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Research Article

Microsatellite evidence of common partridge *(Alectoris chukar)* genetic diversity in the western parts of Iran

Asaad Sarshar[1], Seyed Mahmoud Ghasempouri[2]*, Mansour Aliabadian[3], Morteza Naderi[4]

[1]Department of Environmental Sciences, Faculty of Natural Resources and Environment, Islamic Azad University, Science and Research Branch P.O. Box 14515-775, Tehran, Iran

[2]Department of Environment, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, 46417-76489, Noor, Iran

[3]Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad (FUM), Mashhad, Iran

[4]Department of Environmental Sciences, Faculty of Agriculture and Environment, Arak University, Arak 38156-8-8349, Iran

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Abstract

In this research, the genetic peculiarities of the common partridge *(Alectoris chukar)* have been investigated using a non-invasive sampling method and microsatellite markers in six loci. During 2014 and 2015, one hundred feather samples have been collected from four northwestern provinces of Iran. Our findings indicated that in Ilam, Kermanshah, and Hersin, two distinct subpopulations have diverged from other populations (F_{st} = 0.1). The highest diversity was recorded among the Kordestan populations, which can be related to the traditional culture of target species relocation, releasing in different places by the locals. The highest allelic frequency of 13.15 (and effective allelic frequency of 20) was recorded in Marivan subpopulation, which can be related to winter sampling along with the species' narrow migration routes to a warmer region. Because of this, the later subpopulation also showed deviation from the Hardy-Weinberg equation as well. Finally, two stepwise and two steps mutation models didn't indicate any historical bottleneck, then the species currently face no serious threats.

Keywords: Common partridge, genetic structure, genetic diversity, microsatellite markers

Introduction

Birds are one of the most diverse groups among vertebrates. Based on the latest checklist of Iran's birds, the number of species has been estimated to be around 546 species. Among the Iran birds, Galliformes show high diversity and include families like Tatraonidae, Turnicidae, and Phasianidae. The last family includes eight species which of them, partridges are the most known species. Partridges are of the most globally diverse groups of Galliformes around the world. The Genus *Alectorsi* include 7 polymorphic species belonging to the Phasianus subfamily (Randi 1996; Madge *et al.*, 2002). Common Partridge (*Alectoris chukar*) is a bird with about 33 cm of total body length and is very known for people. This partridge has well marked black and white bars on the flanks and a black band running from the forehead across the eye and running down the head to form a necklace that encloses a white throat. The species has been distributed from the eastern Mediterranean, throughout minor Asia to Himalayas, Mongolia, and china (Fuller *et al.*, 2000). Common partridge commonly can be found in lower latitudes with continental or Mediterranean (usually dry or semidry) climate. It uses hills and mountains even with steep rocky open hillsides with grass or scattered scrub or cultivation. It is one of the most commonly hunted birds all over the world (Barbanera *et al.*, 2007; Madge *et al.*, 2002) as it was hunted 245000 to 600000 individuals each year in Europe (Guerrini *et al.*, 2007). Unfortunately, the population trend of the species is not satisfactory in Iran as well (Abbasi *et al.*, 2010) and it may cause a severe situation in the future in case of any conservation actions and controlling illegal hunting (Fuller *et al*. 2004). We tried to investigate the genetic diversity of the common partridge in western Iran using six microsatellite markers.

Material and methods

Sampling: Common partridge has been sampled using non-invasive methods and collecting feathers (mainly caudal feathers) in western Azerbaijan, Kurdistan, and Kermanshah provinces (Fig. 1). Using feathers as a genetic sample had been already used by different researchers like Speller *et al.*, (2011) and Malago *et al.*, (2008). The geographic coordinates of the sampling location have been recorded as well.

Figure 1. Sampling locations in western Iran

DNA extraction: We used two protocol of DNA extraction from feathers as salting-out (Rudbeck and Dissing 1998, Malago *et al.*, 2002) and Organic (Phenol–Chloroform) (Yue and Orban 2001) methods as follow:

To use the salting-out method feather follicle from the tip to the upper part of the quill was cut in a sterile condition and placed in a 1.5 ml microtubes and were dissected by a sterile sizer (Fig. 2). After that, we added 20 µl NaOH with a concentration of 0.2 normal. The microtubes have been placed in a 75-celsius degree incubator for 20 minutes and then 180 µl 0.04 molar Tris-Hcl (pH= 7.5) has been added. The final solution was separated by micro samples and transferred to the new microtubes. A 0.5–1-cm section was cut from the terminal portion of the feather quill and placed in a 1.5-ml Eppendorf tube containing 500 ml of lysis buffer (50 mM Tris-HCl, pH 8, 20 mM ethylenediaminetetraacetic acid [EDTA], pH 8, 2% sodium dodecyl sulfate) and proteinase K at a final concentration of 175 mg/ml. Lysis temperatures and incubation times were different depending on the feather size. For feather quill contained soft tissue or blood (as in new growing

feathers), the lysis was performed at 37 C overnight with gentle shaking. The primers which were used in amplifying the related markers have been listed in table 1.

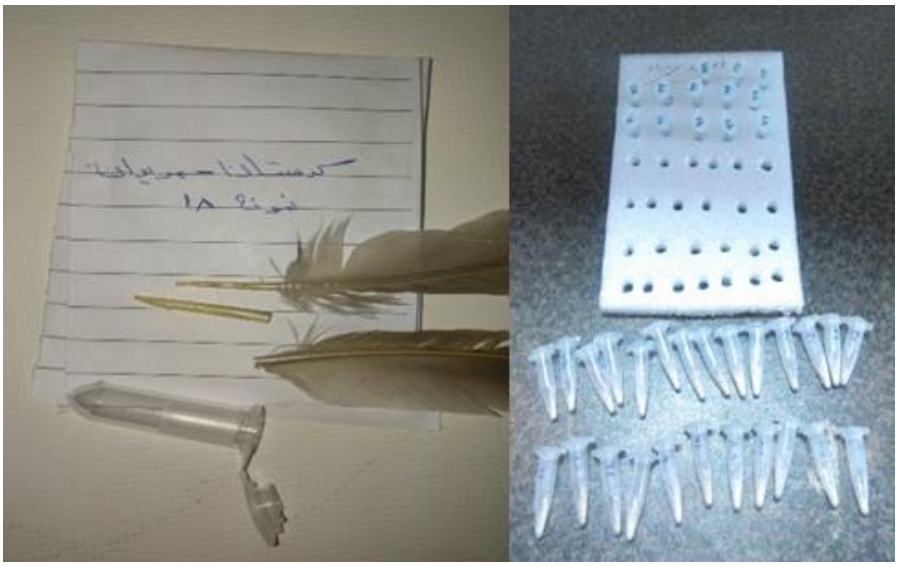

Figure 2. Preparation of samples for DNA extraction

Table 1. Forward and reverse primers used for microsatellites study (John *et al.*, 2006)

Position	Sequence (5'-3')	Annealing temperature (°C)	Size
TSP.1.20.16	F:TCTGCCAAGCATGTTCAAAG R: GGTGAGCGAATTACAAATCCA	58	155–168
TSP.1.20.46	F: TGCACTAGCAATAGCATCACG R: TGCGAATTTCAAAAGCTGTG	58	173-161
TSP.1.20.9	F:ACCATTCCCCAGCTGAATTT R: CTTCCTGAGTCTGGCTCTGC	58	180-194
TSP.1.20.5	F:GGTTCTTATCTTTTGGTTCTTCC R: CCTGCCTCAATTTCAAGGTT	59	187–191
TSP.1.20.2B	F:-TCTCCACTGCCCTGTTCTC R: GTAAGGTCTGCAGTGGCAC	59	228-230
TSP.1.20.43	F:CAGGAGCTCGAGGCTACAGT R: TGCAGGGAACACTTCTTGTG	58	223-229

Data analysis

After getting band pictures and reading them using Gel pro software, we used GenALEx ver 6.5 to determine the allelic frequency, the number of effective alleles, heterozygosity, Hardy-Weinberg ratios, Shannon index, and the relatedness of the individuals to the populations or genetic distances have been calculated. The phylogenetic relationships among the populations based on genetic distance have been checked with UPGMA trees. The correlation between geographic distances and genetic isolation was checked using the Mantel test in GenALEx software. Using F_{st} and AMOVA analysis we checked the inter and intrapopulation genetic diversity as well. Bottleneck analysis was used to see any possible shrinkage in the genetic reservoir. The population structure was identified by STRUCTURE after getting delta K (ΔK) for the target populations in Western Iran. For PCR and thermal cycles, we used the best cycles suggested for possibly low-quality DNA samples (Hogan *et al.*, 2008, Harvey *et al.*, 2006, Volo *et al.*, 2005) (Table 2).

Table 2. Thermal cycles used for PCR

Phase		Temperature	Time (min)
Initial phase		95	11
Denaturation	38 cycles	95	40
Annealing		58	1
Extension		72	2
	Final extension	72	10

PCR products were transferred to 6% acrylamide Gel with silver staining method (Fig. 3). After taking pictures from gels and reading the bands by Gel-Pro Analyzer, the bands have been analyzed by GenALEx software.

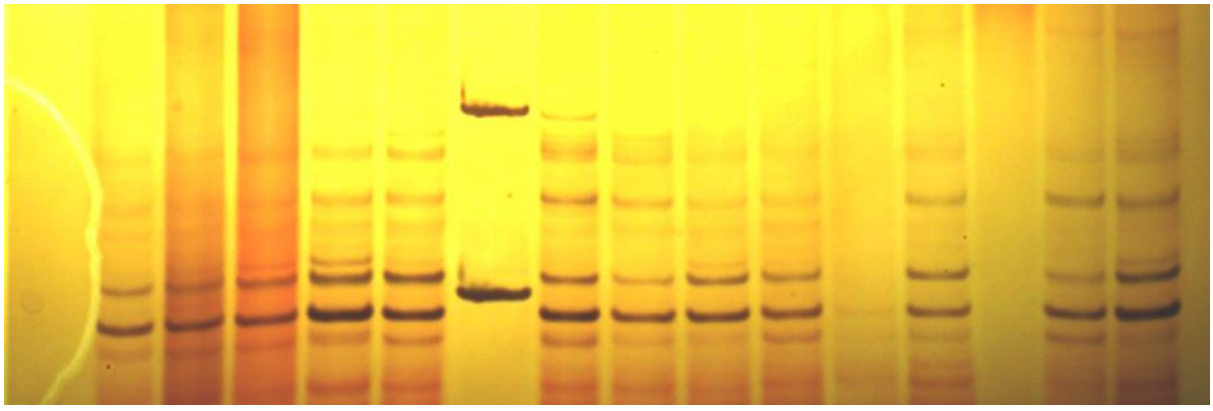

Figure 3. Gels image after silver staining

Results

Observed alleles and effective alleles

Concerning table 3, the number of observed alleles and effective ones showed the highest frequency among the Marivan samples. Since most of the Marivan samples have been collected from different habitats and in different periods, it can be concluded that the vicinity to the migration routes from Iraq Kurdistan to Marivan has been affected by this diversity. Therefore, mixing the populations in the contact zones resulted in such a higher allelic diversity among the Marivan population. The Marivan and Hersin populations showed the highest and lowest observed alleles and frequency of effective alleles respectively (13.15 and 20 for Marivan and 5.1 and 5.83 for Hersin respectively).

Observed heterozygosity (Ho) and Expected Heterozygosity (He)

Observed and expected heterozygosity is sued as a criterion for assessing genetic diversity. However, the higher value of heterozygosity is a positive point for the health status of the population, but not every population and species show high heterozygosity. There is no standard heterozygosity to define as a threshold for genetic diversity and the lower values for these statistics can not necessarily indicate unnatural events but the long-term trend of the lower heterozygosity can be a worrying issue. The observed and expected heterozygosity has been presented in table 4. As this table shows, the average observed and expected heterozygosity changes from 0.769-1 to 0.792-0.85 respectively while for the total populations and all loci it was 0.9 and 0.85 respectively. The *Ho* to *He* in Azerbaijan was lower than 1 for all locus and the mean *Ho* and *He* for Marivan population was 0.920 and 0.933 respectively. As a result, based on *Ho* to *He* ratio, the genetic diversity was lower than 1 in Azerbaijan's 1 and 2 and was higher than 1 in Sardasht, Baneh, Marivan, Kermanshah, Hersin, and Ilam. This ratio was nearly 1 in Sanandaj. Despite higher allelic frequency in common partridge in Marivan, the *Ho* of Hersin and Ilam populations was higher than it. Sampling from various habitats with different mean population densities in Marivan and surrounding villages can justify the higher allelic frequency. On the other side, since most of the Marivan population has been sampled in different periods of the year and may include migrant individuals from Marivan to Iraq Kurdistan. This may increase the allelic frequency of the Marivan population.

Table 3. The number of observed and effective alleles in the common partridg

Locus	Azarbaijan n1		Azarbaijan 2		Sardasht and Baneh		Marivan		Sanandaj		Kermansha h		Hersin		Ilam	
	Observed allels	Effective alleles	Observed allels	Effective alleles	Observed allels	Effective alleles	Observed allels	Effective alleles	Observed allels	Effective alleles	Observed allels	Effective alleles	Observed allels	Effective alleles	Observed allels	Effective alleles
TSP.1.20.16	8.99	10	5.44	9	6.40	7	9.19	18	9.14	10	3.84	5	4.57	5	6.00	7
TSP.1.20.46	6.54	8	7.00	10	6.40	7	10.76	18.	7.74	10	8.00	9	5.33	6	4.80	6
TSP.1.20.9	6.25	7	7.53	9	5.56	6	16.40	23	5.58	9	9.80	11	3.27	4	5.14	6
TSP.1.20.5	7.00	9	6.75	8	5.33	6	13.33	23	11.76	14	10.26	11	5.33	6	5.55	7
TSP.1.20.2B	9.80	11	9.13	11	5.00	0	12.00	17	10.80	12	7.53	9	5.14	7	8.33	9
TSP.1.20.43	5.33	6	9.13	11	6.40	7	17.11	21	12.10	15	8.17	10	6.40	7	7.14	8
Average	**7.30**	**8.5**	**7.50**	**9.67**	**5.84**	**6.50**	**13.15**	**20**	**9.51**	**11.67**	**7.93**	**9.16**	**5.01**	**5.83**	**6.16**	**7.16**

Table 4. Observed and expected heterozygosity in different localities

Loci	Azerbaijan 1		Azerbaijan 2		Sarbaneh		Marivan		Sanandaj		Kermansha h		Hersin		Ilam	
	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He
TSP.1.20. 16	0.714	0.888	1.000	0.816	1.000	0.844	1.000	0.891	0.875	0.891	1.000	0.740	1.000	0.781	1.000	0.833
TSP.1.20. 46	0.833	0.847	0.857	0.857	1.000	0.844	0.917	0.907	1.000	0.870	1.000	0.875	1.000	0.813	1.000	0.792
TSP.1.20. 9	0.600	0.840	0.571	0.867	0.400	0.820	0.759	0.939	0.778	0.821	0.571	0.898	1.000	0.694	0.833	0.806
TSP.1.20. 5	0.857	0.857	0.778	0.852	0.750	0.813	0.967	0.925	0.900	0.915	1.000	0.903	1.000	0.813	1.000	0.820
TSP.1.20. 2B	0.857	0.898	0.875	0.891	1.000	0.800	0.958	0.917	1.000	0.907	1.000	0.867	1.000	0.806	1.000	0.880
TSP.1.20. 43	0.750	0.813	1.000	0.891	1.000	0.844	1.000	0.942	0.818	0.917	1.000	0.878	1.000	0.844	1.000	0.860
Mean	0.769	0.857	0.847	0.862	0.858	0.827	0.933	0.920	0.895	0.887	0.929	0.860	1.000	0.792	0.972	0.832

Shanon index in western populations

Table 5 shows the data on Shannon index for each population. As can be inferred from this table, the mean Shannon index corresponds with He. The mean value of this index shows that the highest value belongs to the Marivan and the lowest for Hersin. The overall mean for populations and examined loci was estimated as 2.108. Since the Shannon index can be used as a criterion for

genetic diversity and those with higher, He, has the highest Shannon index. On the other side, this index indicates the allelic diversity and dispersal among the analyzed markers. Concerning the Shannon index, the highest index was recorded in TSP1.20.2B and the lowest one in TSP1.20.16 locus. Therefore, TSP1.20.2B can be regarded as a suitable marker as shows higher allelic diversity and polymorphism.

Table 5. Shannon index for examined samples

Locus	*Azarbaijan 1*	*Azarbaijan 2*	*Sarbaneh*	*Marivan*	*Sanandaj*	*Kermanshah*	*Hersin*	*Ilam*
TSP.1.20.16	2.243	1.965	1.965	2.542	2.253	1.471	1.560	1.864
TSP.1.20.46	1.979	2.144	1.965	2.613	2.168	2.138	1.733	1.661
TSP.1.20.9	1.887	2.107	1.965	2.948	1.956	2.342	1.265	1.705
TSP.1.20.5	2.069	1.985	1.965	2.845	2.554	2.369	1.733	1.834
TSP.1.20.2B	2.342	2.307	1.965	2.638	2.428	2.107	1.792	2.164
TSP.1.20.43	1.733	2.307	1.965	2.925	2.602	2.206	1.906	2.025
Mean	2.420	2.135	1.965	2.571	2.328	2.105	1.664	1.875

Hardy-Weinberg Equilibrium

Based on Hardy-Weinberg Equilibrium the allelic frequency and genotype stay fix from one generation to another one in case of any disturbances. Mutation, natural selection, gene flow, and nonrandom mating can make nonequilibrium. The Hardy-Weinberg Equilibrium states the most ideal conditions but the important point is that there is a disturbing factor in every population. In this study, six loci (John *et al.*, 2005, Oyler-McCance *et al.*, 2007) were used to compare the common partridge population's genetic diversity. In John *et al.*, (2005) study, 16 loci were used to assess the genetic structure of the Trumpet swans and only two loci were different from others and showed nonequilibrium which was related to the null alleles.

Table 6. Qui square and Hardy-Weinberg Equilibrium

Population	*Locus*	*Qui squer*	*Significant*	*Population*	*Locus*	*Qui squer*	*Significant*
Azarbaijan 1	TSP.1.20.16	52.5	Ns	Sanandaj	TSP.1.20.16	56	Ns

Azarbaijan 1	TSP.1.20.46	28	Ns	Sanandaj	TSP.1.20.46	48	Ns
Azarbaijan 1	TSP.1.20.9	25	Ns	Sanandaj	TSP.1.20.9	39.5	Ns
Azarbaijan 1	TSP.1.20.5	33.4	Ns	Sanandaj	TSP.1.20.5	92.78	Ns
Azarbaijan 1	TSP.1.20.2B	56	Ns	Sanandaj	TSP.1.20.2B	63	Ns
Azarbaijan 1	TSP.1.20.43	16	Ns	Sanandaj	TSP.1.20.43	123.44	Ns
Azarbaijan 2	TSP.1.20.16	35	Ns	Kermanshah	TSP.1.20.16	8.75	Ns
Azarbaijan 2	TSP.1.20.46	43.75	Ns	Kermanshah	TSP.1.20.46	33	Ns
Azarbaijan 2	TSP.1.20.9	47.44	Ns	Kermanshah	TSP.1.20.9	70	Ns
Azarbaijan 2	TSP.1.20.5	28.75	Ns	Kermanshah	TSP.1.20.5	54	Ns
Azarbaijan 2	TSP.1.20.2B	52	Ns	Kermanshah	TSP.1.20.2B	39.67	Ns
Azarbaijan 2	TSP.1.20.43	61.3	Ns	Kermanshah	TSP.1.20.43	43.17	Ns
Sarbaneh	TSP.1.20.16	20	Ns	Hersin	TSP.1.20.16	12	Ns
Sarbaneh	TSP.1.20.46	20	Ns	Hersin	TSP.1.20.46	12	Ns
Sarbaneh	TSP.1.20.9	20	Ns	Hersin	TSP.1.20.9	8.4	Ns
Sarbaneh	TSP.1.20.5	16	Ns	Hersin	TSP.1.20.5	20	Ns
Sarbaneh	TSP.1.20.2B	11.67	Ns	Hersin	TSP.1.20.2B	13.5	Ns
Sarbaneh	TSP.1.20.43	20	Ns	Hersin	TSP.1.20.43	20	Ns
Marivan	TSP.1.20.16	228.29	***	Ilam	TSP.1.20.16	20	Ns
Marivan	TSP.1.20.46	143.1	Ns	Ilam	TSP.1.20.46	12.67	Ns
Marivan	TSP.1.20.9	313.81	**	Ilam	TSP.1.20.9	17.33	Ns
Marivan	TSP.1.20.5	319.3	**	Ilam	TSP.1.20.5	18.33	Ns
Marivan	TSP.1.20.2B	152.63	Ns	Ilam	TSP.1.20.2B	35	Ns
Marivan	TSP.1.20.43	189.22	Ns	Ilam	TSP.1.20.43	25	Ns

P<0.01= **, P<0.001= ***, Not significant=Ns

The information about the Hardy-Weinberg Equilibrium has been presented in Table 6. Three loci of TSP.1.20.16, TSP.1.20.9, and TSP.1.20.05 don't correspond to the Hardy-Weinberg Equilibrium which can be related to the human interventions, seasonal migration in winter, and relocation of the species in Marivan.

F statistics and gene flow among the populations

F statistics use the inbreeding ratio to estimate the genetic differences and the state of genetic diversity distribution among the groups in three levels. The F_{IS} statistics estimate the inbreeding amount of the relatives in relation to other individuals. The second variable, F_{st} which knowns as a fixation index shows the genetic difference. F_{IT} make an overall estimation of inbreeding concerning the heterozygosity. The information on F statistics and gene flow have been presented in table 7. Concerning the mentioned table, the most genetic isolation can be seen at the TSP.1.2043 locus and the lowest one in TSP.1.20.9. The average of indices in the total population and all 6 studied loci was 0.097. Based on Wright (1978), F_{st} varied between 0-0.05 which indicates low discrimination and a lack of subpopulations while the range of 0.15-0.25 indicates moderate genetic difference and upper 0.25 shows higher genetic isolation among the populations. Zhi-min *et al.*, (2010) stated that the range of 0.15-0.25 for F_{st} indicates high differentiation, however, the polymorphism stemmed from mutation can effectively reduce F_{st} and almost is lower than 1 (Charlesworth 1998). Therefore, we can claim that the genetic discrimination of our studied populations is obvious. Since the F_{st} values from microsatellites are being calculated based on allelic frequencies then the F_{st} can be justified based on the allelic frequency of this study. McCance-Oyler *et al.*, (2007) found that F_{st} of Trumpet swans located in different areas of the Raki Mountains and Pacific Ocean doesn't show any significant differences while the amount of this index had been shown significant difference among the populations except in four of them. These authors stated that the presence of a physical barrier like the Raki Mountains can be responsible for this discrepancy. A similar study by Ransler *et al.*, (2011) indicated significant differences among the Trumpet swans populations along with the sampling locations and the origin of the populations except for those of groups raised in a captive breeding site where the inbreeding rate was very considerable. Arruga *et al.*, (2007) showed that low gene flow and high genetic isolation among the partridges can be justified by geographic distance. Based on our study, the highest rate of inbreeding (F_{IS}) and gene flow (Nm) was found in TSP1.20.9 and TSP1.20.9 loci (Table 7). Concerning the calculated average which is lower than 0.1, we can conclude that

Western Iran's partridges' populations can be classified at the higher kin relationship category. In table 8 the rate of kinship has been shown in different locations. As can be seen, the highest amount of F_{st} was recorded for Ilam and Hersin.

Table 7. F values and gene flow among the common partridge populations in Western Iran

Locus	F_{st}	*Total inbreeding* (F_{IT})	*Inbreeding* (F_{IS})	*Gene flow* (*Nm*)
TSP.1.20.16	0.111	0.0009	-0.135	*2.004*
TSP.1.20.46	0.095	-0.012	-0.138	*2.377*
TSP.1.20.9	0.121	0.275	0.175	*1.817*
TSP.1.20.5	0.084	0.37	0.051	*2.715*
TSP.1.20.2B	0.090	0.005	0.104	*2.530*
TSP.1.20.43	0.082	0.006	0.083	*2.801*
Average	0.097	0.049	0.053	*2.374*

Table 8. F_{st} values in sampling localities

Ilam	*Hersin*	*Kermanshah*	*Sanandaj*	*marivan*	*Serbaneh*	*Azerbaijan 2*	*Azerbaijan 1*	
							0.000	*Azerbaijan 1*
						0.000	0.006	*Azerbaijan 2*
					0.000	0.000	0.000	*Serbaneh*
				0.000	0.003	0.009	0.019	*Marivan*
			0.000	0.007	0.000	0.010	0.022	*Sanandaj*
		0.000	0.010	0.013	0.001	0.000	0.022	*Kermanshah*
	0.000	0.009	0.048	0.036	0.020	0.046	0.046	*Hersin*
0.000	0.032	0.036	0.036	0.033	0.030	0.046	0.046	*Ilam*

Bottleneck event in different habitats

Overexploitation, habitat destruction, and population shrinkage will lead to the genetic bottleneck and will be expressed as lower genetic diversity and higher inbreeding (John *et al.*, 2006, Su Ying *et al.*, 2007). Overall, three mutation models are used to explore the genetic bottleneck including

IAM (unlimited allele), TPM (two processes mutation), and SMM (step by step mutation). We ignored the first one as it applied to allozymes. Oyler-McCance *et al.*, (2007) used the same test while investigating the Trumpet swans in the Raki Mountains and the Pacific Ocean using 19 microsatellite markers. Based on considering it as a single population, they concluded the genetic bottleneck in the target species. Then they divided the target group into two different populations and found that the Raki population has passed the genetic bottleneck but this was not the case in the Pacific Ocean population. These researchers stated that the Trumpet Swans probably experienced the genetic bottleneck because of reducing breeding population abundance.

Tej Edor *et al.*, (2005) used microsatellite markers to investigate common partridge genetic diversity. This study also used the Wilcoxon test to explore the genetic bottleneck. This research emphasized the hunting pressure and founder effects on the genetic bottleneck. Delpasand *et al.*, (2015) also used microsatellite markers and TPM and SMM models instead of IAM in this regard. Comparison of Heterozygosity showed any concerns based on the Wilcoxon test. We also used TPM and SMM models using Wilcoxon and Sign test and resulted that the expected Heterozygous loci were suitable (Table 9). However, we didn't result in high genetic bottleneck events in the target populations but we predict that with the current illegal hunting rate and too many hunting guns we may fell into this process.

Table 9. The results from Genetic bottleneck analysis with TPM and SMM model test

	Test/Model	**TMP**		**SMM**	
Azerbaijan n1	Observation	Expectancy	Probability	Expectancy	**Probability**
	Sign Test	3.26	0.05126	3.44	**0.00272**
	Wilcoxon test	0.98438	0.06250	1.00000	**0.03125**
Azerbaijan n2	Sign Test	3.20	0.00574	3.48	**0.00244**
	Wilcoxon test	0.01563	1.00000	0.01563	**1.00000**
Serbaneh	Sign Test	3.01	0.00000	3.21	**0.00000**
	Wilcoxon test	0.01563	1.00000	0.01563	**1.00000**
Marivan	Sign Test	2.94	0.00976	3.08	**0.00686**
	Wilcoxon test	0.01563	1.00000	0.01563	**1.00000**
Sanandaj	Sign Test	3.08	0.00000	2.70	**0.00000**

	Wilcoxon test	0.01563	1.00000	0.01563	**1.00000**
Kermanshah	Sign Test	2.75	0.40156	2.78	**0.11108**
	Wilcoxon test	0.10938	0.92188	0.07813	**0.95313**
Ilam	Sign Test	4.01	0.34819	30.49	**0.4961**
	Wilcoxon test	0.03906	0.9765	0.4219	**0.6562**

UPGMA phylogeny, Neighbor-Joining with Nei molecular distance

To do phylogenetic analysis, we need to detect the genetic distances among the populations using common methods like the Nei method (Nei 1972). We calculated the phylogenetic relationship among the target populations using UPGMA and NJ trees. To this aim, we first calculated the Nei distance matrix and get the UPGMA tree. In the second step, we used the Newick tree to draw the NJ phylogenetic tree (Saitou and Nei 1987). Finally, the last figure has been produced by TreeView software (Fig. 4 and 5). We found that the NJ tree has better coincide with the PCoA graph. Ying Su et al. 2007 indicated that NJ can better indicate the genetic structure of the ducks. The same method has been applied for Trumpet swans as well (Oyler-McCance et al. 2007). We found that Ilam and Azerbaijan 1 have the highest distance based on the Nei distance matrix (Fig. 4). UPGMA tree also confirmed the NJ tree as well (Fig. 5).

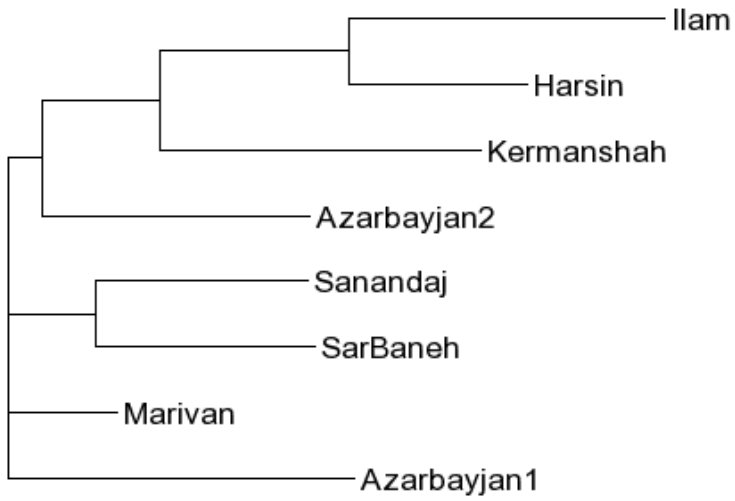

Figure 4. Allelic similarity tree among the target populations

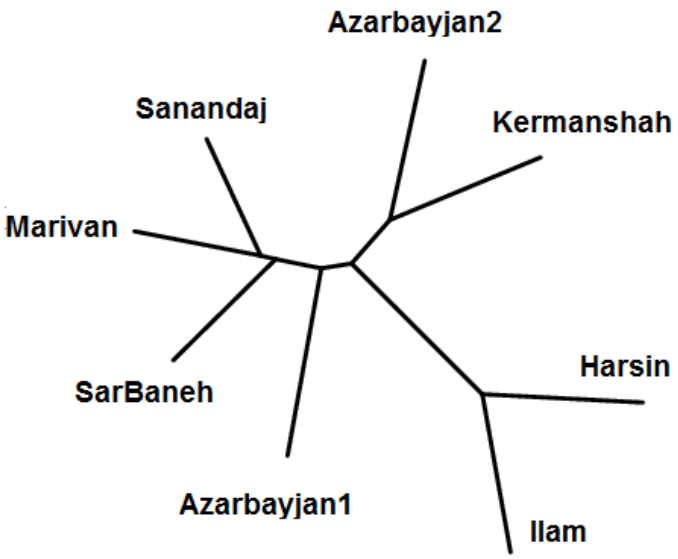

Figure 5. Allelic similarity tree among the target populations using UPGMA method

Genetic permeability in the common partridges in different habitats

STRUCTURE software is one of the common software which usually uses to distinguish species differentiation and individual clustering without considering the sampling localities (Wilson 2013, Oyler-McCance *et al.*, 2007). This software works based on Bayesian inference and detects the homogenous genetic units. Bayesian inference is a statistical approach to explore the best estimation of a random variable from input data and its combination using probability distribution (Smith and Bernardo 1994). In Bayesian inference, the uncertainty value about the value of a real parameter can be explained using probability which is the uncertainty value of the target parameter. This program cluster the individuals with maximum similarity and genetic homogeneity. Nasiri *et al.*, 2014 used the F_{st} parameter to study the wild ass genetic distance which resulted in a unique population based on STRUCTURE results. Ransler *et al.*, . 2011 could successfully use this program to find the origin of the Trumpet swans in the sampling localities. These researchers resulted that using this program, they can discriminate the swans' populations relying on allelic frequency. Oyler-McCance *et al.*, (2007) could classify the swan populations sampled from different stations. They found that two studied populations grouped in one cluster.

We used also this program and got the bar plot which in it each column indicates one individual's allele. In figure 6, from left to right, codes 1 to 8 shows Azerbaijan 1, Azerbaijan 2, Baneh, Marivan, Sanandaj, Kermanshah, Hersin, and Ilam respectively.

Figure 6. Structure software output based on sampling locations

The number of present populations in the study area has been calculated from ΔK as K shows the number of the populations. In this study, 8 chukar habitats in the west of Iran, resulted K was equal with 5 which shows the studied populations can be classified into 5 populations. Then we used this value to draw the barplot (Fig. 7).

Table 10. ΔK statistics to find the actual number of populations

K	Ln P(D)	Var[LnP(D)]	SD	L'(K)	L''(K)	[L''(K)]	Delta K
1	-3230.4	76.5	8.7464	-	-	-	-
2	-3236.3	332.8	18.243	-5.9	-672.2	672.2	36.847
3	-3914.4	1896.8	43.552	-678.1	-5144.6	5144.6	118.12
4	-9737.1	13376.1	115.66	-5822.7	12295	12295	106.31
5	-3265	875	29.58	6472.1	-6729.7	6729.7	227.51
6	-3522.6	1465.9	38.287	-257.6	371.3	371.3	9.6978
7	-3408.9	1298.3	36.032	113.7	-193.1	193.1	5.3591
8	-3488.3	1478.4	38.45	-79.4	-210.5	210.5	5.4746
9	-3778.2	2189.5	46.792	-289.9	-7267	7267	155.3
10	-11335	17193.5	131.12	-7556.9	14356	14356	109.49
11	-4535.7	3708.1	60.894	6799.4	-6510.3	6510.3	106.91
12	-4246.6	3144.8	56.079	289.1	-99.6	99.6	1.7761
13	-4057.1	2762.7	52.561	189.5	-299.8	299.8	5.7038
14	-4167.4	3051.6	55.241	-110.3	687.5	687.5	12.445
15	-3590.2	1820.6	42.668	577.2	-577.2	577.2	13.528

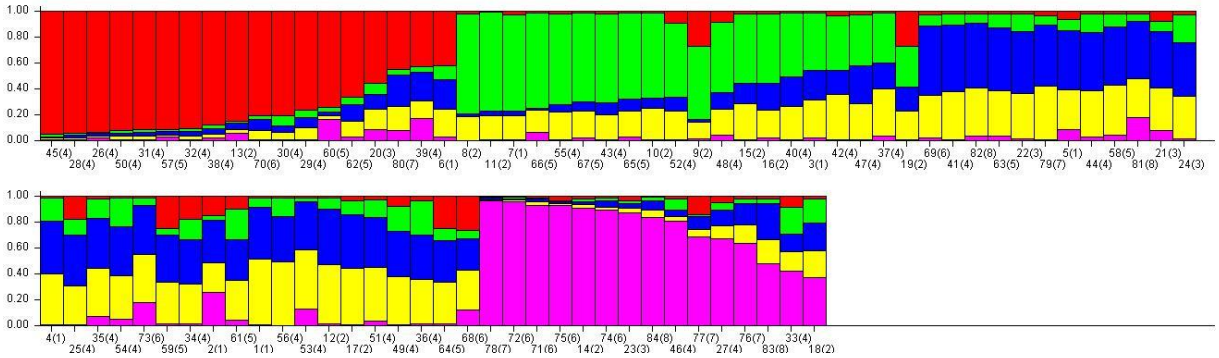

Figure 7. Suggested populations based on allelic structure

Conclusion

Protection of genetic diversity is a necessity in ecosystems and nature viability. Therefore, we need to identify and study the species from different aspects like morphology, genetics, habitat, and population dynamics and apply these studies in the conservation plans and programs. Common partridges have an influential position in Iran tradition and culture and there are many stories about this species in the cultural literature as well. Nowadays, releasing the partridges from captivity to

nature is a symbol of environmentalism, but it can entail some negative effects on the genetic diversity of the species. For instance, releasing the individuals that have been captured in Kermanshah and taken to Baneh and Marivan can affect the genetic purity of the releasing points. Therefore, we strongly recommend that all captured individuals should be released at the same locality where they live captured.

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