Full Length Research Paper

Population genetic structure of Rufous-Vented Prinia (*Prinia burnesii*) in Pakistan

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The objective of the study is to ascertain genetic variation within Rufous-vented Prinia, *Prinia burnesii* an endemic species, by DNA fingerprinting applying random amplified polymorphic DNA (RAPD) technique. Genetic material was obtained from three distant sites along western bank of River Indus. These sites include Chashma barrage (32°50'N, 71°20'E), in the north; Taunsa barrage (30°45'N, 70°45'E) and the southern population from Guddu barrage (28°26'N, 69°44'E). In total, 14 RAPD primers were selected to determine the genetic variability between three populations of the species. The Nei's (1973) genetic diversity in three populations ranged from 0.190 to 0.320. The genetic distance between Populations ranged from 0.149 to 0.265. The polymorphism levels ranged from 48 to 83% in three populations and at species level, polymorphism were 93.07%. The results further indicate that they are genetically isolated populations.

Key words: Random Amplified Polymorphic DNA, Prinia, genetic variation, river Indus.

INTRODUCTION

The Rufous-vented Prinia is an endemic species of the Indian subcontinent that is listed as "vulnerable" (Collar et al., 1994) and "near threatened" (BirdLife International, 2003). The nominate race *Prinia burnesii*-Blyth, occurs along the River Indus and its tributaries in Pakistan (Roberts, 1992) and adjacent northwest India (Collar et al., 1994). In Pakistan, it is historically spanning much of Punjab along the Indus, Jhelum, Chenab, Ravi and Sutlej rivers, and extending down the Indus into southern Sindh. In the northern part of its range in Khyber-Pakhtonkhawa Province, it occurs around the town of Dera Ismail Khan on the banks of the Indus, to Chashma Barrage (Ripley, 1982; Showler and Davidson, 1999). In the past, the Eastern population was locally common (Ali and Ripley, 1987) but there are few recently published records (Collar et al., 1994) which is an indication of dwindling population of this species in Pakistan. With changing environment, the extinction rate has risen considerably and the biggest threat is to the species that are either endemic or threatened (Manuel, 2006). In case of *P. burnesii*, both of these attributes are present. Such species needs immediate attention and conservation.

In order to initiate any conservation program, it is utmost important to identify the conservation units to get the desired results. It is a well establish fact that different populations have adapted to different regions due to different genetic and ecological needs. To assess the true genetic nature of different populations, DNA fingerprinting has helped tremendously (Haig et al., 2006). In this study, we utilized the random amplified polymorphic DNA (RAPD) markers. RAPD generate DNA fingerprints with a single synthetic nucleotide primer (Williams et al., 1990; Welsh et al., 1990), which could efficiently detect polymorphism based on comparison throughout the genome. It does not require any prior knowledge of DNA sequence but still revealed a high level of polymorphism (Karp et al., 1997).

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Figure 1. The map showing the three study sites.

MATERIALS AND METHODS

Sampling

This species is endemic and near threatened to its recent status, hence is of concern. Owing to the special conservation status of the species and hard to find, our study remained confined to a sum of only fifteen samples from three distantly located sites situated at the vicinity of River Indus. The DNA samples were collected during extensive surveys taken in breeding season from March 2007 to April 2008, when males exhibit territorial songs and are easy to spot. These sites included Chashma barrage 32°50'N, 71°20'E, Taunsa barrage 30°45' N, 70°45'E and Guddu barrage 28°26'N, 69°44'E (Figure 1).

DNA extraction

Only a few numbers of tail-feathers (rectrices) were plucked, and plunged in 95% ethanol before they were stored at -20°C. Total genomic DNA from individual feather was extracted following Bello et al. (2001). A fragment (0.5 to 1 cm long) from the base of the quill was used. 500 µl of lysis buffer (50 mM Tris-HCl at pH 8, 20 mM EDTA at pH 8, 2% SDS) was added and then 10 µl proteinase K (final concentration, 175 µg/ml. Each sample was incubated at 55°C overnight. Then, a phenol:chloroform protocol for DNA extrac-tion was employed (Sambrook et al., 1989). DNA concentration and purity were determined spectrophotometrically.

RAPD-polymerase chain reaction (PCR) amplification

RAPD analysis (William et al., 1990) was conducted. This was done using a total of 14 decamer primers selected out of 40 examined primers (Kits A, B, C and H from Genelink) as they showed the reproducible banding pattern for screening three populations of Rufous-vented Prinia comprising five samples each. Total volume of each PCR reaction was 15 µl consisting of 2 µl of 2.5 mM MgCl$_2$, 2 µl 10 x PCR buffer, 1 µl of 2.5mM of each dNTP, 1 µl of 50 ng/ µl of primer, 1.25 U.I. of Taq DNA polymerase, and 1 µl of 50 ng / µl of template DNA; de-ionized water was added to the final volume of 15 µl. RAPD-PCR amplification was performed in Thermal Cycler (gene amplifications PCR system 9700 of Applied Biosystem) using the following PCR conditions: 5 min at 94°C, 45 cycles of 30 s at 94°C, 45 s at 37°C and 45 s at 72°C; then, a final extension of 10 min at 72°C. Bands were separated at 8% denaturing poly-acrylamide gels and stained with AgNO3 (Heukeshoven and Dernick, 1985; Budowle, 1991).

RAPD data analysis

After gel electrophoresis, good quality gel photographs were used to score the all visible and unambiguously fragments/bands amplified by RAPD primers. The presence and the absence of each band were generated by the 14 selected primers (Genelink, GLA2, GLA3, GLA4, GLA5 GLA7, GLA8, GLA9, GLA10 GLA11, GLA12 GLA13, GLA14, GLB1 and GLB2). The resulting matrix was imported into different programs for data interpretation. Each locus was treated as a two-allele system, with only one of the allele per locus being amplifiable by the PCR under the Hardy-Weinberg Equilibrium (Lynch and Milligan, 1994). The Nei's (1973) average gene diversity (H) and the Shannon Index (S) (Lewontin, 1972) (Figure 2) were used for statistical analysis.

$$H = - \sum P_i \log_2 P_i$$

Where, P_i is the frequency of a given RAPD band calculated using POPGENE (v. 1.31: Yeh et al., 1999). The genetic distance is
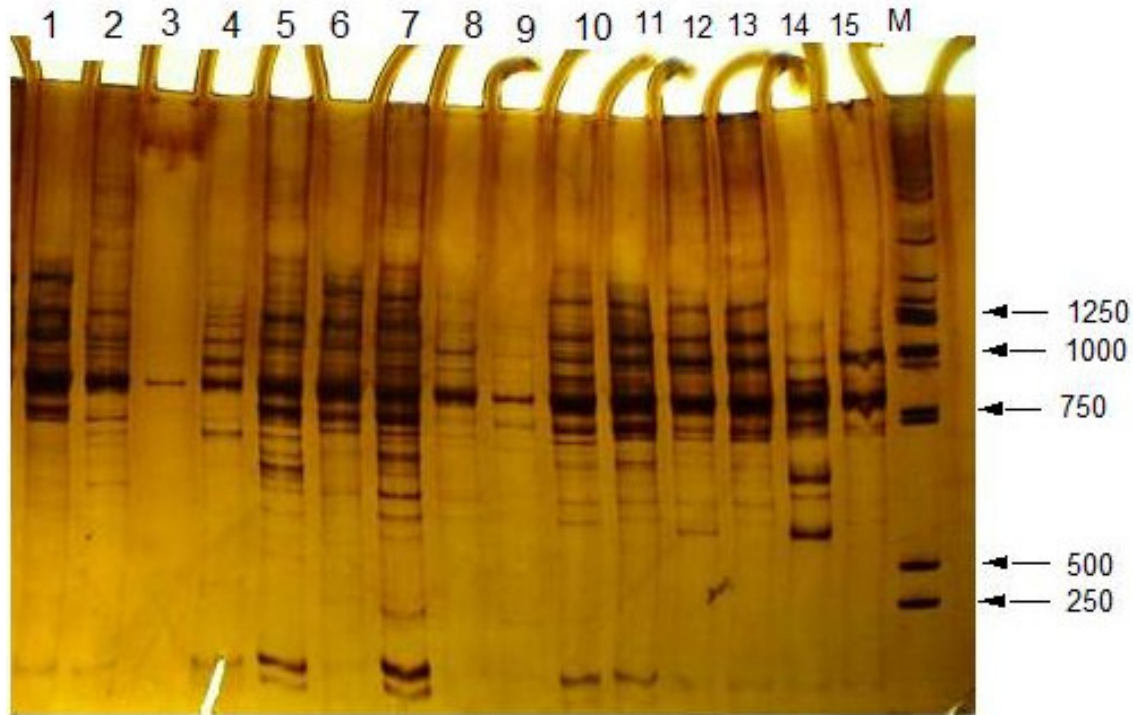
Figure 2. Showing the silver stained banding pattern amplified by the RAPD primer GLA9 of all fifteen samples (1 to 5 Taunsa, 6 to 10 Guddu and 11 to 15 Chasma) resolved on polyacrylamide gel electrophoresis (PAGE).

Table 1. Genetic variation within population.

Population	N_p	P	H	S
Taunsa	192	83.24	0.3209	0.4724
Guddu	131	56.76	0.2137	0.3152
Chashma	111	48.11	0.1908	0.2728
Overall	215	93.07	0.3433	0.5162

N_p, Number of polymorphic loci; P, percentage of polymorphic loci; H, Nei's (1973) gene diversity; S, Shannon's Information index.

Table 2. Showing pair wise Nei's genetic identity (above diagonal) and genetic distance (D) (1978) (below diagonal).

Population	Taunsa	Guddu	Chashma
Taunsa	****	0.8384	0.7671
Guddu	0.1763	****	0.8826
Chashma	0.2652	0.1249	****

calculated by TFPGA software (v. 1.3: Miller, 1997) with Lynch and Milligan's (1994) correction. The similarity matrix was calculated by using unweighted pair- group method with arithmetic average (UPGMA) through NTSYSpc Ver. 2.11 (Rohlf, 2002).

RESULTS AND DISCUSSION

The 14 primers amplified DNA fragments varied from 11 to 25 in a size range from 150 to 1300 bp. The total number of fragments obtained using all these primers was 231 of which 215 were variable. The index of population polymorphism, which was estimated as the proportion of variable RAPD loci in the total number of loci studied, was P = 93.07%. Nei's genetic diversity (H) (1973) and Shannon's Information Index (S) ranged among three populations from 0.1908 to 0.3209 and 0.2728 to 0.4724, respectively. Nei's unbiased (1978)

genetic distance (D) was calculated with Lynch and Milligan's (1994) correction. The calculation of genetic distance (D) yielded values ranging from 0.1249 to 0.2652 (Table 2). Dendrograms among the specimens were constructed using the Jaccard (J) coefficient and the UPGMA cluster analysis algorithm and similarity co-efficient varied from 0.51 to 0.84 (Figure 3).

This is the first report about the genetic structure of Rufous-vented Prinia (*P. burnesii*) from Pakistan (BirdLife, 2003). We sampled from three geographically isolated populations (Chashma, Taunsa and Gudu) (Figure 1) and using the RAPD markers genetically assessed all three populations. The level of polymorphism is comparatively very high among these three populations (P = 93.07%) (Table 1). Similar high levels of polymorphism have been reported in *Ammoperdix griseogularis* (P = 94%) from Pakistan (Khaliq et al., 2010). Other avian studies have also reported high levels of polymorphism while using the RAPD techniques (Giesel et al., 1997; Kulikova et al.,
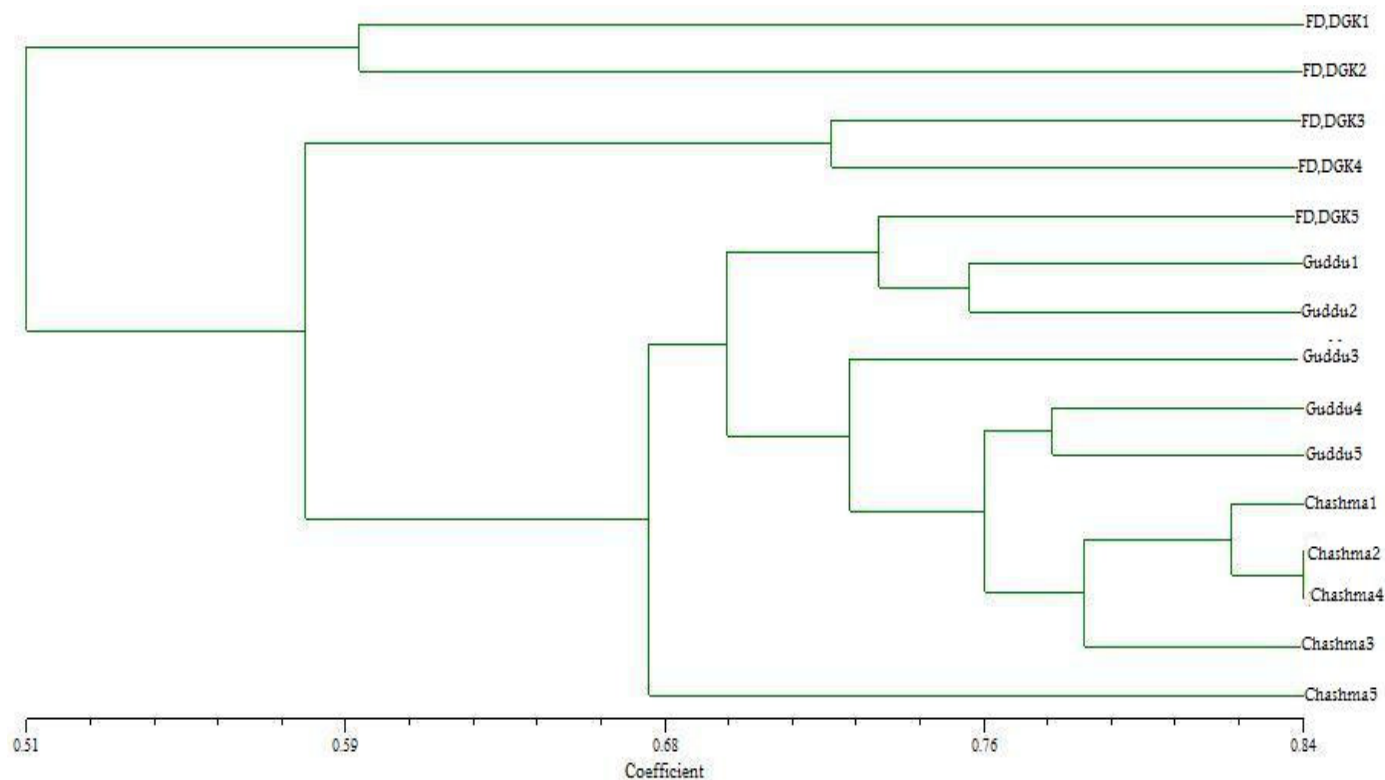
Figure 3. Genetic similarity dendrogram generated with the Jaccard coefficient and UPGMA algorithm.

2002). Interestingly, the polymorphism in two of the three sampled populations (that is, Chashma, P = 48.11% and Guddu, P = 56.76%) (Table 1) were significantly low which is an indication that these populations might be facing the effects of inbreeding owing the species as sedentary in nature.

One cannot ignore the possibility of sampling error but sampling was designed to avoid sampling kins. So, such bias of sampling has been countered. The Nei's genetic diversity suggests low to moderate levels of diversity in these two populations (Chashma H = 0.190, Guddu H = 0.213) (Table 1). Whereas, the level of Nei's genetic diversity was high in Taunsa population (H = 0.3209) (Table 1) that is very near to overall genetic diversity (H = 0.3433) (Table 1). A high level of genetic diversity at Taunsa might be attributed to larger habitat still intact that can be utilized as a good conservation locality for *P. burnesii* and number of other resident bird species as well as migratory bird species. This high level of genetic diversity is comparable to the other studies including *Francolinus francolinus* where genetic diversity is reported around H = 0.276 (Padilla et al., 2000; Khaliq et al., 2010; Riaz et al., 2010). Apparently, high genetic diversity at species level (H = 0.3433, Table 1) at Taunsa, suggests this species harbor a rich genetic diversity that will help in future survival under rapidly changing environment but low genetic diversity in two of the populations and high genetic distance (Table 2) between all

populations is indicative that these populations are pretty much structured and do not exchange much distant genetic material thus two populations out of three are not that much diverse. Our study also throws light on the point that whenever any conservation program is to be taken overall genetic diversity might indicate wrong esti-mates. Therefore, it is very important to assess the genetic diversity at population levels, as population is the true conservation unit.

The genetic distance among three populations showed interesting results as the highest level of genetic distance was recorded between the Taunsa and Chashma (D = 0.2652) (Table 2) population whereas the longest distant populations are Chashma and Guddu. This finding is in accordance with other avian studies that have shown that if species is of sedentary nature then geographical distance does not hold much significance (Bates, 2000; Francisco et al., 2007) The dendrogram clearly showed that each population is genetically differentiated from the other populations (Figure 3).

In conclusion, the low polymorphism seen in popu-lations of Guddu and Chashma might be a result of inbreeding caused by dwindling intra-population numbers of this species. High polymorphism and gene diversity in Taunsa population indicates good signs of rich genetic resource of this endemic vulnerable species which is still available and may be used for any future studies. Such genetically differentiated and diverse spots are of special

importance as they could be the ideal conservation unit for species like *P. burnesii*.

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