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A Study of Genetic Diversity of Common Buzzard (*Buteo buteo*) from Iran Using Microsatellite Markers

Research Article

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ABSTRACT

The common buzzard (*Buteo buteo*) is a bird of prey with Eurasia distribution. *Buteo buteo* from Iran, has an important role in the ecological cycle of the north and the northwest parts of Iran, now is suffering from illegal hunting. Blood samples from 50 *Buteo buteo* birds from rehabilitation center of Pardisan Park, Tehran, Iran were taken to evaluate and study the genetic diversity of these birds using 10 microsatellite markers. The population was a random population involved birds from different habitats of *Buteo buteo* from Iran. The results of this study showed that the population had a high genetic diversity and the highest and lowest heterozygosity was observed at Bbu30 and Bbu33 markers (0.860 and 0.514), respectively. Expected heterozigosity (H_e) was ranged from 0.638 in Bbu22 to 0.832 in Bbu30. The lowest number of alleles (N_a) and effective number of alleles (N_e) were observed at Bbu22 and Bbu30, respectively. The Bbu17 had the highest and Bbu22 had the lowest F-statistics value (0.313 and 0.104). This population did not show significant deviation from Hardy-Weinberg equilibrium. Results indicated that the *Buteo buteo* from Iran population from rehabilitation center of Pardisan Park harbors, showed adequate amount of genetic variation and the microsatellite information effectively evaluate the genetic diversity of this species needed for conservation genetic management.

KEY WORDS *Buteo buteo*, common buzzard from Iran, genetic diversity, microsatellite.

INTRODUCTION

The family Accipitridae (such as hawks and eagles) represents a large radiation of predatory bird with an almost global distribution (Raposo do Amaral *et al*. 2006). The common buzzard (*Buteo buteo*) is one of the most numerous and widespread raptor species, especially in Eurasia (Anonymous, 2017). Among them, *Buteo buteo* from Iran, is one of the most broadly distributed birds throughout of the north and northwest Iran, but has been experiencing a significant decline in population size during the past century (Mansoori, 2013). Furthermore, it is very popular as captive birds in Arabian countries around the Persian Gulf, which makes it a suitable object for illegal hunting. Molecular genetic investigations of wide-ranging species with several recognized subspecies provide an opportunity to understand the ecological, behavioral and evolutionary patterns responsible for differentiation and evaluate the support for sub-specific status (Hull *et al*. 2008). The conservation genetic is an interface science with the aim of using molecular genetic techniques to determine and maintain biodiversity (Diz and Presa, 2009). Microsatellite analysis makes it possible to determine the genotypes of individuals by examining the variation of the number of repetitions of sequences that are several nucleotides long (Mahmut *et al*. 2001). The *Buteo buteo* from Iran is under conservation but

there are no evidence of microsatellite markers used to evaluate population genetic parameters in this species. Using microsatellites to determine the genetic diversity of *Buteo buteo* from Iran as one of the important species of bio-cycle of wilderness, will provide important information that can be applied in conservation genetic strategies. In order to investigate genetic diversity of this population, 10 microsatellite markers were amplified and the population genetic parameters were estimated.

MATERIALS AND METHODS

Characteristics and habitats of *Buteo buteo* from Iran

Common buzzard from Iran (genus *Buteo*, species *Buteo buteo*) has a variety of coat color (light brown to dark brown) with an average height of 50 cm (Figure 1).

Figure 1 Common Buzzard (*Buteo buteo*)

It is a medium body size rapt or similar to eagle, which is localized in south areas of Caspian Sea and also distributed in North-East of Iran.

The nests are usually found on the shore rocks, trees and scaly lands (Mansoori, 2013). The International Union for Conservation of Nature (IUCN) red list has been mentioned this bird as least concern but nowadays, illegal hunting and disturbance of the natural habitats are issues about this species.

Sampling and DNA extraction

Blood samples were collected from 50 *Buteo buteo*birdkept in the rehabilitation center of Pardisan Park, Tehran, managed by the Department of Environment of Iran. DNA was extracted from samples using Bioneer Extraction Mini Kit.

DNA amplification

In the present study, 10 sets of primers were used to evaluate microsatellite markers Bbu10, Bbu14, Bbu17, Bbu22, Bbu30, Bbu33, Bbu34, Bbu49, Bbu51 and Bbu59 (Johanson *et al*. 2005). The accession number, length, sequence of primers, annealing temperature and number of plexes from the microsatellite markers are displayed in Table 1. The PCR reactions were conducted in 25 µL (DNA 50 ng/µL) using AmpliqonTaq Polymerase 2X master mix Red. The PCR program was 95 ˚C for 15 minutes, followed by 35 cycles of 95 ˚C for 30 seconds, annealing temperature for 40 seconds and 30 seconds of 72 ˚C, and one cycle of 72 ˚C for 5 minutes. Genotyping was done on an automatic capillary sequence (genetic analyzer ABI, 3130) and analyzed with the Gene Mapper v3.2 software. All individuals were separately screened at least twice and if there was problem with the data, the process was done again. Only genotypes with unambiguous results were included in the final data set.

Data analysis

The dataset was checked with Micro-checker 2.23. (Van Oosterhount *et al*. 2004). Estimating observed (H_o) and expected (H_e) heterozygosities, potential deviations from Hardy-Weinberg equilibrium (HWE) and AMOVA based on conventional F-statistics (Fst) were calculated with gene ALE × 6.2 software (Peakall and Smouse, 2012). The number of alleles (N_a) and effective number of alleles (N_e) were estimated by gene ALE × 6.2 software (Peakall and Smouse, 2012).

RESULTS AND DISCUSSION

The allele length, N_a, N_e, H_o, H_e and Fst estimated for the microsatellite markers of *Buteo buteo* from Iran are displayed in Table 2.

Genetic polymorphism

The total data set of 50 successfully genotyped individuals of *Buteo buteo* from Iran yielded an average N_a of 11.8 and average N_e of 8.7. The Bbu30 locus had the highest N_a (18) and Bbu59 had the lowest N_a (6); Johanson *et al*. (2005) reported 17 and 5 alleles for the same loci, respectively on 90 individuals of the same species from Germany. The frequency of the alleles per locus are summarized in Table 3 and nearly all alleles have frequency lower than 0.95, demonstrating widespread genetic polymorphism in these loci. The Fst is shown in Table 2; the average Fst was 0.21 ranging from 0.104 in Bbu22 to 0.313 in Bbu17.

Genetic diversity

Genetic diversity in a population could be estimated by H_o and H_e.

Table 1 Locus name, accession number, length, primers, annealing temperature and plex number of *Buteo buteo* microsatellite markers (Johanson *et al.* 2005)

Locus	Accession number	Length	Primers	T_a	Label	Plex
Bbu10	AJ715882	206	F: 5'-TGAAGATTTTTACCTCCTCAG-3' R: 5'-GATCTCCTTGGTACCAGAAA-3'	56	6-FAM	1
Bbu17	AJ715888	174	F: 5'-ACCAGGTTGCAGCTGAGTG-3' R: 5'-CAAATGTTCTCAACAGCTTAAGTCC-3'	56	HEX	1
Bbu51	AJ715921	164	F: 5'-TTTCCCTAACATTTACTGACTCCTG-3' R: 5'-GAGGTCACTGGCTCGAGATG-3'	56	6-FAM	1
Bbu33	AJ715903	167	F: 5'-GACGCTTGAGCAAAGAGAGG-3' R: 5'-TGCAAGAACACTTCCAAAGC-3'	56	HEX	1
Bbu14	AJ715885	147	F: 5'-GACCAGAAGCCTTGACTTGC-3' R: 5'-TTTGCTTCCTGAATAGGATGG-3'	54	HEX	2
Bbu30	AJ715900	151	F: 5'-TGCCGCCATCTTACTGAAG-3' R: 5'-ATCACAAGATAGCCAGCTATGG-3'	54	6-FAM	2
Bbu49	AJ715919	184	F: 5'-AGACCAGCAAACCCAAACAG-3' R: 5'-TTGATATATCTTGCTCCATGCTG-3'	54	HEX	2
Bbu22	AJ715892	124	F: 5'-GAGAACATTTTCACTTACG-3' R: 5'-GATCCTTCACCTGTACATA-3'	58	6-FAM	3
Bbu34	AJ715904	159	F: 5'-GACCTGGTGCTCTGCATTC-3' R: 5'-TGAAACAGATTTGATTCTGGATG-3'	58	HEX	3
Bbu59	AJ715928	137	F: 5'-CCTGCCACAGGGTATTACTATGAC-3' R: 5'-AGGCTCGCTAAAGGAACAAG-3'	58	HEX	3

Table 2 Amplified length, N_a, N_e, H_o, H_e and Fst estimated for the microsatellite markers of *Buteo buteo* from Iran

Locus	Number	Length (bp)	N_a	N_e	H_o	H_e	Fst
Bbu10	48	205-213	10	7.126	0.668	0.744	0.256
Bbu17	50	153-181	11	9.763	0.764	0.794	0.313
Bbu51	50	162-180	7	7.236	0.651	0.680	0.106
Bbu33	50	167-181	12	10.332	0.514	0.800	0.189
Bbu14	50	130-142	9	6.210	0.631	0.716	0.148
Bbu30	50	158-166	18	14.232	0.860	0.832	0.141
Bbu49	50	182-196	14	9.210	0.664	0.756	0.290
Bbu22	49	132-144	5	4.705	0.700	0.638	0.104
Bbu34	49	160-174	10	8.578	0.714	0.738	0.241
Bbu59	50	146-158	6	8.125	0.651	0.820	0.119
Average	-	-	11.82	8.74	0.691	0.754	0.210
SE	-	-	2.11	2.82	0.108	0.060	0.020

N_a: number of alleles; N_e: effective number of alleles; H_o: observed heterozygosity and H_e: expected heterozygosity.

The average H_o and H_e in *Buteo buteo* from Iran were 0.691 and 0.754, respectively. The highest H_o was 0.860 and the lowest was 0.514 in Bbu30 and Bbu33 locus, respectively. In addition, Bbu30 and Bbu22 had the highest and the lowest H_e, 0.830 and 0.638, respectively. Johanson *et al.* (2005) reported H_o ranged from 0.133 to 0.99 in a population of *Buteo buteo*. None of the loci showed significant deviation from Hardy-Weinberg equilibrium.

Genetic diversity is a base material for the evolution of a population (Norris *et al.* 1999), so maintenance of the genetic diversity plays the main role in most conservation programs.

Microsatellite markers are genetic markers that are broadly used in conservation management strategies (Liu *et al.* 2009). These markers are particularly valuable due to their co-dominance nature and they are therefore more useful than genetic mutations (Liu and Cordes, 2004).

In a population, allele enrichment is more valuable than heterozigosity (Diz and Presa, 2009), and microsatellite markers could determine the allele number and frequency in a population. In the present study, all 10 primer sets were amplified successfully and nearly all of the loci had multiple alleles. Locus number Bbu30 had 18 alleles and Bbu22 had 5 alleles; the most and the least numbers of alleles respectively. Johanson *et al.* (2005) evaluated 60 microsatellite markers in 90 samples of *Buteo buteo* from different parts of Eastern West-Phalia and not all of the primers had reliable results, but most of the loci were genetically polymorphic. Twenty six novel microsatellite markers were characterized by Hull *et al.* (2007) in Swainson's hawks. The number of alleles per locus ranged from six to 49 with an average of 18.9. Honnen *et al.* (2010) evaluated nuclear microsatellite markers in white-tailed sea eagle from central Europe.

Table 3 Allele frequency per locus of microsatellite markers from *Buteo buteo* from Iran

Allele	Bbu10	Bbu14	Bbu17	Bbu22	Bbu30	Bbu33	Bbu34	Bbu49	Bbu51	Bbu59
A	1.0000	0.4800	0.0833	0.2320	0.0196	0.0196	0.0196	0.0097	0.3918	0.0417
B	0.3697	0.1431	0.7899	0.0101	0.0097	0.0196	0.0505	0.0097	0.0722	0.1530
C	0.0097	0.0097	0.0297	0.3067	1.0000	0.2342	0.0947	0.0196	0.1875	0.3846
D	0.0196	0.1431	0.1954	0.4493	0.0097	0.1429	0.0196	0.1431	0.0297	0.7899
E	0.3282	0.2088	0.0341	01949	0.0196	0.3846	0.1530	0.0097	0.0097	0.1096
F	0.0196	0.0679	0.0341	***	0.0097	0.6568	0.5574	0.1431	0.4800	0.1431
G	0.1530	0.0749	0.1183	***	0.0097	0.6463	0.0101	0.2088	0.1431	***
H	0.5574	0.0297	0.3918	***	0.0196	0.5574	0.3067	0.0505	***	***
I	0.1506	0.0196	0.0722	***	0.0505	0.4348	0.6463	0.0297	***	***
J	0.0097	***	0.1875	***	0.0947	0.5405	0.5574	0.0722	***	***
K	***	***	0.0297	***	0.1304	0.3282	***	0.1685	***	***
L	***	***	***	***	0.0196	0.1149	***	0.1064	***	***
M	***	***	***	***	0.0097	***	***	0.0400	***	***
N	***	***	***	***	0.1875	***	***	0.0196	***	***
O	***	***	***	***	0.0722	***	***	***	***	***
P	***	***	***	***	0.0505	***	***	***	***	***
Q	***	***	***	***	0.0297	***	***	***	***	***
R	***	***	***	***	0.0722	***	***	***	***	***

***: no allele.

The results showed that the population could be subdivided into two main genetic clusters. The total data set yielded 65 different alleles with an average number of 9.3 per locus.

The amount of heterozygosity was estimated in the two forms of observed and expected heterozigosity (H_o and H_e, respectively). Nine loci out of the 10 microsatellite markers had H_o higher than 0.5 with an overall H_o of 0.69. The overall H_e was 0.75. Honnen *et al*. (2010) reported 0.59 and 0.70 for the overall H_o and H_e, respectively. The amount indicated that *Buteo buteo* population of Iran, have high variability. The H_e reported for *Buteo swainsensis* ranged from 0.44 to 0.96 while the H_o ranged from 0.40 to 0.95 (Hull *et al*. 2008). In the present study the H_o ranged from 0.51 to 0.86 and H_e ranged from 0.63 to 0.83. The overall Fst estimated 0.21, ranged from 0.104 (Bbu22) to 0.290 (Bbu49), the Fst showed that there were genetic differences between individuals from *Buteo buteo* from Iran. Hull *et al*. (2008b) evaluated 21 microsatellite markers from different populations of *Buteo lineatus* and reported that Fst was 0.18, which means the population had genetic differences. In another study, 19 microsatellite loci were analyzed to determine genetic diversity and structure of *Buteo swainson* and Fst estimate suggested limited differentiation among Swainson's Hawks with isolation by distance (Hull *et al*. 2008c). Otherwise 17 loci revealed obvious differences between subpopulations of *Buteo jamaicensis*, which would be the consequence of geographic distances (Hull *et al*. 2008d). *Buteo galapagoensis* is one the most inbred hawk in the world living in a small isolated island. The analysis of microsatellite showed low Fst, which indicated a high resemblance between individuals of the studied populations (Bollmer *et al*. 2006).

CONCLUSION

The results of the present study showed that polymorphic microsatellite markers are able to evaluate genetic diversity in *Buteo buteo* from Iran. They had high genetic polymorphism with heterozygosity, besides overall and individual Fst indicated differentiation of markers in the population. The microsatellite markers had not been suffered from natural selection or small population size, and all loci were in Hardy Weinberg Equilibrium. The common buzzard population of the rehabilitation center of Pardisan Park included individuals from different parts of the North and North-East Iran, which is likely the main reason for the high genetic diversity between individuals in the population. Because of the genetic structure and diversity of this population, it seems that there is good opportunity to establish a base population, by recording and labeling birds to provide information to determine conservation strategies. The data obtained from the present study could be merged with data from other populations of Common buzzard from Iran, also by morphological and other genetic markers such as mitochondria genome, it could make a useful dataset for conservation genetic management.

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REFERENCES

Anonymous. (2017). Handbook of the Birds of the World and Bird Life. International digital checklist of the birds of the world. Available at: http://datazone.birdlife.org/species/taxonomy.

Bollmer J.L., Kimball R.T., Whiteman N.K., Sarasola J.H. and Parker P.G. (2006). Phylogeography of the Galápagos hawk (*Buteo galapagoensis*): A recent arrival to the Galápagos Islands. *Mol. Phylogen. Evol*. **39,** 237-247.

Diz P.A. and Presa P. (2009). The genetic diversity pattern of Mytilusallo provincialis in Galician Rías (NW Iberian estuaries). *Aquaculture*. **287,** 278-285.

Honnen A.C., Hailer F., Kenntner N., Literak I., Dubska I. and Zachos F.E. (2010). Mitochondrial DNA and nuclear microsatellites reveal high diversity and genetic structure in an avian top predator, the white-tailed sea eagle, in central Europe. *Biol. J. Linn. Soc*. **99,** 727-737.

Hull J.M., Strobel B.N., Boal C.W., Hull A.C., Dykstra C.R., Irish A.M., Fish A.M. and Ernest H.B. (2008). Comparative phylogeography and population genetics within *Buteo lineatus* reveals evidence of distinct evolutionary lineages. *Mol. Phylogen. Evol*. **49,** 988-996.

Hull J.M., Anderson R., Bradbury M., Estep J.A. and Ernest H.B. (2008b). Population structure and genetic diversity in Swainson'showks (*Buteo swainsoni*): Implications for conservation. *Conserv. Genet*. **9,** 305-316.

Hull J.M., Savage W.K., Bollmer J.L., Kimball R.T., Papker P.G., Whiteman N.K. and Ernest H.B. (2008c). On the origin of the Galápagos hawk: an examination of phenotypic differentiation and mitochondrial paraphyly. *Biol. J. Linn. Soc*. **95,** 779-789.

Hull J.M., Hull A.C., Sacks B.N., Smith J.P. and Ernest H.B. (2008d). Landscape characteristics influence morphological and genetic differentiation in a widespread raptor (*Buteojamaicensis*). *Mol. Ecol*. **17,** 810-824.

Hull J.M., Tufts D., Topinka J.R., May B. and Ernest H.B. (2007). Development of 19 microsatellite loci for Swainson'showks (*Buteo swainsoni*) and other buteos. *Mol. Ecol. Notes*. **7,** 346-349.

Johanson P.C.D., Fowlie M.K. and Amos W. (2005). Isolation of microsatellite loci from the common buzzard, Aves: Accipitridae (*Buteo buteo*). *Mol. Ecol. Notes*. **5,** 208-211.

Liu F., Xia J.H., Bai Z.H., Fu J.J., Li J.L. and Yue G.H. (2009). High genetic diversity and substantial population differentiation in grass carp (*Ctenopharyn godonidella*) revealed by microsatellite analysis. *Aquaculture*. **297,** 51-56.

Liu Z. and Cordes J.F. (2004). DNA marker technologies and their applications in aquaculture genetics. *Aquaculture*. **238,** 1-37.

Mahmut H., Gnnzoring S., Onima M., Masuda R., Suzuki M. and Ohtaishi N. (2001). A preliminary study of the genetic diversity of Xinjiang Tarim red deer (*Cervus elaphus yarkandensis*) using the microsatellite DNA method. *Japanese J. Vet. Res*. **49(3),** 231-237.

Mansoori J. (2013). Handbook of Iranian Birds. Farzane Ppress, Tehran, Iran.

Norris A.T., Bradley D.G. and Cunningham E.P. (1999). Microsatellite genetic variation between and within farmed and wild Atlantic salmon (*Salmo salar*) populations. *Aquaculture*. **180,** 247-264.

Peakall R. and Smouse P.E. (2006). GENEALEX6: Genetic analysis in excel. Population genetic software for teaching and research. *Mol. Ecol. Notes*. **6(1),** 288-295.

Raposo do Amaral F.S., Miller M.J., Silveria L.F., Bermingham E. and Wajntal A. (2006). Polyphyly of the hawk genera *Leucopternis* and *Buteogallus* (Aves: Accipitridae): Multiple habitat shifts during the Neotropical buteonine diversification. *BMC Evol. Biol*. **6,** 10-17.

Van Oosterhount C., Hutchinson W.F., Wills D.P.M. and Shipley P. (2004). Micro-checker: Software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes*. **4,** 535-538.