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Assessment of genetic variation and evolutionary history of Caucasian Grouse *(Lyrurus mlokosiewiczi*)

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Caucasian Grouse (*Lyrurus mlokosiewiczi)* is an endemic species found in the Caucasus whose population is declining. Initial assessment of genetic variation and phylogenetic status of the species confirmed the monophyly of *L. mlokosiewiczi*as and indicated a sister relationship between *L. mlokosiewiczi* and *L. tetrix* (Black grouse). Further the Caucasian grouse from Georgia, Caucasus and Iran created three genetic groups with no shared haplotype. This separation could be the result of different evolutionary events or geographic distances between them. Four different haplotypes were identified in north-western Iran, distributed inside and outside Arasbaran protected area (APA), suggesting the expansion of APA to include Caucasian grouse habitats in the Kalibar Mountains (western APA) and enhance the protection of the species in the region.

1. Introduction

Levels of genetic diversity in a particular species are determined by various factors, including its evolutionary history, past climatic events, and current habitat configurations such as fragmentation, continuity, and isolation (Freeland 2005, Sork & Waits 2010). Partitioning of genetic diversity within a species is correlated with life-history characteristics and the degree of differentiation among populations (Epperson 2003). Key historical events such as Pleistocene cold periods have changed the spatial-temporal patterns of genetic variation (Hewitt 2000). Harsh climate fluctuations during the cold periods of the Pleistocene produced changes in species distributions and most species have only persisted in places described as refugia (Stewart *et al.* 2010). With the amelioration of climate, species expanded from one or several refugia and colonised uninhabited areas (Hewitt 2004). Comparative studies of genetic variability have revealed significant changes in species distributions, demonstrating the locations of glacial refugia and postglacial colonization routes (Taberlet *et al.* 1998, Hewitt 2004). In Asia, refugia have been suggested in the Middle East and the Caspian Sea (e.g., Hansson *et al.* 2008, Naderi *et al.* 2014, Khalilzadeh *et al.* 2016). Caucasus is a biodiversity hotspot of plant and animal species, important for the conservation of biodiversity on a global scale (Tarkhnishvili *et al.* 2012).

Genetic differences between populations may also result from isolation due to ecological mechanisms such as the timing of reproduction, physical barriers or geographical distance. In addition, individuals of most species are also limited in their movements by habitat features and by their degree of vagility (Scribner *et al*. 2005). Declines in population sizes and an increase of genetic isolation are a major concern in conservation of many species including phasianid galliforms (e.g., Höglund *et al.* 2007, Larsson *et al.* 2008). Determining the genetic structure of a species in its distribution range helps developing conservation strategies based on the current genetic differentiation (e.g., Caizergues *et al.* 2003).

Caucasian Grouse (*Lyrusus mlokosiewiczi;* Taczanowskai, 1875) is a large bird in the grouse family (Phasianidae), endemic to the Greater and Lesser Caucasian Mountains. Caucasian grouse are distributed in, Georgia, Turkey, Armenia, Azerbaijan and Iran (BirdLife International 2016). The majority of its distribution range is in Georgia, while Iranian populations are the southernmost part of the distribution in the Caucasus with less than 500 individuals in the Kalibar Mountains in North-Western Iran (BirdLife International 2016). Arasbaran protected area (APA) has been established and legally protected since 1976 for the conservation of the species; however, a large portion of its distribution in Kalibar Mountains (western APA) has remained unprotected.

The species is classified as near threatened in the IUCN Red List; however, population estimates, status, and trends have been very variable across its range and data are patchy, which has hindered data collection on populations, trends and threats (BirdLife International 2016). The population of Caucasian grouse is decreasing due to human construction, grazing, and hunting (BirdLife International 2016). In Iran, hunting, habitat loss and degradation due to livestock grazing, mine excavation and tourism (altering nest sites and breeding habitats) are considered as the most threatening factors (Masoud & Mehdizadeh-Fanid 2006).

No published data are currently available on the phylogenetic status and population structure of the Caucasian Grouse. The only previous genetic study used microsatellite loci to obtain genotypes from feather samples and found moderate levels of genetic diversity (observed heterozygosity was 0.6) within the species (Segelbacher & Storch 2003).

Iranian populations represent the southernmost population of the Caucasian Grouse in the Caucasus, which probably has no contact with populations further northward. Range-edge dynamics and fragmentation are expected to impact genetic diversity, decreasing genetic diversity and increasing differentiation (Vucetich & Waite 2003, Eckert *et al.* 2008). Research on Iranian populations has previously focused only on the species' ecology and habitat requirements (Masoud & Mehdizadeh-Fanid 2006, Habibzadeh *et al.* 2010, Habibzadeh *et al.* 2013, Habibzadeh & Rafieyan 2016). Phylogenetic analyses have revealed that Black Grouse *Lyrurus tetrix* (an endemic species in Europe) is a sister species to Caucasian Grouse (Dimcheff *et al.* 2002, Drovetski 2002). The effects of colonisation out of glacial refugia on the genetic diversity and genetic structure was demonstrated through phylogeographic research on Black grouse (Corrales *et al.* 2014).

Caucasian Grouse is one of the least studied grouse in the world, mainly due to its small range, the difficulty of access to its high mountain and remote habitats, and its relatively small population size (GCCW 2004). Unlike the Black Grouse, (Corrales *et al.* 2014), the phylogenetic status and genetic diversity of the Caucasian grouse is unknown and only limited information is available on the species ecology and habitat requirements (Habibzadeh *et al.* 2010, Habibzadeh *et al.* 2013). The current study aimed to (i) assess the phylogenetic relationships of the Caucasian Grouse in its three major population in Iran, Georgia, and Russia and (ii) investigate the genetic diversity of the Iranian population in APA (the southern-most population of Caucasian Grouse) and assessing whether it was related to decreasing genetic diversity and increasing differentiation.

2. Methods

Caucasian grouse occupy high mountain habitats, in altitudes of 1,300–3,000 m.a.s.l. (Gokhelashvili *et al.* 2003, Masoud & Mehdizadeh-Fanid 2006). This situation makes its habitat hard to access for collecting samples such as feather and feces. Further, due to low population size, observation of the

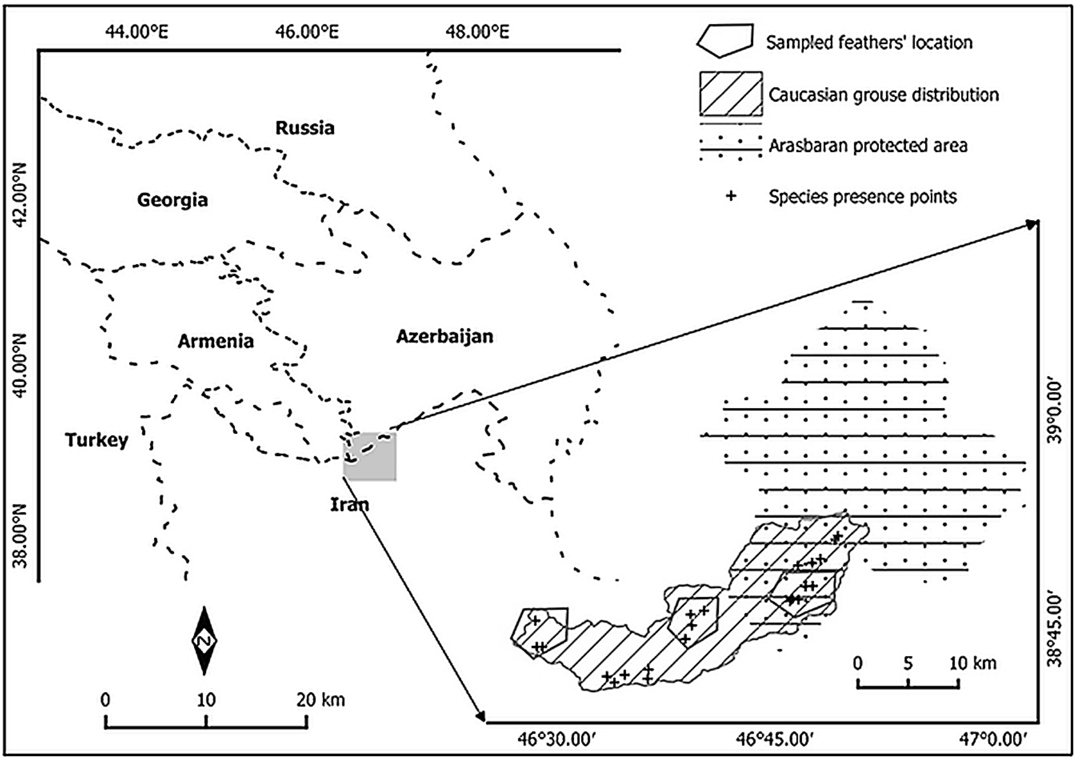

Fig. 1. Study area and sampling sites of the Caucasian Grouse in Arasbaran region, northwestern Iran.

species is difficult except when males display on lek sites during the mating season (Gottschalk *et al.* 2007). During field surveys in North-Western Iran, about 25 presence points were recorded inside and outside the established protected area (APA). Three lekking sites (within the APA and its western unprotected areas, Fig. 1) were identified and used to collect genetic materials. In total, 14 feather samples were collected from North-Western Iran, four tissue samples from the Greater and Lesser Caucasus Mountains and six tissue samples from Caucasus (Table 1, Fig. 1).

DNA was extracted using Qiagen DNeasy blood and tissue kit (Qiagen) according to the manufacture protocol specialized for feathers. A 600 bp fragment of the NADH dehydrogenase subunit 2 (ND2) gene was amplified using forward and reverse primers L5216 and F5766 respectively (Sorenson 2003). Amplifications were performed in 20µl volumes, including 2µl DNA, 1µm of each primer, 3 mM MgCl, 0.1ul dNTP, 1 × Fisher Taq buffer (containing 100 mm Tris-HCl, pH 8.3, 500 ml KCl) and 1 U Fisher Taq buffer (Applied Biosystems). Thermocycling was performed using an initial denaturation cycle of 95 for 3 min, 35 cycles of 93°C for 42 s, 55°C for 32 s and 72°C for 2 min and 30 s with a final extension of 72°C for 10 min. PCR products were purified using PEG 8000 protocol and sequenced on an automated DNA sequencer (ABI 3130 Genetic Analyzer).

Sequences were aligned using the Clustal W algorithm implemented in Mega6, and checked visually. Nucleotide diversity (π) and haplotype diversity (h) were estimated, using ARLEQUIN v3.5.2.2 (Excoffier & Lischer 2010). The HKY+G model was selected as the most appropriate model of nucleotide change using jModeltest v0.1.1 (Posada 2008), according to the Akaike information criterion, with gamma-distributed (G) rate variation across the sites. Phylogenetic reconstruction was performed using Bayesian inference of phylogeny (BI) in MrBayes v3.2 (Ronquist & Huelsenbeck 2003), using the HKY+G model of sequence evolution and two independent runs of four Markov chains over 10,000,000 generations

Table 1. Taxon sample list including, identification number (ID), species, location/origin, haplotype number (H) and reference.

ID	Species	Location / Origin	H	Reference
CBGI1	*L. mlokosiewiczi*	Iran, Azerbaijan, outside Arasbaran protected area	H4	This study
CBGI2	*L. mlokosiewiczi*	Iran, Azerbaijan Sharghi, Arasbaran protected area	H6	This study
CBGI3	*L. mlokosiewiczi*	Iran, Azerbaijan, outside Arasbaran protected area	H5	This study
CBGI5	*L. mlokosiewiczi*	Iran, Azerbaijan Sharghi, Arasbarn protected area	H6	This study
CBGI4	*L. mlokosiewiczi*	Iran, Azerbaijan Sharghi, Arasbarn protected area	H7	This study
CBGI6	*L. mlokosiewiczi*	Iran, Azerbaijan Sharghi, Arasbarn protected area	H7	This study
GCBG23	*L. mlokosiewiczi*	Georgia, Great Caucasus Mountains	H2	This study
GCBG25	*L. mlokosiewiczi*	Georgia, Great Caucasus Mountains	H3	This study
LCBG66	*L. mlokosiewiczi*	Georgia, Lesser Caucasus Mountains	H2	This study
LCBG67	*L. mlokosiewiczi*	Georgia, Lesser Caucasus Mountains	H2	This study
CBGR1771	*L. mlokosiewiczi*	Russian Caucasus	H1	This study
CBGR1772	*L. mlokosiewiczi*	Russian Caucasus	H1	This study
CBGR1720	*L. mlokosiewiczi*	Russian Caucasus	H1	This study
CBGR1758	*L. mlokosiewiczi*	Russian Caucasus	H1	This study
CBGR1781	*L. mlokosiewiczi*	Russian Caucasus	H1	This study
CBGR2074	*L. mlokosiewiczi*	Russian Caucasus	H1	This study
AF222562	*L. mlokosiewiczi*	GenBank, AF222562	H1	Dimcheff *et al*. (2002)
AF230119	*L. mlokosiewiczi*	GenBank, AF222564	H2	Lucchini *et al*. (2001)
AF222564	*L. tetrix*	GenBank, AF222564		Dimcheff *et al*. (2002)
NC024554	*L. tetrix*	GenBank, NC024554		Li *et al*. (2010)
AF230120	*L. tetrix*	GenBank, AF230120		Lucchini *et al*. (2001)
AF222565	*Tetrao urogallus*	GenBank, AF222565		(Dimcheff *et al*. 2002)
AF230122	*Tetrao urogallus*	GenBank, AF230122		Lucchini *et al*. (2010)
AF222563	*Tetrao parvirostris*	GenBank, AF222563		(Dimcheff *et al*. 2002)
A230121	*Tetrao parvirostris*	GenBank, AF222563		Lucchini *et al*. (2001)
AF222567	*Tympanuchus cupido*	GenBank, AF222567		(Dimcheff *et al*. 2002)

and sampling every 100 generations. Sampling trees and estimated parameters generated early in the chain (the first 25%) were discarded as burn-in. Phylogenetic analysis was performed in MrBayes v3.2 (Ronquist & Huelsenbeck 2003) with two independent runs of four Markov chains over 10,000,000 generations and sampling every 100 generations. Sequences of other grouse species were included in the phylogenetic analysis (Table 1) and a sequence of Greater Prairie Chicken *Tympanuchus cupido* (GenBank accession number AF222567) was used as an outgroup. In total 26 sequences were used for the phylogenetic analysis (see below).

A median-joining (MJ) network was constructed using NETWORK v4.1.0 (Bandelt *et al.* 1999). Finally, to explore signs of historical population expansions, we performed a mismatch distribution analysis (Rogers & Harpending 1992) for the Iranian and Georgian samples using DnaSP v.5 (Librado & Rozas 2009). A diagram of haplotype frequencies of pairwise genetic differences was drawn using DnaSP v.5 (Librado & Rozas 2009). Finally, we performed an analysis of molecular variance (AMOVA) to test significant differences between populations using, ARLEQUIN v3.5.2.2 (Excoffier & Lischer 2010).

3. Results

Sequences for the ND2 fragment were obtained for Caucasian grousesamples, including six sequences from Iran, four from Georgia and six from Caucasus (Table 1). All new sequences have been submitted to the Genbank (accession numbers MK617323–MK617338). Eight of the feather samples collected in north-western Iran were unsuccessful in providing DNA or PCR products. The ND2 sequences had open reading frames in all sequences, suggesting they are functional genes and unlikely to be nuclear copies of mtDNA

Table 2: Estimates of evolutionary divergence between sequences. The number of base differences (above diagonal) and pairwise genetic distances between sequences (below diagonal).

	Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	AF222565		12	7	24	27	27	22	29	29	29	29	29	29	30	12	30	30	30	30	29	29	30	27	27	27	37
2	AF230121	0.06		13	7	13	14	12	18	18	18	18	18	18	18	18	14	18	18	18	16	16	16	16	13	13	26
3	AF230122	0.04	0.07		22	23	20	28	28	28	28	28	28	28	28	30	13	29	29	29	27	28	2	27	26	26	41
4	AF222563	0.07	0.03	0.08		1	6	10	10	10	10	10	10	10	12	6	11	11	11	11	11	14	14	11	14	14	39
5	NC024554	0.08	0.06	0.03	0.06		5	11	11	11	11	11	11	11	11	13	7	12	12	12	12	15	15	12	15	15	39
6	AF222564	0.08	0.07	0.03	0.07	0.00		5	11	11	11	11	11	11	11	13	7	12	12	12	12	15	15	12	15	15	39
7	AF230120	0.07	0.06	0.04	0.06	0.02	0.01		12	12	12	12	12	12	12	14	9	13	13	13	13	16	16	13	14	14	38
8	CBGR1781	0.09	0.09	0.06	0.08	0.03	0.03	0.03		0	1	0	1	1	0	0	1	1	1	1	3	8	8	5	8	8	35
9	CBGR2074	0.09	0.09	0.06	0.08	0.03	0.03	0.03	0.00			1	1	1	0	0	1	1	1	1	3	8	8	5	8	8	35
10	CBGR1771	0.09	0.09	0.06	0.08	0.03	0.03	0.03	0.00	0.00		0	1	1	0	0	1	1	1	1	3	8	8	5	8	8	35
11	CBGR1772	0.09	0.09	0.06	0.08	0.03	0.03	0.03	0.00	0.00	0.00		1	1	0	0	1	1	1	1	3	8	8	5	8	8	35
12	CBGR1720	0.09	0.09	0.06	0.08	0.03	0.03	0.03	0.00	0.00	0.00	0.00		1	0	0	1	1	1	1	3	8	8	5	8	8	35
13	CBGR1758	0.09	0.09	0.06	0.08	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00		0	0	1	1	1	1	3	8	8	5	8	8	35
14	AF222562	0.09	0.09	0.06	0.08	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00		0	1	1	1	1	3	8	8	5	8	8	35
15	GCBG25	0.09	0.09	0.06	0.09	0.03	0.04	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.01		1	1	1	1	3	8	8	5	8	8	37
16	AF230119	0.10	0.12	0.08	0.11	0.05	0.05	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		1	1	1	2	4	4	4	6	6	14
17	LCBG66	0.09	0.09	0.06	0.09	0.03	0.03	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		1	1	2	7	7	4	7	7	36
18	LCBG67	0.09	0.09	0.06	0.09	0.03	0.03	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		1	2	7	7	4	7	7	36
19	GCBG23	0.09	0.09	0.06	0.09	0.03	0.03	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		2	7	7	4	7	7	36
20	CBGI1	0.09	0.08	0.06	0.08	0.03	0.03	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		5	5	2	5	5	36
21	CBGI2	0.09	0.08	0.06	0.08	0.04	0.04	0.05	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.01		1	3	8	8	35
22	CBGI5	0.09	0.08	0.06	0.08	0.04	0.04	0.05	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.01	0.00		3	8	8	35
23	CBGI3	0.09	0.08	0.06	0.08	0.03	0.03	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01		5	5	36
24	CBGI4	0.08	0.06	0.06	0.08	0.04	0.04	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.05	0.02	0.02	0.02	0.01	0.02	0.02	0.01		5	35
25	CBGI6	0.08	0.06	0.06	0.08	0.04	0.04	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.05	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.00		35
26	AF222567	0.12	0.14	0.11	0.13	0.12	0.12	0.12	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.12	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	

(numts). Further, no evidence for double PCR-amplification peaks and ambiguities in the sequence data was found to suggest the presence of numts in the mtDNA data set.

On average, the ND2 sequence was found to be A–C rich (A = 31%, C = 31%, G = 11% and T = 27%). Transitions were more frequent than transversions with a majority of changes between C and T. A total of 13 variable sites and 12 parsimony informative sites were observed among Caucasian Grouse sequences (Table 2). Seven haplotypes were identified within the dataset (Table 1, Fig. 2A) including four haplotypes (H4, H5, H6, H7) from Iran (*n* = 6), two haplotypes (H2 and H3) from Georgia (*n* = 4) and one (H1) from Russian Caucasus (*n* = 6). All haplotypes were unique with no shared haplotype between the three regions (Iran, Georgia and Russian Caucasus). Haplotype diversity was 0.80 ± 0.09 and nucleotide diversity was 0.008 ± 0.02.

Intra-specific sequence divergence obtained by applying the HKY+G model to the dataset (proportion of invariable sites = 0.48, Gamma distribution with shape parameter = 1.2) ranged from 0 to 2%. The Bayesian tree (Fig. 2B) confirmed the monophyly of *L. mlokosiewiczi*as, as all the specimens share the same clade. Results also indicated a sister relationship between Caucasian Grouse and Black Grouse, therefore, this region of ND2 is appropriate for the species-level recognition of grouse species. In an analysis of molecular variance (AMOVA), significant FST was obtained for populations, with 77% of the variation was partitioned among populations and 23% within populations. Pairwise FST values were statistically significant between Iran-Russia (FST = 0.807, *P* = 0.002), Iran-Georgia (FST = 0.67, *P* = 0.01) and Russia-Georgia (FST =0.845, *P* = 0.001).

4. Discussion

4.1. Regional differences

The current study is the first attempt to provide phylogenetic information about the Caucasian Grouse and to include genetic samples from the southernmost part of the species range. Although we used the small sample size, our work is the only available data on the species.

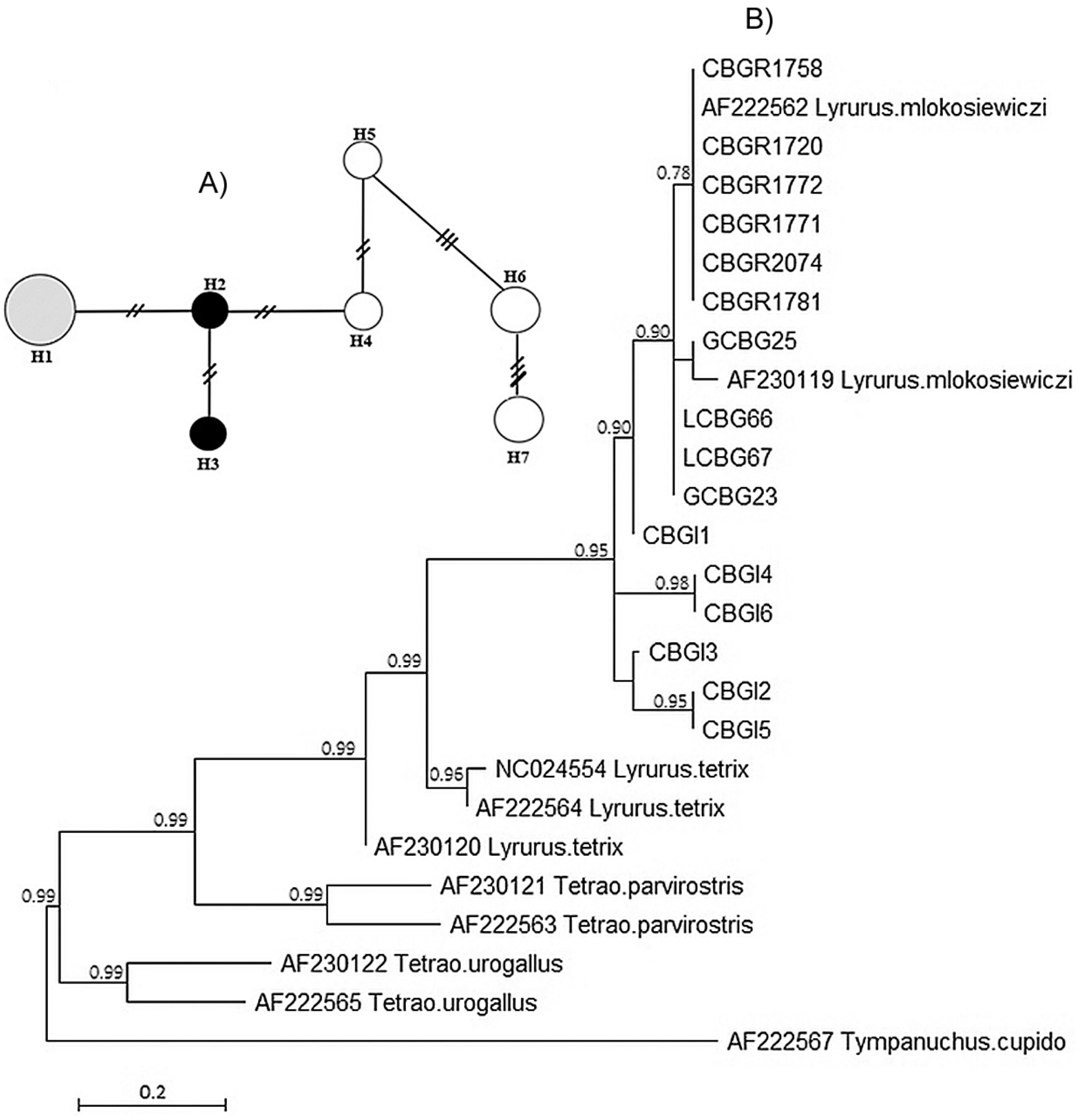

Fig. 2. A) Median-joining network based on the ND2 sequences depicting the relationships among seven Caucasian Grouse haplotypes (H1–H7). Number of mutations between haplotypes are indicated with a dashed line. Circle sizes are proportional to the haplotype frequencies. Black and white colours represent Georgian and Iranian samples, and the gray pattern represents Russian samples. C: bayesian phylogenetic tree of ND2 sequences of Caucasian Grouse inferred using HKY+ G substitution model. Numbers on nodes show Bayesian posterior probabilities. Accession numbers are given for GenBank sequences of Black Grouse *Tetrao tetrix*, Western Capercaillie *Tetrao urogallus,* Spotted Capercaillie *Tetrao parvirostris* and Greater Prairie Chicken.

Haplotype diversity of the species was relatively high (0.80) and similar to values previously reported for other grouse species such as Black Grouse (0.82, Corrales *et al.* 2014) and European Capercaillie (*Tetrao urogallus*) (0.79, Segelbacher & Piertney 2007). Genetic diversity in Black Grouse is suggested to be due to the admixture of lineages from different refugia (Corrales *et al.* 2014). Patterns of phylogeographic structure in Black grouse and European Capercaillie indicated that extant European populations are derived from a few refugia. Populations restricted to refugia expanded their range to a variety of unoccupied habitats, following the retreat of glacial ice sheets (Segelbacher & Piertney 2007, Corrales *et al.* 2014). Caucasus is recognized as a biodiversity hotspot, having the largest forest refugium in the Western Asian/Near Eastern region (e.g., Tarkhnishvili *et al.* 2012). Hyrcanian forest in the north of Iran is suggested to be a refugia for several species such as fat dormouse (*Glis glis*) and wild boar (*Sus scrofa*) (Naderi *et al.* 2014, Khalilzadeh *et al.* 2016). Limited number of haplotypes in the current study prevented us from further analyses to explore signs of historical population expansions in Caucasian Grouse. Therefore, larger sample size would be helpful to clarify phylogeographic patterns of the species.

The three regions examined in this study (Georgia, Caucasus, and Iran) created three genetic groups within the Caucasian Grouse clade with no shared haplotype (a genetically linked sequences of alleles with greater power for discrimination between genomic regions). This separation could be the result of different evolutionary events or genetic distances among them. The previous genetic study indicated that the populations of Armenia, Azerbaijan, and Georgia are separated from each other which might be a result of geographical distances and fragmentation (Segelbacher & Storch 2003). Despite the limited number of samples, the current data can be a starting point for revealing genetic relationships between the Caucasian Grouse populations and serves as a baseline for future studies.

4.2. Caucasian Grouse in Iran

Iranian populations represent the southern-most part of Caucasian Grouse range in the Caucasus with less than 500 individuals in the Kalibar Mountains in North-Western Iran (BirdLife International 2016). Four haplotypes were identified within the six samples collected from north-western Iran. It has been suggested that range-edge dynamics and isolation decrease genetic diversity and increase differentiation (Vucetich & Waite 2003, Eckert *et al.* 2008). Iranian populations could be remnants of a larger ancestral population which probably retained much of its former genetic diversity. The existence of natural barriers such as valleys and habitat fragmentation could have prevented contact with other populations. A fine genetic study is required to further define the genetic structure and the isolation of Caucasian grouse populations in Iran.

Two (H4 and H5) of the four haplotypes identified in North-Western Iran were located outside the established APA, suggesting that parts of the Iranian populations are currently unprotected. Field surveys also indicate that a large portion of the current Caucasian grouse population in Iran is outside the established APA. In 2009, for example, 350 individuals were recorded in north-western Iran, including 190 individuals in APA and 160 individuals outside the APA (M. Masoud, unpubl. data).

Our genetic data are consistent with this information as further evidence that only parts of the Iranian populations are currently protected. Hunting and habitat loss and degradation, occurring due to livestock grazing, tourism industry and mine excavation (e.g., deforestation of 800 hectares of the species habitat in Kalibar Mountains due to Sungun copper mine excavation) are major threats to the species nesting and breeding sites (Masoud & Mehdizadeh-Fanid 2006). From a conservation perspective, it is recommended to expand APA to include Caucasian grouse habitats outside the current protected area (western APA) and enhance the protection of the species in the region.

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Kaukasianteeren geneettinen variaatio ja evoluutiohistoria

Kaukaasianteeri (*Lyrurus mlokosiewiczi)* on Kaukasuksen alueen endeeminen laji, jonka populaatiokoko on laskussa. Alustava fylogenian ja geneettisen vaihtelun analyysi osoitti, että laji on monofyleettinen, ja sisarlaji teerelle (*L. tetrix*). Kaukasianteeret Georgiasta, Venäjän Kaukasukselta ja Iranista muodostivat kolme geneettistä ryhmää, joilla ei ollut yhteisiä haplotyyppejä. Nämä erot voivat johtua esimerkiksi maantieteellisestä etäisyydestä tai evolutiivisesta historiasta. Pohjois-Iranin populaatioissa havaittiin neljä haplotyyppiä. Haplotyypit sijaitsivat sekä suojelualueen (Arasbara) sisällä että ulkopuolella (Kalibar-vuoristo), mikä viittaa siihen, että suojelualuetta suositellaan laajennettavaksi lajin elinkykyisten populaatioiden turvaamiseksi.

References

Bandelt, H.J., Forster, P. & Rohl, A. 1999: Median-joining networks for inferring intraspecific phylogenies. — Molecular Biology & Evolution 16: 37–48.

BirdLife International 2016: Lyrurus mlokosiewiczi. The IUCN Red List of Threatened Species 2016: e.T22679483A92815595. http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22679483A92815595.en. Downloaded on 29 May 2018.

Caizergues, A., Rätti, O., Helle, P., Rotelli, L., Ellison, L. & Rasplus, J.-Y. 2003: Population genetic structure of male black grouse (*Tetrao tetrix* L.) in fragmented vs. continuous landscapes. — Molecular Ecology 12: 2297–2305.

Corrales, C., Pavlovska, M. & Höglund, J. 2014: Phylogeography and subspecies status of Black Grouse. — Journal of Ornithology 155: 13–25.

Dimcheff, D.E., Drovetski, S.V. & Mindell, D.P. 2002: Phylogeny of Tetraoninae and other galliform birds using mitochondrial 12S and ND2 genes. — Molecular Phylogenetic & Evolution 24: 203–215.

Drovetski, S.V. 2002: Molecular phylogeny of grouse: individual and combined performance of W-linked, autosomal, and mitochondrial loci. — Systematic Biology 51: 930–945.

Eckert, C.G., Samis, K.E. & Lougheed, S.C. 2008: Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. — Molecular Ecology 17: 1170–1188.

Epperson, B.K., 2003: Geographical genetics. — Princeton, Princeton University Press.

Excoffier, L. & Lischer, H.L. 2010: Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. — Molecular Ecology Resources 10: 564–567.

Freeland, J.R., 2005: Molecular ecology. — Chichester, Wiley & Sons Ltd.

GCCW 2004: Caucasian Black Grouse Research, Monitoring, and Conservation Management in Georgia. Deliverable no 1: Data Collation and Identification of Study Areas. Tbilisi, Georgian Center for the Conservation of Wildlife.

Gokhelashvili, R., Reese, K.P. & Gavashelishvili, L. 2003: How much do we know about the Caucasian black grouse *Tetrao mlokosiewiczi*? — Sandgrouse 25: 32–40.

Gottschalk, T.K., Ekschmitt, K., Isfendiyaroglu, S., Gem, E. & Wolters, V. 2007: Assessing the potential distribution of the Caucasian black grouse *Tetrao mlokosiewiczi* in Turkey through spatial modelling. — Journal of Ornithology 148: 427–434.

Habibzadeh, N., Karami, M., Alavipanah, S.K. & Riazi, B. 2013: Landscape requirements of Caucasian grouse (*Lyrurus mlokosiewiczi*) in Arasbaran region, East Azerbaijan, Iran. — Wilson Journal of Ornithology 125: 140–149.

Habibzadeh, N., Karami, M. & Tarinejad, A. 2010: Caucasian black grouse (*Tetrao mlokosiewiczi*) breeding display sites selection in Arasbaran region, East Azerbaijan, Iran. — Russian Journal of Ecology 41: 450–457.

Habibzadeh, N. & Rafieyan, O. 2016: Land-cover patterns surrounding Caucasian grouse leks in Arasbaran region, East Azerbaijan, Iran. — Wildlife Research 43: 267–275.

Hansson, B., Hasselquist, D., Tarka, M., Zehtindjiev, P. & Bensch, S. 2008: Postglacial colonisation patterns and the role of isolation and expansion in driving diversification in a passerine bird. — PLoS ONE 3: e2794.

Hewitt, G.M. 2000: The genetic legacy of the ice ages. — Nature 405: 907–913.

Hewitt, G.M. 2004: Genetic consequences of climatic oscillations in the Quaternary. — Philosophical transactions of the Royal Society of London. Series B, Biological sciences 359: 183–195.

Höglund, J., Larsson, J.K., Jansman, H.A.H. & Segelbacher, G. 2007: Genetic variability in European black grouse (*Tetrao tetrix*). — Conservation Genetics 8: 239–243.

Khalilzadeh, P., Rezaei, H.R., Fadakar, D., Serati, M., Aliabadian, M., Haile, J. & Goshtasb, H. 2016: Contact Zone of Asian and European Wild Boar at North West of Iran. — PLoS One 11: e0159499.

Larsson, J.K., Jansman, H.A.H., Segelbacher, G., Höglund, J. & Koelewijn, H.P. 2008: Genetic impoverishment of the last black grouse (*Tetrao tetrix*) population in the Netherlands: detectable only with a reference from the past. — Molecular Ecology 17: 1897–1904.

Librado, P. & Rozas, J. 2009: DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. — Bioinformatics 25: 1451–1452.

Masoud, M. & Mehdizadeh-Fanid, L. 2006: A study of Caucasian black grouse (*Tetrao mlokosiewiczi*) population dispersion confined in Iran. — Grouse News 31: 5–8.

Naderi, G., Kaboli, M., Koren, T., Karami, M., Zupan, S., Rezaei, H.R. & Krystufek, B. 2014: Mitochondrial evidence uncovers a refugium for the fat dormouse (*Glis glis* Linnaeus, 1766) in Hyrcanian forests of northern Iran. — Mammalian Biology 79: 202–207.

Posada, D. 2008: jModelTest: phylogenetic model averaging. — Molecular Biology & Evolution 25: 1253–1256.

Rogers, A.R. & Harpending, H. 1992: Population growth makes waves in the distribution of pairwise genetic differences. — Molecular Biology & Evolution 9: 552–569.

Ronquist, F. & Huelsenbeck, J.P. 2003: MrBayes 3: Bayesian phylogenetic inference under mixed models. — Bioinformatics 19: 1572–1574.

Scribner, K., Blanchong, J.A., Bruggeman, D.J., Epperson, B.K., Lee, C.Y., Pan, Y.W., Shorey, R.I., Prince, H.H., Winterstein, S.R. & Luukkonen, D.R. 2005: Geographical genetics: conceptual foundations and empirical applications of spatial genetic data in wildlife management. — Journal of Wildlife management 69: 1434–1453.

Segelbacher, G. & Piertney, S. 2007: Phylogeography of the European capercaillie (*Tetrao urogallus*) and its implications for conservation. — Journal of Ornithology 148: 269–274.

Segelbacher, G. & Storch, I. 2003: Testing existing markers for studying genetic variability in Caucasian Black Grouse (*Tetrao mlokosiewiczi*), a pilot study. — Grouse News 28: 12–13.

Sorenson, M.D., 2003: Avian mtDNA primers. — Boston, Boston University Press.

Sork, V.L. & Waits, L. 2010: Contributions of landscape genetics – approaches, insights, and future potential. — Molecular Ecology 19: 3489–3495.

Stewart, J.R., Lister, A.M., Barnes, I. & Dalen, L. 2010: Refugia revisited: individualistic responses of species in space and time. — Proceedings of the Royal Society B 277: 661–671.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.G. & Cosson, J.F. 1998: Comparative phylogeography and postglacial colonization routes in Europe. — Molecular Ecology 7: 453–464.

Tarkhnishvili, D., Gavashelishvili, A. & Mumladze, L. 2012: Palaeoclimatic models help to understand current distribution of Caucasian forest species. — Biological Journal of the Linnean Society 105: 231–248.

Vucetich, J.A. & Waite, T.A. 2003: Spatial patterns of demography and genetic processes across the species' range: Null hypotheses for landscape. — Conservation Genetics 4: 639–645.