Biochem Genet (2010) 48:954–961
DOI 10.1007/s10528-010-9377-5

Analysis of Genetic Diversity of Chukar Partridge (*Alectoris chukar*) Populations in Khorasan-e-Razavi Province of Iran by RAPD-PCR

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Received: 8 November 2009 / Accepted: 29 July 2010 / Published online: 5 September 2010
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Abstract RAPD markers were used to investigate population genetic parameters of an endangered partridge, *Alectoris chukar*, in four areas of Iran, as a part of a genetic conservation program. The aim of this study was to analyze the genetic similarity among these populations. Blood samples from 75 birds were used for DNA extraction and RAPD-PCR analysis of 67 loci, with 28 polymorphic bands (41.79%). The populations of Kalat-e-Nader and Mashhad were found to be closely related, as were the Torbat-e-Jaam and the Quchan populations. Mean heterozygosity for all populations was 0.4405 ± 0.0755. The results indicate that chukar partridge genetic diversity in Khorasan-e-Razavi province is sufficient and the amount of gene flow among populations is acceptable.

Keywords Chukar partridge · Genetic diversity · RAPD-PCR (random amplified polymorphic DNA-polymerase chain reaction) · Khorasan-e-Razavi province · Gene flow

Introduction

The partridge commonly referred to as chukar (*Alectoris chukar*, Galliformes) is one of the most common and popular game birds in Khorasan-e-Razavi province, of northeastern Iran. Wild populations of this partridge have decreased dramatically because of destruction of their habitat, excessive hunting, migration, and other factors, causing them to be placed on lists of conservation concern in recent years. This most widespread species of partridge is distributed from the Balkans to the eastern Mediterranean islands and across Asia Minor to the Himalayas, Mongolia, and China. Moreover, this species has been successfully introduced into the western

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USA, Canada, and New Zealand (Madge and McGowan 2002). Little is known about the genetic structure of the chukar. The lack of information is potentially harmful for its conservation (Frankham 2005).

Conservation attempts have been limited; management practices are based almost solely on controlled burning, leaving areas of varying post-fire age to maintain optimal habitat availability (McFarland 1991). The analysis of genetic variability is an essential ingredient for conservation programs, and the approach must be based on a combination of phenotypic and genetic data (Hetzel and Drinkwater 1992). The use of molecular markers can aid in the choice of breeds and populations to be conserved, when there is a shortage of resources, as well as the estimation of genetic variability of species breeds and populations (Egito et al. 2005). Random amplified polymorphic DNA (RAPD) is a useful approach to assessing genetic variation for conservation of wild populations; it is based on PCR amplification of genomic DNA with arbitrary nucleotide sequence primers. The RAPD marker can detect high levels of DNA polymorphism and can produce fine genetic markers (Williams et al. 1990; Welsh and McClelland 1990). This method is simple and quick to perform when there is no prior knowledge about the genetic make-up of the organism (Hadrys et al. 1992). Nevertheless, RAPD analysis has some limitations. It shows dominant inheritance, and marker/marker homozygotes cannot be distinguished from marker/null heterozygotes (Williams et al. 1990). This peculiarity of RAPDs impedes direct estimations of allele frequency and can bias calculations of population differentiation (Lynch and Milligan 1994). This problem can be overcome by appropriate statistical methods, such as analysis of molecular variance, AMOVA (Excoffier et al. 1992), which is not influenced by the dominance problem (Huff et al. 1993; Diaz et al. 2001). In addition, it is unable to assign bands to specific loci unless a previous pedigree analysis is performed. In applying this method, it is assumed that populations are in Hardy–Weinberg equilibrium, that polymorphic bands segregate in the Mendelian way, and that marker alleles from different loci do not comigrate to the same position in the gel (D'Amato and Corach 1996). The problem of low reproducibility in early RAPD analyses can now be overcome through improved laboratory techniques and band scoring procedures (Nybom 2004; Volis et al. 2001). RAPD markers, despite their limitations, provide a useful approach for evaluating a population's genetic differentiation, particularly in species that are poorly known genetically (Nybom 2004).

The aim of this study was to investigate the genetic diversity of the chukar partridge in four areas of Khorasan-e-Razavi province of Iran, as a part of a genetic conservation program.

Materials and Methods

Study Area

Khorasan-e-Razavi province, in northeastern Iran, has an area of 118,854 km^2, including many areas such as Kalat-e-Nader, Mashhad, Shirvan, Torbat-e-Jaam, and Quchan (Fig. 1a). The climate is typically characterized by cold rainy winters and

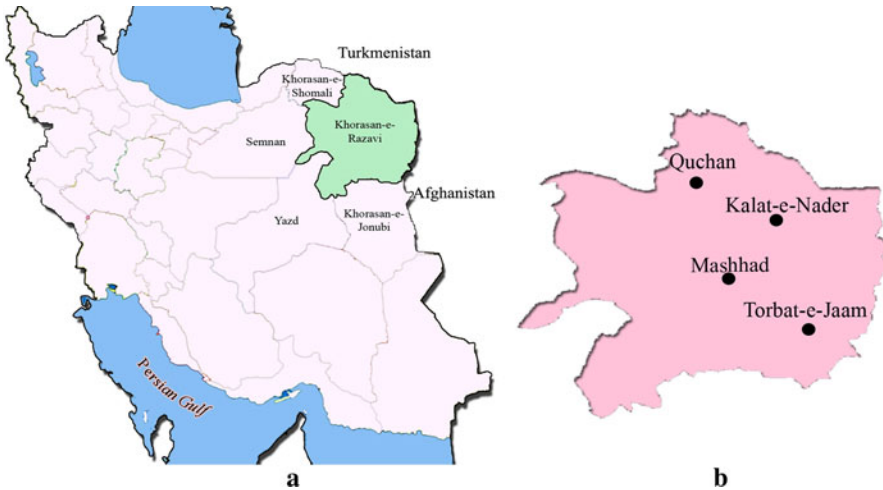

Fig. 1 The study area: **a** Location of Khorasan-e-Razavi province within Iran. **b** Sampling locations in Khorasan-e-Razavi province

relatively hot summers, depending on Siberian and Mediterranean high-pressure circulations. The average annual rainfall is around 200 mm. According to rainfall records, a year can be divided into a dry season (summer and autumn) and a wet season (winter and spring) with about 75% of the total rainfall.

Sample Collection and DNA Extraction

Blood samples were collected from 75 chukar in four areas: Kalat-e-Nader (19 samples), Mashhad (21), Torbat-e-Jaam (18), and Quchan (17) (Fig. 1b). Individual blood samples (1 ml) were taken from the brachial vein under the wing using disposable syringes and transferred immediately into tubes containing 15% EDTA as an anticoagulant agent. Total genomic DNA was extracted from blood using the guanidinium isothiocyanate-silica gel method described by Boom et al. (1990), with minor modification. The total DNA concentration and its purity were measured with a Nano-Drope ND-2000 spectrophotometer (Thermo, Wilmington, USA), the DNA was diluted to a final concentration of 50 ng/µl in dH_2O and stored at 4°C.

PCR and Data Analysis

Amplifications for RAPD markers were performed according to Negro et al. (2001) and Barbanera et al. (2005). The reaction mixture contained 20 ng template DNA, 25 pM (PH primers) or 18.75 pM (OP primers) of a single decanucleotide (Table 1), 0.2 mM each of dNTPs (Pharmacia, Uppsala, Sweden), 3 mM $MgCl_2$, and 1 U *Taq* polymerase (Invitrogen, USA) in the reaction buffer provided by the manufacturer; sterile distilled water was added for a final volume of 20 µl. Amplification was performed in an ABI 9700 thermal cycler (Applied Biosystems, Calif., USA) with the following cycling conditions: initial denaturation step at 94°C

Table 1 Primer sequences and band pattern analysis of eight markers in chukar partridge

Primer	Sequence (5′–3′)	G + C%	Total scored bands	Polymorphic bands	% Polymorphic loci	Band size range (bp)
OP-C-08	TGGACCGGTG	70	8	4	50.00	300–1500
OP-C-09	CTCACCGTCC	70	11	5	45.45	200–1450
OP-C-20	ACTTCGCCAC	60	7	3	42.85	350–1250
OP-H-12	ACGCGCATGT	60	7	3	42.85	300–1350
PH-05	AACGCGCAAC	60	11	4	36.36	250–1500
PH-04	AAGAGCCCGT	60	7	3	42.85	350–1200
PH-03	GTAGACCCGT	60	5	2	40.00	300–1200
OP-C-15	GACGGATCAG	60	11	4	36.36	250–1400

for 3 min; 45 cycles of 94°C for 30 s, 36°C for 1 min, and 72°C for 1 min; and a final extension step at 72°C for 5 min. The PCR amplifications were duplicated for each locus at different times to decrease the risk of obtaining different RAPD bands for the same individuals, which has been reported by some researchers as a defect of the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique (Ellsworth et al. 1993). PCR products were run on a 2% agarose gel containing ethidium bromide at 70 V for 2 h, along with a Gene-Ruler DNA ladder mix as the molecular weight marker (Fermentas), and visualized using a UVdoc gel documentation system. Band patterns were detected visually on agarose gel and analyzed using Popgen32 software, version 1.31 (Yeh and Boyle 1997).

Results and Discussion

All random primers showed clear and polymorphic bands in all samples (Fig. 2). To score the band pattern, we assumed that one band corresponded to one locus. After the duplication tests, it was concluded that the RAPD bands acquired in this study are reproducible. The number of polymorphic bands varied from 2 to 5, with a range of 200–1500 bp (Table 1). Primers of OP-C-09 and PH-03 showed the highest and lowest number of polymorphic bands, respectively. Primer OP-C-08 showed the highest percentage of polymorphic loci (50%), and primers PH-05 and OP-C-15 had the lowest (36.36%).

Statistical analysis of genetic variation for all loci revealed maximum gene diversity for OP08-1 and minimum gene diversity for PH05-2, with a mean of 0.4405 ± 0.0755 (Table 2). The 67 loci amplified produced 28 polymorphic bands (42%) among all investigated populations. All primers used in this study were highly informative, producing an average of eight bands per primer. The percentage of polymorphic loci in these populations ranged from 36 to 50%. Our results indicate that the majority of genetic diversity (50%) is within populations.

To study the genetic relationships between populations, we constructed a dendrogram (Fig. 3) using Nei's (1972, 1978) genetic identity and the genetic distance data (Table 3). The Kalat-e-Nader and Mashhad populations appear to be

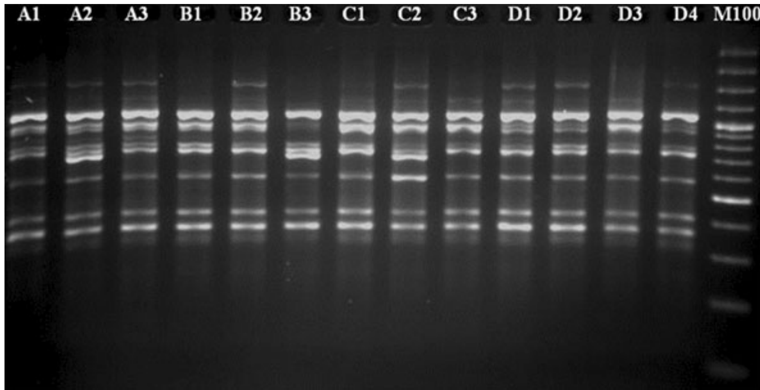

Fig. 2 RAPD banding pattern of marker OP-H-12. *Lanes A1–3* samples from Kalat-e-Nader, *lanes B1–3* samples from Mashhad, *lanes C1–3* samples from Torbat-e-Jaam, *lanes D1–4* samples from Quchan, *lanes M100* 100 bp ladder

the most closely related, probably because of migration and introgression between these populations. Furthermore, Kalat-e-Nader has the same climate as Mashhad, and Quchan and Torbat-e-Jaam have similar climates. Climate change affects animal genetic diversity (Hoffmann 2010). Although many existing technologies in animal genetic resource characterization, conservation, and breeding will be crucial for climate change adaptation and mitigation, research gaps do exist, especially with regard to the physiology and genetics of adaptation (Hoffmann 2010). Our results indicate that the mean genetic diversity among the populations studied is sufficient (0.4405 ± 0.0755), and there is probably enough gene flow between populations. So, the results show that genetic diversity in these populations is acceptable (Xiao et al. 2004).

Selected RAPD markers can be highly effective in disclosing hybridization and genetic diversity of a given specimen (Negro et al. 2001; Barbanera et al. 2005). A considerable number of primers, however, are required to demonstrate genetic purity, or the absence of any introgressive event (Boecklen and Howard 1997). In this study, the use of eight primers together offered complementary information for their detection, and the set of eight markers met the condition that they were present in all *Alectoris chukar* tested.

The results demonstrate that optimized RAPD methodology can be used to obtain genetic band patterns that are reproducible in partridges and is an effective way to detect hybrids. This information can be complemented with other markers, such as SSCPs and SNPs, to increase the reliability of detecting hybrids (Garcia and Arruga 2006).

The majority of RAPD bands are common for most populations. Some RAPD loci were not found in particular populations. No population-specific loci were obtained. The results demonstrate the usefulness of the RAPD approach for detecting DNA polymorphism and establishing relationships among populations of partridge.

Table 2 Genetic variation for all loci[a]

Locus	Sample size	Number of alleles		Nei's gene diversity[c]	Shannon's information index[d]
		Observed	Effective[b]		
OP08-1	75	2.0000	1.9999	0.5000	0.6931
OP08-2	75	2.0000	1.9941	0.4985	0.6917
OP08-3	75	2.0000	1.9273	0.4811	0.6742
OP08-4	75	2.0000	1.9975	0.4994	0.6925
OP09-1	75	2.0000	1.5720	0.3639	0.5501
OP09-2	75	2.0000	1.8427	0.4573	0.6498
OP09-3	75	2.0000	1.9724	0.4930	0.6231
OP09-4	75	2.0000	1.9723	0.4930	0.6861
OP09-5	75	2.0000	1.8632	0.4930	0.6426
OP20-1	75	2.0000	1.9968	0.4992	0.6923
OP20-2	75	2.0000	1.8813	0.4685	0.6613
OP20-3	75	2.0000	1.7161	0.4173	0.6080
OP12-1	75	2.0000	1.7632	0.4329	0.6244
OP12-2	75	2.0000	1.9941	0.4985	0.6917
OP12-3	75	2.0000	1.9991	0.4998	0.6929
PH05-1	75	2.0000	1.7130	0.4162	0.6069
PH05-2	75	2.0000	1.3684	0.2692	0.4402
PH05-3	75	2.0000	1.5816	0.3677	0.5543
PH05-4	75	2.0000	1.8716	0.4687	0.6641
PH04-1	75	2.0000	1.8360	0.4553	0.6478
PH04-2	75	2.0000	1.7412	0.4911	0.5261
PH04-3	75	2.0000	1.8710	0.4635	0.6519
PH03-1	75	2.0000	1.8322	0.4542	0.6466
PH03-2	75	2.0000	1.7514	0.4290	0.6204
PH15-1	75	2.0000	1.2884	0.2239	0.3834
OP15-2	75	2.0000	1.6258	0.3849	0.5732
OP15-3	75	2.0000	1.6242	0.3843	0.5725
OP15-4	75	2.0000	1.7524	0.4325	0.6571
Mean	75	2.0000	0.6220	0.4405	1.7954
SD		0.0000	0.0831	0.0755	0.2084

[a] Nei (1987)

[b] Kimura and Crow (1964)

[c] Nei (1973)

[d] Lewontin (1972)

The selection of animals with the least genetic similarity can help to conserve the maximum variability within a population and can even optimize the choice of breeders for ex situ conservation programs.

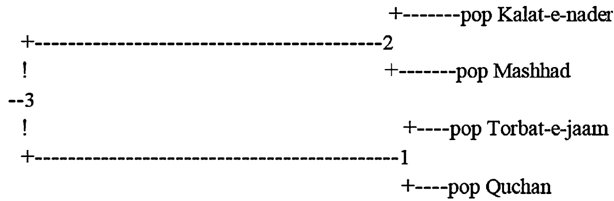

Fig. 3 Dendrogram constructed using Nei's coefficient of similarity between populations

Table 3 Genetic identity of four chukar populations

Population	Kalat-e-nader	Mashhad	Torbat-e-jaam	Quchan
Kalat-e-nader	–	0.9842	0.9299	0.8615
Mashhad	0.0159	–	0.9401	0.8787
Torbat-e-jaam	0.0727	0.0617	–	0.9892
Quchan	0.1491	0.1293	0.0108	–

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Acknowledgments The authors extend special thanks to the excellent Centre in Animal Science of Ferdowsi University of Mashhad, Iran, and the Environment Protection Organization of Khorasan-e-Razavi, Iran, for providing necessary facilities, equipments and partridge blood samples. This work was supported by a grant of the agricultural faculty of Ferdowsi University of Mashhad, Iran.

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