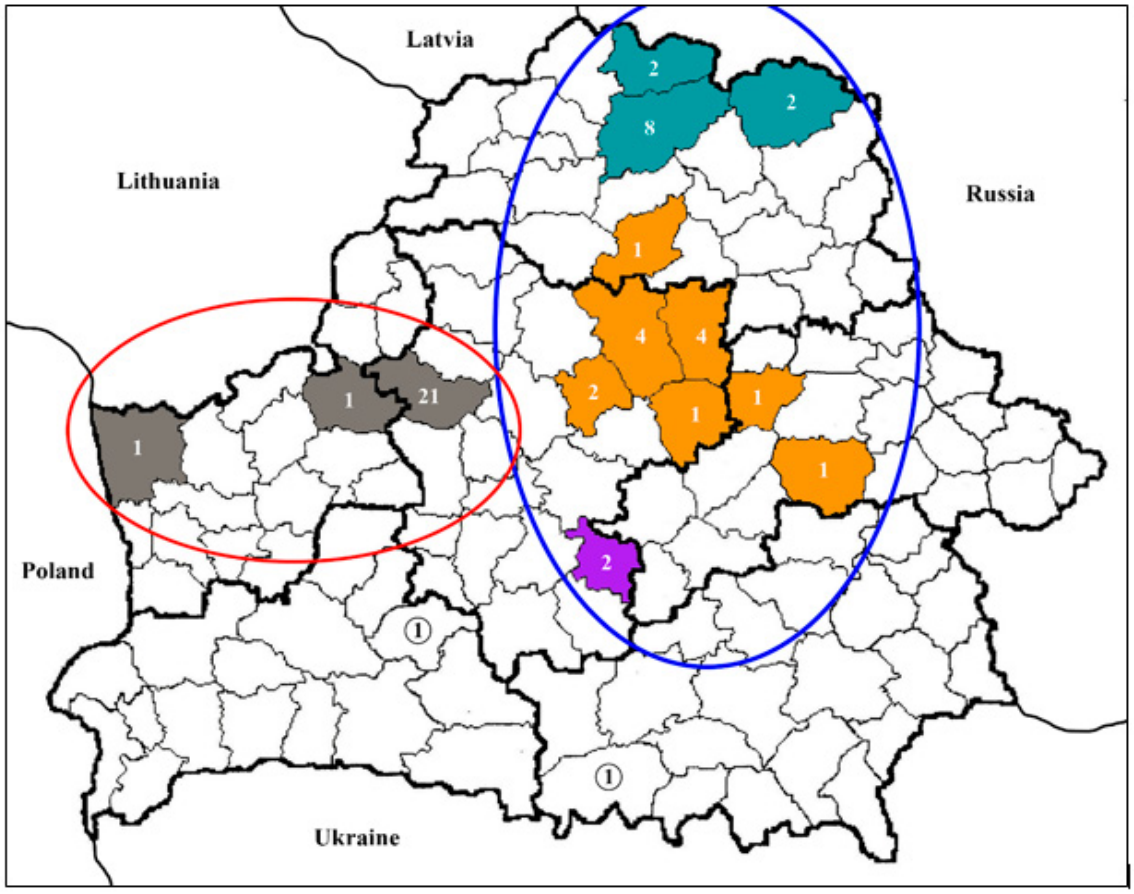

Fig. 1. Spatial distribution of capercaillie specimens included in the study of genetic structure of the species' population in Belarus: red oval — capercaillie specimens from the range of the western subspecies in Belarus (western subpopulation), blue oval — capercaillie specimens from the range of the eastern subspecies in Belarus (eastern subpopulation), turquoise fill — northern local population group (hereafter — population group) of capercaillie of the eastern subpopulation, orange fill — central population group of capercaillie of the eastern subpopulation, purple fill — central-southern population group of capercaillie of the eastern subpopulation, grey fill — capercaillie subpopulation from the range of the western subspecies, figures — number of specimens, figures on white fill — capercaillie specimens from the range of the eastern subspecies not included into the analysis of genetic structure of the species at the level of population groups.

Рис. 1. Просторовий розподіл проб глушця, включених у дослідження генетичної структурованості популяції цього виду в Білорусі: червоний овал — проби глушця з ареалу західного підвиду в Білорусі (західна субпопуляція), синій овал — проби глушця з ареалу проживання східного підвиду бірюзова заливка — північне локальне популяційне угруповання (далі *популяційне угруповання*) глушця східної субпуляції, помаранчева заливка — центральне популяційне угрупування глушця східної субпопуляції, бузкова заливка — центрально-південне популяційне угруповання глушця східної субпопуляції, сіра заливка — субпопуляція глушця з ареалу проживання західного підвиду, цифри — кількість проб, цифри на білій заливці — проби глушця з ареалу східного підвиду, не включені в аналіз генетичної структурованості виду на рівні популяційних угруповань.

from muscle tissue and 4 μl of DNA extract from non-invasively collected feathers. The reaction mix was made up to 25 μl final volume with ultrapure water (Purelab Option Q7BP, Elga, UK).

PCR was performed under the following conditions: 3 min at 94˚C, 35 cycles of 30 s at 94˚C, 30 s at specified temperature for loci (see Supplement 3), 45 s at 72˚C, final cycle 10 min at 72˚C.

PCR products were genotyped using commercial protocols, reagents, and software for the GenomeLab GeXP Genetic Analysis System (Beckman Coulter, USA).

Statistical analysis. Standardization of microsatellite fragment sizes was performed using the TANDEM v 1.07 software [Matschiner & Salzburger 2009].

Fragment analysis data was evaluated for genotyping errors (null alleles, stuttering, large allele dropout) using the software Micro-Checker version 2.2.3 [Chakraborty *et al.* 1992; Brookfield 1996]. An additional estimate of the frequency of null alleles was carried out in Genepop version 4.3 [Raymond & Rousset 1995; Rousset 2008]. Linkage disequilibrium (LD) between loci and deviation of the studied loci from the Hardy–Weinberg equilibrium (HWE) was carried out in Genepop.

Analysis of the genotypes matching was done using GenAlEx v. 6.501 [Peakall & Smouse 2012]. Samples with absolute genotype similarity were excluded from further analysis.

Evidence of recent reductions in the sizes of capercaillie subpopulations was investigated using Bottleneck 1.2.02 [Cornuet & Luikart 1996]. Microsatellite data were tested using the TPM (Two-phase Model) with 95% SMM and variance of 12% [Rutkowski *et al.* 2017b]. IAM (infinite allele model) and SMM (stepwise mutational model) were used as well. The significance of heterozygote excess was assessed using sign test, standardized differences test, and Wilcoxon's sign-rank test.

The number of alleles, allelic richness (AR), inbreeding coefficient estimator (F_{is}, Weir & Cockerham, 1984), observed (H_o) and expected (H_e) heterozygosity, genetic differentiation between capercaillie subpopulations and population groups (F_{st}, G_{st}, D_{Jost}) were calculated using R package diveRsity v1.9.90 [Keenan *et al.* 2013]. The D_{Jost} index was calculated due to the fact that F_{st} and G_{st} can be unreliable when the genetic diversity of the studied populations is very high (Jost, 2008, cited in [Pavlovska 2012]). An additional check for genetic differentiation was performed in Arlequin version 3.5.2.2 [Excoffier & Lischer 2010] using an exact test of population differentiation between samples based on genotype frequencies (number of steps in Markov chain = 100 000, number of dememorization steps = 10 000).

Bayesian inference of population structure was performed using the software STRUCTURE version 2.3.4 [Pritchard *et al.* 2000]. STRUCTURE parameters were admixture model, correlated allele frequencies among populations, length of burning period = 50 000, number of MCMC (Markov chain Monte Carlo) = 100 000. STRUCTURE was run 15 times for each putative genetic cluster (K = 1–5). To visualize the STRUCTURE results we used STRUCTURE HARVESTER 0.6.94 [Earl & vonHoldt 2012]. Graphic representation of the established genetic clusters was carried out in the CLUMPAK web application [Kopelman *et al.* 2015].

Additional analysis of the genetic structure of the capercaillie population in Belarus was carried out in the Genetix 4.05.2 program using the factorial correspondence analysis (FCA) [Belkir *et al.* 2004]. Visualization of FCA was carried out in PAST 4.0 [Hammer *et al.* 2001].

Results and Discussion

Fragment size standardization in TANDEM 1.07 revealed the presence of potential outlier fragment sizes in significant quantities (> 20) for loci TUD4, TUD1, and TUD5.

Genotyping error analysis using Micro-Checker 2.2.3 did not outright confirm the presence of stuttering or large allele dropout for any of the loci utilized in the STR analysis, while indicating the possible presence of null alleles for loci TUD4, BG14, and BG16. Null allele frequencies analysis via the Genepop 4.3 software additionally showed possible null alleles presence for loci TUT1, BG15, BG18, and TUD5. Despite the presence of null alleles, we estimate their frequency sufficiently low (<0.2) as not to skew the further analysis. Indeed, it can be argued that evidence of null alleles is specific to the analysed capercaillie subpopulations rather than loci.

Estimation of the fragment size data for linkage disequilibrium (LD) indicated the absence of any linked loci in the analysis. The eastern subpopulation of capercaillie displayed a significant deviation from the HWE according to loci BG16, BG18, and BG14, while the western subpopulation displayed deviated loci TUT2, BG16, and BG18. While being a cause of concern, the deviation from the HWE is definitely not a sufficient cause for exclusion of the marker from the analysed dataset [Selkoe & Toonen 2006].

Table 1. **Genetic diversity and inbreeding coefficient estimates for the eastern and western capercaillie subpopulations in Belarus**

Таблиця 1. **Показники генетичної різноманітності та коефіцієнта інбридингу для східної та західної субпопуляцій глушця у Білорусі**

Locus	N_a	H_o	H_e	AR	F_{is}
Eastern capercaillie subpopulation					
TUT1	7	0.57	0.65	5.77	0.1252
TUT2	7	0.79	0.77	6.20	–0.0224
TUT3	6	0.83	0.74	5.52	–0.1228
TUT4	7	0.80	0.70	5.61	–0.1492
BG15	5	0.76	0.77	4.98	0.0109
BG16	13	0.89	0.89	11.12	0.0014
BG18	11	0.74	0.81	8.96	0.0894
TTT1	5	0.70	0.68	4.80	–0.0374
BG14	8	0.63	0.83	6.89	0.2387
Mean	7.67	0.75	0.76	6.65 (6.0…7.22)	0.0181 (–0.0547…0.0901)
Western capercaillie subpopulation					
TUT1	6	0.64	0.77	5.74	0.1787
TUT2	5	0.35	0.49	4.41	0.2964
TUT3	4	0.74	0.69	4.00	–0.0698
TUT4	5	0.57	0.69	4.62	0.1853
BG15	4	0.83	0.73	4.00	–0.1277
BG16	9	0.61	0.81	7.87	0.2520
BG18	12	0.68	0.74	9.28	0.0833
TTT1	5	0.86	0.67	4.92	–0.2802
BG14	4	1.00	0.70	3.83	–0.4382
Mean	6.00	0.70	0.70	5.41 (4.78…5.89)	0.0071 (–0.0688…0.0746)

Note: N_a— number of alleles; H_o— observed heterozygosity; H_e— expected heterozygosity; AR — allelic richness; F_{is}— inbreeding coefficient; the 95% CI (confidence interval) for the mean values of AR and F_{is} is shown in parentheses.

In sum, the abovementioned issues served as ground for exclusion the loci TUD4, TUD1, and TUD5 from the further analysis, generally due to the high presence of dubious outlier STR fragment sizes. The final genetic diversity and inbreeding coefficients data for the studied subpopulations is presented in Table 1.

The present data allows us to consider the genetic diversity of the studied subpopulations as sufficiently high. The eastern subpopulation is characterised by slightly higher values of all of the utilized measures, including inbreeding coefficient. The observed high values of genetic diversity for the western subpopulation are most peculiar. The possible explanations for this occurrence include sampling bias, as well as presence of gene flow from the eastern subpopulation. Another option is the lack of any separation between the studied subpopulations, possibly allowing us to consider them as a single population.

The obtained estimates are compatible with the results of prior studies of the species in Poland. Strzała *et al.* (2015) provides the following genetic diversity estimates for the capercaillie population of the Capercaillie Breeding Centre in Wisła Forest District: $H_e = 0.755$, $Ho = 0.772$, $N_a = 6.3$, as well as similar estimations for the capercaillie population of the Bory Dolnośląskie Forest: $H_e = 0.675$, $H_o = 0.863$, $N_a = 5.3$ [Strzała *et al.* 2015]. In general, the genetic diversity estimates for the studied capercaillie subpopulations in Belarus are similar to or slightly higher than the estimates for the Polish populations [Strzała *et al.* 2015; Rutkowski *et al.* 2017b], the boreal forests area [Segelbacher *et al.* 2003b], or Southern Europe (the Balkans) [Klinga et al. 2020], and higher than the estimates for the populations of Central (Germany, Austria, Italy, and Slovenia) [Segelbacher & Storch 2002; Segelbacher *et al.* 2008] and Western (Cantabrian, Pyrenees, and Vosges mountains) Europe [Rodríguez-Muñoz *et al.* 2007; Morán-Luis *et al.* 2014; Fameli *et al.* 2017; Cayuela *et al.* 2021]. The obtained inbreeding coefficient estimates for the Belarusian subpopulations of capercaillie match those of the

Polish populations (in both cases, the values are not statistically significant) [Strzała *et al.* 2015; Rutkowski *et al.* 2017b], but this estimate does not show high values across the capercaillie range in general [Segelbacher *et al.* 2003a; Rodríguez-Muñoz *et al.* 2007], with the exception of several populations that experienced known dramatic population declines in the past [Cayuela *et al.* 2021].

Genetic analysis for the presence of bottleneck events did not reveal any evidence of those in the demographic history of the studied Belarusian subpopulations. For the eastern subpopulation of capercaillie, its high and stable abundance, as well as the absence of barriers that could facilitate isolation of population groups within the subpopulation can explain this outcome. For the western capercaillie subpopulation the situation can be explained either by the presence of gene flow from the eastern subpopulation or by insufficient resolving power of the STR loci set used for the analysis. Additionally, the utilized test for past demographic declines from genetic data is known to give false negative results when applied to populations with low effective population sizes [Höglund 2009]. Although the latter explanation seems unlikely due to high genetic diversity indices shown for the studied subpopulations.

Bayesian analysis of genetic structure has indicated the presence of two clusters in the total dataset, corresponding to the eastern and western subpopulations of capercaillie in Belarus (Fig. 2).

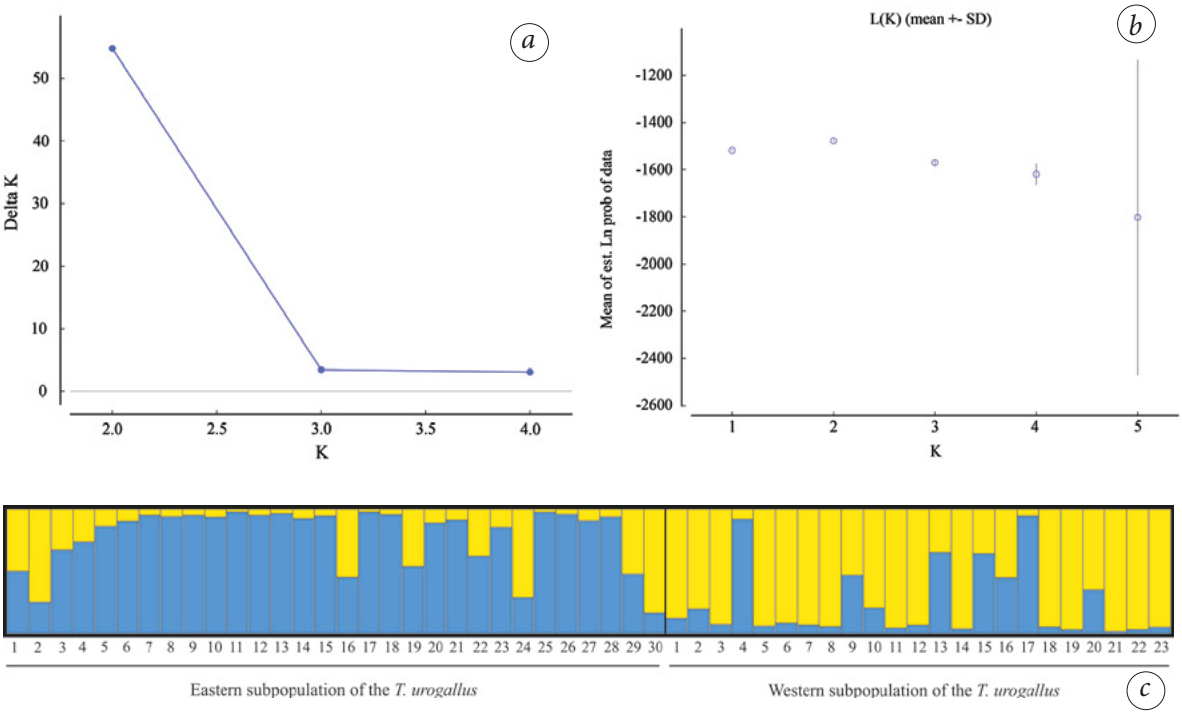

Fig. 2. Results of STRUCTURE analysis for the capercaillie population in Belarus: *a* — ΔK; *b* — estimated mean likelihoods of each number of genetic clusters (SD shown by whiskers); *c* — bar plots; each individual is represented by a vertical bar partitioned into segments; the height of each segment corresponds to the estimated membership proportions for each of the genetic clusters; the number of genetic clusters (K) for the studied sample equals two (ΔK = 54.80, Mean LnPr(2K) = -1478.693). Mean LnPr(2K) — posterior probability estimate for a given number of clusters; values on the horizontal axis in C correspond to the individuals from Supplement 1.

Рис. 2. Результати аналізу STRUCTURE для популяції глушця у Білорусі: *a* — ΔK; *b* — середня оцінка правдоподібності кожного числа генетичних кластерів зі стандартним відхиленням (SD); *c* — графік генетичних кластерів, кожен стовпець представляє особину, включену у вибірку; сегменти стовпців відбивають частку генетичних кластерів кожної особини; кількість виділених генетичних кластерів (K) для досліджуваної вибірки дорівнює двом (ΔK = 54.80 при Mean LnPr(2K) = -1478.693); Mean Ln Pr (2K) — апостеріорна оцінка ймовірності для даного числа кластерів; цифри по осі абсцис C відповідають особин Додатка 1.

Among the individuals sampled in the eastern subpopulation (n = 30), 20 specimens were estimated to belong to the eastern genetic cluster (coloured blue in Fig. 2), while 9 specimens display a mixed character of ancestry (qi < 75), and 1 specimen was assigned to the western genetic cluster (coloured yellow in Fig. 2, Supplement 1). Of the individuals sampled in the western subpopulation (n = 23), 16 were assigned to the western genetic cluster, 2 individuals assigned to the eastern genetic cluster, and 5 more were estimated to have intermediate ancestry relative to the two clusters present (Supplement 1).

The presented data on the genetic structure of the capercaillie population in Belarus provides evidence for the presence of gene flow between the studied subpopulations, which serves as a preferable explanation of the high values of the estimated genetic diversity indices seen not only in the successful eastern subpopulation, but in the less steady western subpopulation as well. This also provides valuable evidence supporting the importance of preservation of the western subpopulation, as it is characterised by a distinguishable genotype composition, and thus serves as a source of genetic diversity for the Belarusian capercaillie population contributing to its stability. It should acknowledged that the eastern subpopulation also carries a significant portion of the species' genetic diversity pool. We present a list of all observed alleles as well as alleles unique to each subpopulation in Supplement 4.

A total of 35 unique alleles in the studied capercaillie population in Belarus were detected for 9 STR loci. Of those 35, 10 unique alleles (29%) were discovered in specimens sampled from the western subpopulation. The lower quantity of unique alleles found in the western subpopulation of capercaillie could be caused by the limited sample size for that subpopulation, as well as its smaller size. Additionally, the low number of unique alleles could be a consequence of an undetected past demographic decline in the subpopulation.

The obtained genetic structure of the capercaillie population in Belarus is also supported by the FCA results, obtained from the same microsatellite fragment size data (Fig. 3).

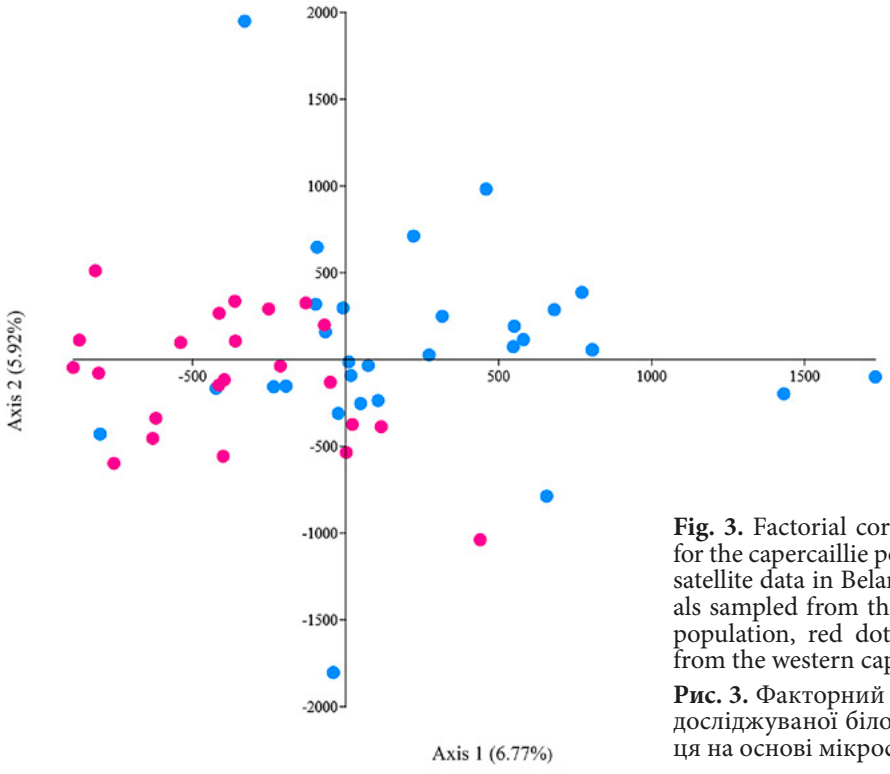

Fig. 3. Factorial correspondence analysis plot for the capercaillie population based on microsatellite data in Belarus: blue dots — individuals sampled from the eastern capercaillie subpopulation, red dots — individuals sampled from the western capercaillie subpopulation.

Рис. 3. Факторний аналіз відповідності для досліджуваної білоруської популяції глушця на основі мікросателітних даних.

Table 2. **Genetic differentiation indices between the eastern and western capercaillie subpopulations in Belarus**

Таблиця 2. **Показники генетичної диференціації між східною та західною субпопуляціями глушця у Білорусі**

Subpopulation / Population group	G_{st}	F_{st}	D_{jost}
Eastern vs western	0.03* (0.02…0.05)	0.06* (0.03…0.09)	0.10* (0.04…0.18)
Northern vs central	0.03* (0.01..0.06)	0.06* (0.01…0.11)	0.12* (0.01…0.25)
Northern vs central-southern	0.02 (–0.03…0.08)	0.02 (–0.05…0.13)	0.01 (–0.1…0.24)
Northern vs western	0.03* (0.01…0.07)	0.07* (0.02…0.13)	0.06 (–0.01…0.15)
Central vs central-southern	0.02 (–0.03…0.07)	0.03 (–0.04…0.12)	0.0003 (–0.08…0.20)
Central vs western	0.04* (0.02…0.07)	0.08* (0.04…0.13)	0.16* (0.07…0.26)
Central-southern vs western	0.07* (0.02…0.14)	0.12* (0.05…0.21)	0.14 (–0.01…0.37)

Note: * — statistically significant, 95% confidence intervals are given in parentheses, capercaillie subpopulations: eastern, western.

The estimation of genetic differentiation indices between the eastern and western capercaillie subpopulations indicates the presence of a moderate genetic differentiation between them (Table 2).

The genetic differentiation between the eastern and western subpopulations of capercaillie is supported by every obtained genetic differentiation estimate, with the maximum value of 0.10 for D_{jost}. Additionally, genetic differentiation between the western subpopulation and the eastern population groups of capercaillie is evident in the maximum D_{jost} value of 0.16 for the central population group. The lowest values of differentiation, lacking statistical significance, can be seen between the northern and central-southern population groups, as well as between the central and central-southern population groups. Limited sample size for the central-southern population group can be the likely cause for that outcome, as well as for the surprisingly high estimates of differentiation between the central-southern population group and the western subpopulation of the capercaillie. The observed genetic differentiation estimates, specifically the F_{st} values (Table 3), are common for capercaillie populations from different parts of the species' range. Like so, the F_{st} distance between the Polish populations and the boreal zone populations equals 0.159 (range from 0.02 to 0.28) [Rutkowski *et al.* 2017b]. For the isolated populations from the French Vosges, the F_{st} distance varied in a range from 0.08 to 0.21 [Cayuela *et al.* 2021], and in a comparison of genetic structure between capercaillie populations inhabiting territories of variable connectivity (boreal forests zone, Central Europe and Western Europe) the F_{st} values varied in a range from 0.019 to 0.237 [Segelbacher *et al.* 2003b]. Thus, the degree of genetic differentiation, as it is shown by neutral nuclear markers, is determined by the demographic history and environmental character of the inhabited range for any given population or a group of populations.

Applying the population groups to the factorial correspondence analysis also supports the presented model of genetic differentiation within the Belarusian capercaillie population (Fig. 4).

This FCA plot supports the presence of two eastern population groups of capercaillie (northern and central) and their distinguishable position relative to the western subpopulation of capercaillie, while the central-southern population group can be considered as part of the central population group. The key element here is the overlap between all clusters, which can indicate a minor degree of genetic structuring. The latter point is validated by the exact test of population differentiation based on genotype frequencies, which showed the lack of significant difference between the studied population groups of the capercaillie, as well as between these groups in general and the western subpopulation (total $P = 0.32$). This test also does not reveal genetic differentiation between the western and eastern subpopulations of the capercaillie (total $P = 0.49$).

A comparative estimation of genetic diversity measures and inbreeding coefficient between the eastern population groups and the western subpopulation of the capercaillie presents us with the same conclusions as the comparison of the western and eastern subpopulations (Table 3), with the only exception being the central-southern population group displaying the lowest genetic diversity values, probably due to the very limited sample size.

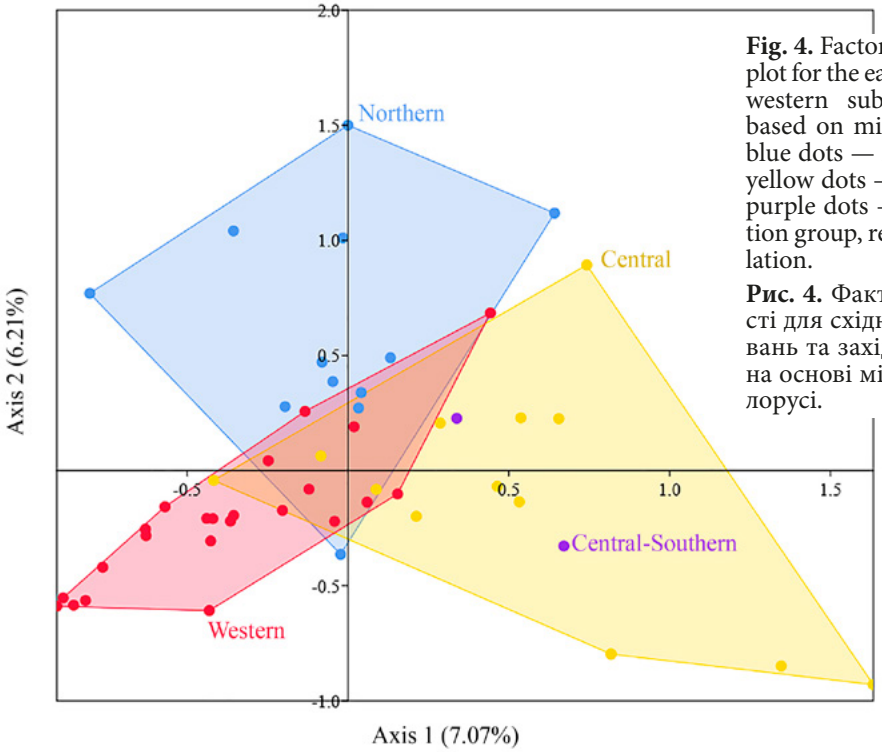

Fig. 4. Factorial correspondence analysis plot for the eastern population groups and western subpopulation of capercaillie based on microsatellite data in Belarus: blue dots — northern population group; yellow dots — central population group; purple dots — central-southern population group, red dots — western subpopulation.

Рис. 4. Факторний аналіз відповідності для східних популяційних угруповань та західної субпопуляції глушця на основі мікросателітних даних у Білорусі.

Table 3. **Genetic diversity and inbreeding coefficient estimates for the eastern population groups and western subpopulation of the capercaillie in Belarus**

Таблиця 3. **Показники генетичної різноманітності та коефіцієнта інбридингу східних популяційних угруповань та західної субпопуляції глушця в Білорусі**

Population groups	H_o	H_e	AR	F_{is}
Northern	0.72	0.74	2.56 (1.6…3.2)	0.03 (–0.12…0.14)
Central	0.77	0.71	2.49 (1.4…3.1)	–0.08 (–0.23…0.06)
Central-southern	0.72	0.51	2.2 (1.7…2.7)	–0.40 (–0.70…-0.10)
Western subpopulation	0.70	0.70	2.41 (1.6…2.9)	0.007 (–0.07…0.08)

Note: H_o—mean observed heterozygosity; H_e—mean expected heterozygosity; AR—mean allelic richness, in parentheses 95% CI; F_{is}—mean inbreeding coefficient, in parentheses 95% CI.

Conclusions

The outcomes of genetic analysis let us conclude that the capercaillie population in Belarus possesses a degree of subpopulation-level genetic differentiation while lacking isolation between the studied subpopulations. The latter can indicate the presence of gene flow between different subpopulations.

The western subpopulation of the capercaillie can be considered as a management unit on its own, fit for being a subject of conservation and monitoring efforts as a group possessing a distinct pool of genetic diversity, which contributes to the total Belarusian capercaillie population, while still retaining a clear reproductive connection with the eastern subpopulation. The same point also means that the western subpopulation cannot be considered an evolutionarily significant unit (ESU), a conclusion that is also supported by earlier findings on genetic diversity of the Belarusian capercaillie population gained from the analysis of mitochondrial control region polymorphism [Homel *et al.* 2019].

Recommendations for conservation of the western subpopulation of the capercaillie in Belarus. Given that the process of conservation breeding of *T. u. major* has already began in Belarus, we suggest considering the proper measures for the successful realization of this project. Genetic monitoring of the newly created population, as well as genetic analysis of the specimens used for breeding are

necessary in order to continuously evaluate the extent of inbreeding and prevent management issues caused by inbreeding and outbreeding depression.

As the next step in the genetic study of the western subpopulation of the capercaillie, we propose long-term evaluation of its effective population size in order to measure the extent and rate of loss of genetic diversity due to genetic drift and inbreeding. This approach is important for maintaining the local subpopulation of the species in Belarus as well as for conducting international efforts for the conservation of capercaillie populations in critical parts of its range (Poland, Lithuania).

Evaluation of the size of the western subpopulation of the capercaillie through genetic tagging also appears promising for efficient monitoring of the group's demographics with minimal disturbance using a non-invasive survey approach. Realization of such measures is possible through BRFFR, UNDP, and the small grants program in Belarus.

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Supplements

Table 1S. **Samples of capercaillie individuals included into the study of the genetic structure and genetic diversity of the species in Belarus**

Таблиця 1S. **Зразки глушця, включені у дослідження генетичної структурованості та генетичної різноманітності виду в Білорусі**

#	Sample code	Administrative region	#	Sample code	Administrative region
Eastern subpopulation of the capercaillie			**Western subpopulation of the capercaillie**		
1	AV00018*	Polotskii	1	AV01998	Volozhinskii
2	AV00019*	Lel'chitskii	2	AV01999	Volozhinskii
3	AV00055*	Polotskii	3	AV03302	Grodnenskii
4	AV00056*	Polotskii	4	AV03252 **(E)**	Iv'evskii
5	AV00243	Borisovskii	5	AV03597	Volozhinskii
6	AV00249	Krupskii	6	AV03598	Volozhinskii
7	AV00255	Berezinskii	7	AV03599	Volozhinskii
8	AV00373	Starodorozhskii	8	AV03251	Volozhinskii
9	AV00374	Smolevichskii	9	AV01180*	Volozhinskii
10	AV00376	Gantsevichskii	10	AV01181	Volozhinskii
11	AV00378	Borisovskii	11	AV01183	Volozhinskii
12	AV00382	Smolevichskii	12	AV01184	Volozhinskii
13	AV00387	Starodorozhskii	13	AV01185*	Volozhinskii
14	AV00390	Krupskii	14	AV03048	Volozhinskii
15	AV00398	Borisovskii	15	AV03052*	Volozhinskii
16	AV00411*	Rossonskii	16	AV03053*	Volozhinskii
17	AV00412	Krupskii	17	AV03063 **(E)**	Volozhinskii
18	AV00417	Krupskii	18	AV03516	Volozhinskii
19	AV00660*	Polotskii	19	AV03650	Volozhinskii
20	AV00665	Polotskii	20	AV03523*	Volozhinskii
21	AV00667	Gorodokskii	21	AV03521	Volozhinskii
22	AV00674*	Belynichskii	22	AV03518	Volozhinskii
23	AV00676	Gorodokskii	23	TeuNL	Volozhinskii
24	AV00687*	Polotskii			
25	AV00710	Novopolotskii			
26	AV00711	Borisovskii			
27	AV00714	Rossonskii			
28	AV00718	Novopolotskii			
29	TeuBh*	Bykhovskii			
30	TeuBr **(W)**	GPU «Berezinskii biosfernyi zapovednik»			

Note: *—s pecimens with mixed ancestry (qi < 75) according to STRUCTURE results; **(W)** — the specimen from the eastern subpopulation of the capercaillie that belongs to the western genetic cluster according to STRUCTURE results; **(E)** — the specimens from the western subpopulation of the capercaillie that belong to the eastern genetic cluster according to STRUCTURE results; the rest of the specimens from the eastern and western subpopulations of the capercaillie belong to the eastern and western genetic clusters, respectively.

Table 2S. **Population groups from the range of** *T. u. pleskei* **in Belarus**

Таблиця 2S. **Популяційні угруповання з території проживання** *T. u. pleskei* **в Білорусі**

#	Sample code	Administrative region	#	Sample code	Administrative region
Northern population group			**Central population group**		
1	AV00018	Polotskii	1	AV00243	Borisovskii
2	AV00055	Polotskii	2	AV00249	Krupskii
3	AV00056	Polotskii	3	AV00255	Berezinskii
4	AV00411	Rossonskii	4	AV00374	Smolevichskii
5	AV00660	Polotskii	5	AV00378	Borisovskii
6	AV00665	Polotskii	6	AV00382	Smolevichskii
7	AV00667	Gorodokskii	7	AV00390	Krupskii
8	AV00676	Gorodokskii	8	AV00398	Borisovskii
9	AV00687	Polotskii	9	AV00412	Krupskii
10	AV00710	Novopolotskii	10	AV00417	Krupskii
11	AV00714	Rossonskii	11	AV00674	Belynichskii
12	AV00718	Novopolotskii	12	AV00711	Borisovskii
Central-southern population group			13	TeuBh	Bykhovskii
1	AV00373	Starodorozhskii	14	TeuBr	GPU 'Berezinskii biosfernyi zapovednik'
2	AV00387	Starodorozhskii			

Table 3S. **Microsatellite panel for the capercaillie**

Таблиця 3S. **Мікросателітна панель для глушця**

Locus	Forward primer (5'–3')	Reverse primer (5'–3')	T_a, (°C)
BG10	ATGTTTCATGTCTTCTGGAATAG	ATTTGGTTAGTAACGCATAAGC	55
BG12	TCTCCTTCTAAACCAGTCATTC	TAGTTTCCACAGAGCACATTG	55
BG15	AAATATGTTTGCTAGGGCTTAC	TACATTTTTCATTGTGGACTTC	54
BG16	GTCATTAGTGCTGTCTGTCTATCT	TGCTAGGTAGGGTAAAAATGG	54
BG18	CCATAACTTAACTTGCACTTTC	CTGATACAAAGATGCCTACAA	53
TUD1	ATTTGCCAGGAAACTTGCTC	AACTACCTGCTTGTTGCTTGG	59
TUT1	GGTCTACATTTGGCTCTGACC	ATATGGCATCCCAGCTATGG	60
TUT2	CCGTGTCAAGTTCTCCAAAC	TTCAAAGCTGTGTTTCATTAGTTG	60
TUT3	CAGGAGGCCTCAACTAATCACC	CGATGCTGGACAGAAGTGAC	60
TUT4	GAGCATCTCCCAGAGTCAGC	TGTGAACCAGCAATCTGAGC	60
TUD4	TTAGCAACCGCAGTGATGTG	GGGAGGACTGTGTAGGAGAGC	60
TUD5	CCTTGCTGCACATTTTCTCC	GGTGCTGAGCATGTACTAGGG	57
TTT1	GCAGTCCAGCCTTATTTCA	TCAGTGCTTCACTAACCTCTT	TD*
BG14	ATCCTACTGAACAAAATATCTGC	TATGCAGGTAGGTAGTGAGAGAG	54

Note: T_a—annealing temperature, TD—touchdown according to [51].

Table 4S. **List of alleles and distribution of unique alleles in the eastern and western capercaillie subpopulations in Belarus**

Таблиця 4S. **Список алелей і розподіл унікальних алелей у східній та західній субпопуляціях глушця в Білорусі**

Allele	Subpopulation (unique allele frequency)	Allele	Subpopulation (unique allele frequency)	Allele	Subpopulation (unique allele frequency)
TUT1		TUT4 (cont.)		BG18 (cont.)	
203	East (0.05)	175		202	
207		179		206	
211		**187**	East (0.02)	**210**	West (0.02)
215		**191**	East (0.02)	**214**	East (0.02)
219		BG15		**218**	East (0.06)
223	West (0.14)	135		222	
227		139		**226**	East (0.11)
275	East (0.03)	143		**238**	East (0.02)
TUT2		147		242	
155	West (0.02)	**151**	East (0.09)	**246**	West (0.02)
159		BG16		**250**	West (0.07)
163		**130**	East (0.04)	**254**	West (0.02)
167	East (0.04)	**150**	East (0.02)	**262**	West (0.02)
171	East (0.07)	**154**	East (0.02)	TTT1	
175		**158**	East (0.05)	209	
179		162		213	
187	East (0.02)	166		217	
TUT3		170		221	
142	East (0.02)	174		**225**	East (0.19)
146		**178**	West (0.02)	**229**	West (0.18)
150		182		BG14	
154		186		**176**	East (0.05)
158		190		**180**	East (0.05)
162	East (0.08)	194		184	
TUT4		**198**	East (0.02)	**188**	East (0.21)
159	East (0.02)	BG18		192	
163	West (0.11)	190		196	
167		194		200	
171		198		**204**	East (0.05)

Note: **Bold** — a unique allele; East — eastern subpopulation of the capercaillie; West — western subpopulation of the capercaillie.