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Full Length Research Paper

Evaluation of the genetic structure of the urban dwelling species of Bank Myna (*Acridotheres ginginisnus*) using random amplified polymorphic DNA (RAPD) analysis

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We used the random amplified polymorphic DNA (RAPD) technique to decipher the genetic structure of the Bank Myna (*Acridotheres ginginisnus*) in Pakistan. The samples were collected from four cities namely: Dera Ghazi Khan, Jahanian, Khanewal and Gujranwala. The analysis showed a high genetic diversity at species level (H = 0.318, S = 0.467) but low levels at population levels (H = 0.047, S = 0.069 to H = 0.195, S = 0.283). The four populations analyzed were genetically distant from each other and hinted the effect of urbanization role in isolating the urban dwelling species. The genetic distances between populations ranged from D = 0.2403 to D = 0.3419 and the similarity coefficient showed low range from 0.66 to 0.70. This study will help in future conservation plans and also help the understanding of the role of urbanization on fragmentation of species' natural distribution and dispersal mechanism.

Key words: *Acridotheres ginginisnus*, Bank Myna, random amplified polymorphic DNA (RAPD), genetic structure.

INTRODUCTION

Bank Myna (*Acridotheres ginginianus* Latham, 1790) is an endemic species to the Indian sub-continent. It is only found in northern India, south to approximate latitudes of Mumbai, Maharashtra and Orissa, except for the drier regions in Rajasthan. Distribution is patchy, but is most commonly found in major river valleys. Normally, this species is resident but with regular seasonal local movements in some areas (Grimett et al., 1998). In Pakistan, *A. ginginianus* is wide spread throughout Sind and Punjab but is uncommon in areas away from the rivers. Waite (1948) considered it as a rare bird in the Punjab salt range, but in the 1980s, small flocks could be seen around salt range lakes. They have not been seen in the Baluchistan but have been found in Khaber Pakhtunkha, only in the well watered cultivated areas around Kohat, Bannu and Peshawar. They also occur abundantly in rice cultivated areas; however, they have not been seen in Swat where rice is extensively cultivated (Whistler, 1930; Ali and Ripley, 1986; Roberts, 1992).

The Bank Myna is often found together with the common Myna (*Acridotheres tristis*) and is very similar to it in habits, but is more gregarious than common Myna. Despite its wide distribution and its status being as Least Concern (Birdlife, 2010), no genetic data of Pakistani populations is available in the literature. In this sense, RAPD technique has proved to be very useful in other avian population genetic studies (Khaliq et al., 2010; Muhammad et al., 2010; Imtiaz et al, 2011; Riaz et al., 2011). RAPD technique requires limited expertise, it is easy to use and reproducible results can be obtained, providing a baseline for further detailed studies. So, we

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Table 1. Genetic polymorphism based on the primers employed.

Primer	Number of loci		Polymorphism (%)
	Total	Polymorphic	
GLA-01	16	13	81.25
GLA-03	14	12	85.71
GLA-04	14	10	71.42
GLA-05	21	14	66.66
GLA-06	23	14	60.87
GLA-07	23	20	86.95
GLA-08	22	16	72.72
GLA-9	24	21	87.50
GLA-10	18	16	88.88
GLA-11	16	14	87.50
Total	191	150	78.5

applied the RAPD markers to analyze the genetic structure of *A. ginginisnus* in Pakistan. This information can help us to identify the conservation units in future.

MATERIALS AND METHODS

Study area

Most of the birds were sampled from Punjab province. These sites included Gujranwala (32.16°N, 74.18°E), Khanewal (30°18'N, 71°56'E), Dera Ghazi Khan (30°03N, 70°38′E) and Jahanian (30°18′N, 71°56′E), which are all urban settlements. The air distance between Gujaranawala and Dera Ghazi Khan is approimately 500 km, Khanawal and Gujeranwala is 360 km, Gujeranwala and Jahanian is 325 km, Dera Ghazi Khan and Khanewal is 140 km, Dera Gazi Khan and Jahanian is 168 km, and Jahanian and Khanawal is 28 km.

DNA extraction

Tail feathers were plucked and stored at -20°C before DNA was extracted using Bello et al. (2001) method with minor modifications. A fragment from the base of the quill was taken and after crushing, it was mixed with 500 µl lysis buffer (50 mM Tris-HCl at pH 8, 20 mM EDTA at pH 8 and 2% SDS). 10 µl of proteinase K (final concentration 175 µg/ml) was added before incubating the samples overnight at 55°C for complete lysis. Then, standard phenol: chloroform method was applied to extract the high-quality DNA (Sambrook et al., 1989). The concentration of DNA was determined spectrophotometrically.

RAPD- polymerase chain reaction (PCR) amplification

We scanned a total of 20 RAPD decamer primers (Williams et al., 1990) of Kit A (Genelink). Out of these primers, 10 (Table 1) showed reproducible results and are thus suitable to decipher the genetic structure of *A. ginginisnus*. Each sample was tested thrice for reproducibility. Total volume of each PCR reaction was 15 µl containing 50 ng/µl of DNA, 2.5 mM $MgCl_2$, 2 µl 10 x PCR buffer, 2.5 mM of each dNTP, 50 ng/µl primer and 1 U.I. of Taq DNA polymerase. RAPD-PCR amplification was performed in a Thermal Cycler (gene amplifications PCR system 9700 of Applied Biosystem) using the following PCR conditions: 5 min at 93°C, 45 cycles of 40 s at 93°C, 45 s at 36°C and 1 min at 72°C; then, a final extension of 10 min at 72°C. Bands were separated at 8% denaturing polyacrylamide gels and stained with $AgNO_3$ (Heukeshoven and Dernick, 1985; Budowle, 1991).

RAPD data analysis

Photographs of the gels were taken and scoring was done manually. Each amplified fragment was considered as dominant and scored '1', whereas the absence of band was scored as '0' and considered as recessive allele. The generated matrix was analyzed through different softwares to understand the genetic structure. The Nei's average genetic diversity (H) and Shannon index (S) (Lewontin, 1972) was calculated through POPGENE (V. 1.13: Yeh et al., 1999). The genetic distance (D) and G-test were calculated by TFPGA software (V. 1.3: Miller, 1997) with Lynch and Milligan's (1994) correction. Genetic similarity dendrogram among the specimens were constructed using the Jaccard (J) coefficient and the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis algorithm in the NTSYS-PC Version 2 (Rohlf, 1992) computer program.

RESULTS AND DISCUSSION

Studies of the population genetic structure provide a window of opportunity into the roles that selection, mutation, gene flow and drift play in processes such as local adaptation and speciation (Barton and Clark, 1990; Avise, 1994; Slatkin, 1994). Previous studies on population genetics of birds have shown that RAPD results are similar to the outcomes of other genetic markers such as isoenzyme, allozymes, RFLP and mitochondrial DNA (Baruffi et al., 1995; Crochet, 2000; Kjolner et al., 2004). In this study, these markers were used to analyze the genetic structure and the genetic variability within and among four populations of *A. ginginisnus*. A total of 20 primers were screened, with 10

Table 2. Genetic variation within populations.

Population	N_p	P	A	A_e	H	S
Dera Ghazi Khan	91	47.64	1.476 (±0.50)	1.356 (±0.42)	0.195 (±0.21)	0.283 (±0.30)
Jahanian	22	11.52	1.115 (±0.32)	1.081 (±0.22)	0.047 (±0.13)	0.069 (±0.19)
Khanawal	82	42.93	1.429 (±0.49)	1.279 (±0.37)	0.161 (±0.20)	0.238 (±0.28)
Gujerawal	95	49.74	1.497 (±0.50)	1.337 (±0.39)	0.191 (±0.20)	0.281 (±0.29)
Overall	150	78.50	1.811 (±0.39)	1.561 (±0.36)	0.318 (±0.18)	0.467 (±0.25)

N_p = Number of polymorphic loci; P = percentage of polymorphic loci; A = observed number of alleles; Ae = effective number of alleles [Kimura and Crow (1964)]; H = Nei's (1973) gene diversity; S = Shannon's information index [Lewontin (1972)].
Values in parenthesis are the standard deviation.

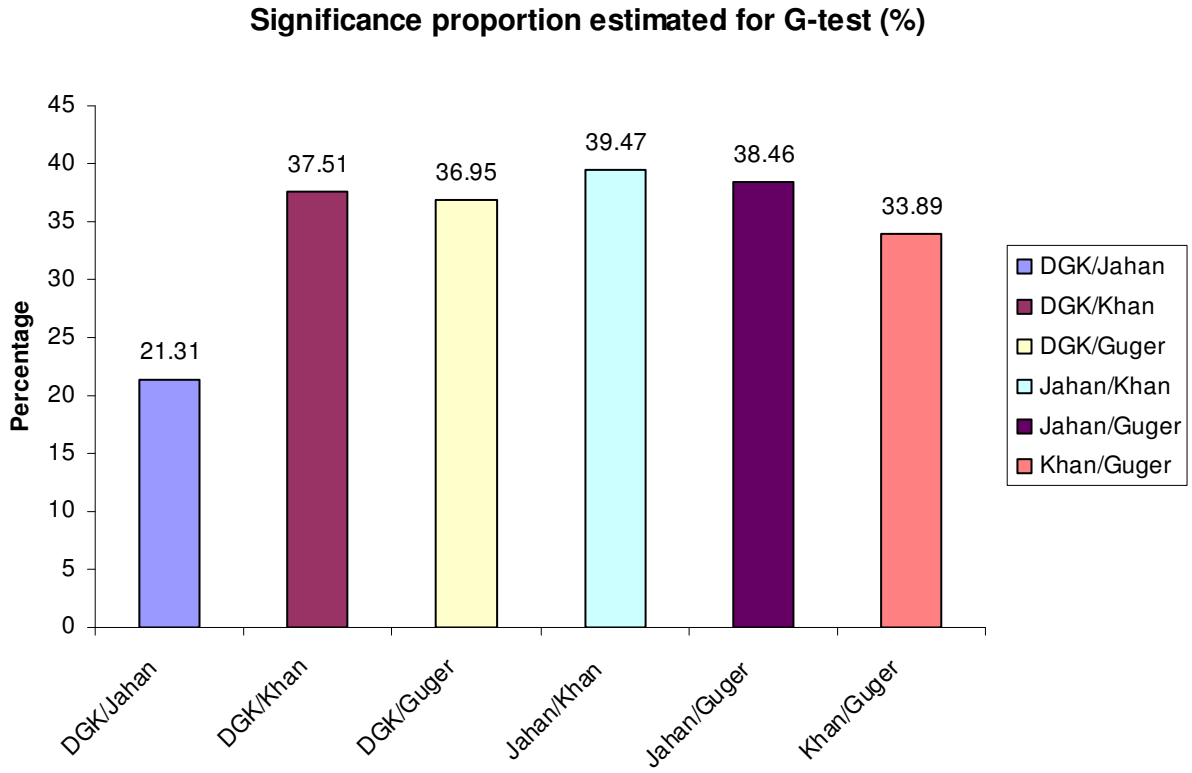

Figure 1. Statistical significance of different proportions of polymorphic loci between pairs of populations (P<0.05).

primers (Table 1) showing reliable and reproducible polymorphic pattern. Analyses showed that polymorphism (P) at species level (P = 78.50%) (Table 2) was high, and was comparable with other avian reports (Khaliq et al., 2010; Muhammad et al., 2010; Imtiaz et al., 2011; Riaz et al., 2011). On the contrary, the polymorphism (P) at the population level was very low as compared to the total, which ranged from P = 11.52%, (Jahanian) to P = 49.74%, (Gujeranwala) (Table 2). The low polymorphism at the population level was comparable with *A. tristis* where polymorphism was calculated as 38.5% (Baker and Moeed, 1987). These low levels might be attributed to high inbreeding or to low sample size. However, the pairwise comparison of populations showed much higher polymorphism. The observed polymorphism between populations ranged from P = 62% in Khanawal/ Gujeranwala to P = 81% in Jahanian and Khanewal. G-test also supported this result where the highest levels of statistically differentiated polymorphic loci were observed between Jahanian and Khanewal (39.47%) (Figure 1). Though the geographical distance is small between these two localities, the high levels of genetic differentiation might be as a result of the urbanization. Although, certain previous reports showed that geographical distance (Bates, 2000, 2002; Lunardi, 2007) may not have any significant contribution in genetic mixing between neighboring populations of the resident and sedentary species, but in our study, urbanization seemed to be a

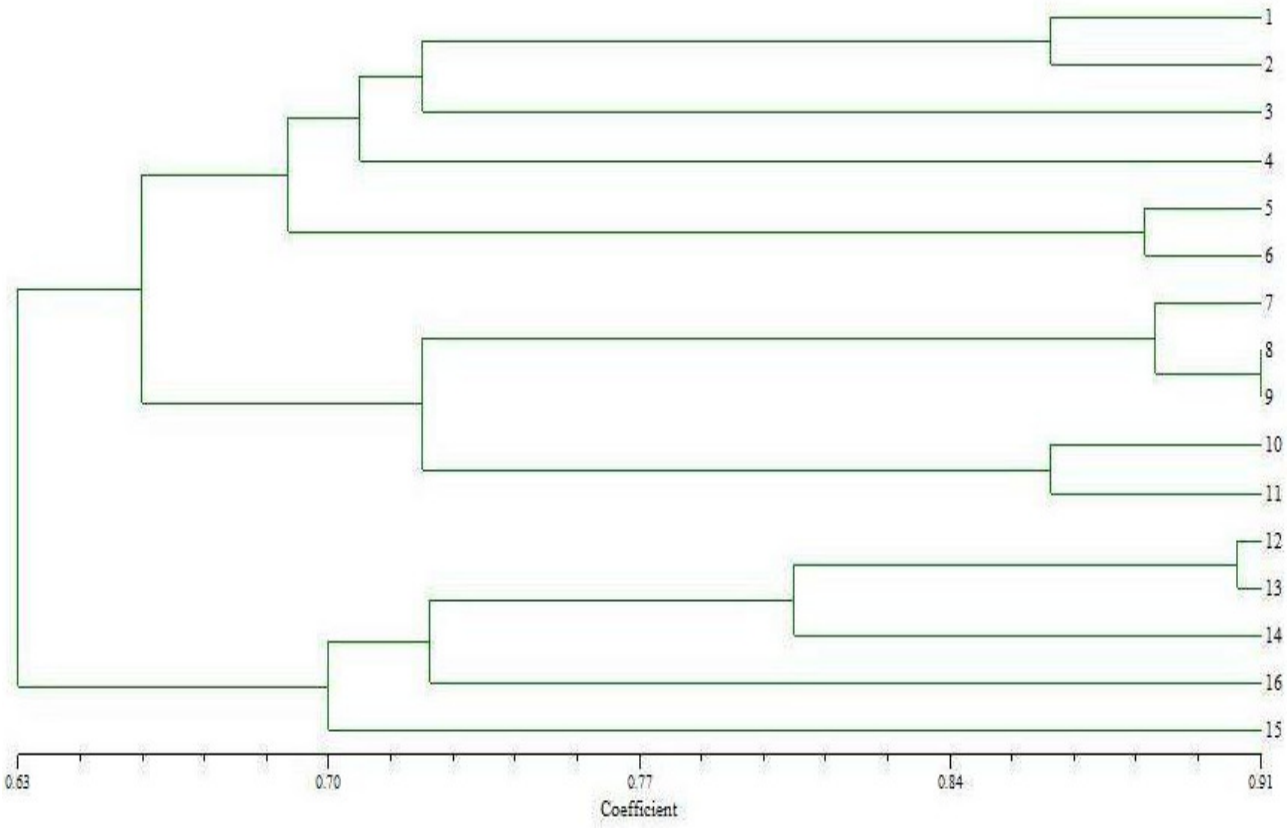

Figure 2. UPGMA dendrogram showing genetic similarity between 16 *A. ginginianus* genotypes (1 to 4, Dera Ghazi Khan; 5 to 6, Jahanian; 7 to 11, Khanewal; 12 to 16, Gujranwala) and between individuals chosen based on 10 RAPD primers. Scale on the bottom corresponds to Nei and Li's (1979) coefficient of similarity.

more logical reason. After the urbanization, the birds of this species tended to remain localized. In urban areas, these birds are protected from natural predators on one hand and food is easily available on the other hand. In addition, these birds find nesting places very easily as a lot suitable spaces are present in buildings.

The genetic diversities of the four populations analyzed were estimated by Nei's genetic diversity (H) and Shannon's information index (S). The value of genetic diversity at species level was high: H = 0.318 and S = 0.467 (Table 2). It can be compared with the values observed in the *Corvus corone* (H = 0.30; Spiridonova et al., 2003). The genetic diversity at the population level was variable, showing a range of H = 0.047, S = 0.069 (Table 2) in Jahanian population to H = 0.195, S = 0.283 (Table 2) in Dera Ghazi Khan population. The level of genetic diversity determines the adaptive potential of a population to environmental changes and a higher variability reflects a higher fitness (Heuertz et al., 2001; Hansson and Westerberg, 2002). This study indicated that the level of genetic diversity at the species level was higher than the average. Among populations, the most genetically diverse was Dera Ghazi Khan. The population genetic diversity of the species was affected by a number of factors including mating system, dispersal, geographic isolation, environmental interaction and urbanization. These results suggest that this species had adapted very well to urbanization and found very favorable conditions around Dera Ghazi Khan city in Pakistan, thus showing a high genetic diversity despite the low sample size.

Population dendrogram also showed that all the populations might be in isolation (Figure 2). The greatest Nei's genetic distance (1978) was found between Gujeranwala and Jahanian populations (D = 0.341) (Table 3) and the lowest genetic distances (0.243) were observed between Dera Ghazi Khan and Jahanian (Table 3). From genetic similarity dendrogram, it was shown that similarity coefficient varied from 0.66 to 0.70 (Figure 2). The similarity dendrogram splits the populations of *A. ginginianus* into two clusters. Interestingly, the Dera Ghazi Khan population clustered with Jahanian and Khanewale, whereas the Gujaranwala population formed a separate cluster. The Gujeranwala population of *A. ginginianus* was in isolation from other three populations. These clusters were in accordance with their geographical distances. Behavioral aspects as well as urbanization seemed to be the major factors that explain the genetic structuring, especially for Khanewal and Jahanian population, since they were only 28 km apart, which showed that they prefer to remain in the native areas.

Table 3. Nei's (1978) unbiased measures of genetic distance (below diagonal) and genetic identity (above diagonal) with Lynch and Milligan's correction (1994), between the *A. ginginianus* samples from the four different populations.

Population	Dera Ghazi Khan	Jahanian	Khanawal	Gujaranwala
Dera Ghazi Khan	****	0.7839	0.7864	0.7726
Jahanian	0.2435	****	0.7501	0.7104
Khanawal	0.2403	0.2875	****	0.7751
Gujaranwala	0.2580	0.3419	0.2547	****

CONCLUSION

Our study reported the low movement of individuals among these populations which is concurrent with field observation. The exchange of genetic material between these populations was very small and it was also reflected by the high levels of genetic distances among the populations. This study also reflected the role of urbanization in fragmentation of this urban dwelling species. It also indicated the impact of urbanization on the natural setup of avian group. Ecological and biological factors and the human impact on habitat alteration could have contributed to the geographically genetic structuring of the species observed. In this respect, RAPD markers have proved to be a useful tool for understanding the *A. ginginianus* genetic variation at the population level. In the future, more molecular markers for deeper analysis and more populations across the Pakistan will be carried out to understand the true genetic structure of this species. We believe that in the future, this study will provide a baseline for the conservation of this species in Pakistan.

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