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Full Length Research Paper

Genetic diversity of Pakistani common myna (*Acridotheres tristis*) revealed by RAPD-PCR

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Acridotheres tristis **is a widely distributed species of Pakistan but no genetic data of this species is available in the literature. In this study, we applied 8 selected RAPD markers from a total of 20 tested markers, to decipher the genetic structure and genetic diversity of different populations. The RAPD markers revealed total polymorphism at P = 87.73%. The overall Nei's genetic diversity (H) and Shannon's index (S) levels were high and remained at H = 0.369 and S = 0.540. The genetic distances (D) between populations were also observed to be high. These distances ranged from D = 0.137 between Dera Gahzi Khan and Khanewal to D = 0.273 between Gujranwala and Khanewal. This study will be useful in conservation plans in future under changing environmental scenario.**

Key words: *Acridotheres tristis*, genetic diversity, genetic distance, RAPD markers.

INTRODUCTION

The Sturnidae (starlings) is a very successful and adaptable passerine family. They are distributed, to a large range, and estimated at a global extent of occurrence of 1,000,000 to 10,000,000 km². Common myna (*Acridotheres tristis*) is a Least Concern (IUCN) species which are tropical member of the family Sturnidae. Native range of common myna (*A. tristis*) originated from central and southern Asia and is widely distributed throughout India (Feare and Craig, 1999, Lim et al., 2003). Their huge populations are present in South Africa, eastern and southeastern Australia, North Island of New Zealand, Solomon Islands, New Caledonia, Fiji, Western Samoa, Cook Islands, Hawaii and Réunion. There is a detail report of breeding of common mynas in northern France (Feare and Craig, 1999). Common mynas (*A. tristis*) occupy a wide range of habitats in warm areas with access to water, about 3000 m (high); average. 1500 m. In their native range, common mynas (*A. tristis*) inhabit open agricultural areas such as farmlands as well as cities. They are often found on the outskirts of towns and also outlying homesteads in desert or forest (Australian Museum, 2003; Invasive Species Specialist Group, 2006; Kannan and James, 2001). In Pakistan, common myna (*A. tristis*) is distributed throughout the country but no genetic data of Pakistani population is available in literature.

Under climate change scenario, for management and protection programs, the genetic structure of species at population level has received special attention in the past few years (Pfenninger et al., 2010). Analyses of genetic diversity can be applied to studies of the evolutionary ecology of populations. The presence of unique genetic characteristics distinguishes members of a given population from those of any other population. High diversity is an indicator of better adaptability of a population and thus more fitness under rapidly changing environment. In addition, genetically differentiated populations are to be treated as separate conservation units. Keeping this in view, the population genetic structure of common myna (*A. tristis*) is characterized by applying the RAPD technique. This technique is easy to use and has

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proven a good choice in population genetic studies in aves (Andayani et al., 2001; Èesonienë et al., 2005; Barbanera et al., 2010a, Khaliq et al., 2010, Muhammad et al., 2010, Riaz et al., 2011). RAPD-PCR can potentially increase the resolution of genetic differences among individuals in population genetic studies if different primers are used (Apostol et al., 1996; Nybom and Bartish, 2000; Franck and Awadhesh, 2006) as done in this study. Hopefully, this study will contribute, extensively to the quality of genetic data available in Pakistani common myna (*A. tristis*). We feel this study will provide a baseline in conservation efforts (Jiang et al., 2005; Groom et al., 2006) in the future.

MATERIALS AND METHODS

Study area

Samples of common myna (*A. tristis*) were collected from different parts of Pakistan. These sites included Jhelum valley in Azad Kashmir, Khanewal, Dera Gazi Khan and Gujranwala from Punjab. The birds sampled in each area were referred to as a single population.

DNA extraction from feathers

Plucked tail feathers were used. These were plunged down in 95% ethanol before storing at -20°C. Modified Bello et al. (2001) procedure for total genomic DNA extraction was followed. A 0.5 to 1 cm long fragment from the base of the quill was used. Then, 500 µl of lysis buffer (50 mM Tris-HCl at pH 8, 20 mM EDTA at pH 8.2% SDS) was mixed together with 10 µl of proteinase K (final concentration, 175 µg/ml. Each sample was incubated at 55°C overnight. The following day, a phenol: chloroform protocol for DNA extraction was employed (Sambrook et al., 1989). DNA concentration and purity were determined by spectrophotometry (Perklin Elmer Ltd, UK).

RAPD- polymerase chain reaction (PCR) amplification

RAPD technique (William et al., 1990) was applied using a total of 20 decamer primers of Kit A (Genelik). Only 8 primers produced the best results and were used for genetic analysis. 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 $Mgcl_2$, 10 x PCR buffer, 2.5 mM of each dNTP, 50 ng/µl of primer and 1 U.I. of Taq DNA polymerase. RAPD-PCR amplification was performed in Thermal Cycler (gene amplifications PCR system 9700 of Applied Biosystem) using the following PCR conditions: 4 min at 94°C, 45 cycles of 45 s at 92°C, 45 s at 37°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

Good quality gel photographs were scored manually. Each amplified band was treated as a dominant allele and scored as '1' and the absence of allele was scored as '0'. The generated matrix was analyzed to quantify the genetic variations through different software. The Nei's average genetic diversity (H) (Nei, 1973) and Shannon index (S) (Lewontin, 1972) was calculated through POPGENE (V. 1.13: Yeh et al., 1999). The genetic distance (D) (Nei, 1978) was calculated by TFPGA software (V. 1.3: Miller, 1997) with Lynch and Milligan's (1994) correction. Genetic similarity dendrogram among the specimens were constructed by using the Jaccard (J) coefficient and the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis algorithm in the NTSYS-PC (Rohlf, 1992) computer program.

RESULTS AND DISCUSSION

Genetic diversity plays an important role in the survival and adaptability of a species. When a species environment changes, slight gene variations are necessary for it to adapt and survive. A species that has a large degree of genetic diversity among its individuals will have more variations from which to choose the most fitting allele. The loss of genetic variability can be a consequence of fragmentation, inbreeding, genetic drift, bottle neck effect and stochastic effects (Nelson and Soule, 1987). Random amplified polymorphic DNA (RAPD) is a PCR-based molecular markers developed by Williams et al. (1990) which have been demonstrated to be useful not only for the study of population genetic variation but also for taxonomic identities, systematic relationships, parentage identifications, identification of interspecific hybridization and introgressive hybridization (Semenova et al., 1996; Giesel et al., 1997; Dolmatova et al., 2000; Barbanera et al., 2010a). The eight selected primers (Table 1) amplified total 106 loci out of which 93 were polymorphic. The amplified fragments ranged from 120 to 1900 bp. The RAPD profile of GLA-04 is given in Figure 1. Maximum number of fragments was produced by GLA-01, 19 and the lowest (11) fragments were produced by three different primers namely, GLA-02, GLA-05 and GLA-09 (Table 1). The level of polymorphism among all samples was P = 87.73% (Table 1). Interestingly, low levels of polymorphism were observed at population levels. Only P = 25% (Table 2) polymorphic loci were observed in Khanewal and Gujranwala populations. In Jhelum and Dera Ghazi Khan, the polymorphism was higher (P = 74 and P = 65% (Table 2), respectively).

The low levels of polymorphism at Khanewal and Gujranwala populations might be attributed to low sample size and sampling error. Samples might have belonged to same covey and thus, showed low levels of polymorphism. These low levels are comparable with other avian species and particularly with the *Phasianus colchicus* where polymorphism was reported to be about P = 34% (Giesel et al., 1997; Bowditch et al., 1993; Haig et al., 1994 and Nussar et al., 1996). The levels of Nei's genetic diversity and Shannon's index were H = 0.3696; S = 0.5400 (Table 2), respectively, in all samples. Similar high levels have already been reported in other avian species as well; like See see partridge (*Ammoperdix griseogularis*), Rufous vented prinia (*Prinia burnesii)* and Black francolin (*Francolinus francolinus*) (Khaliq et al., 2010; Muhammad et al., 2010, Riaz et al., 2011) while

Table 1. Inter-specific polymorphism detected by RAPD primers.

Primer	Sequence 5'-3'	Number of loci		Polymorphism (%)
		Total	Polymorphic	
GLA-01	5'-CAGGCCCTTC-3'	19	18	94.7
GLA-02	5'-TGCCGAGCTG-3'	11	10	90.9
GLA-03	5'-AGTCAGCCAC-3'	15	13	86.6
GLA-04	5'-AATCGGGCTG-3'	13	10	76.9
GLA-05	5'-AGGGGTCTTG-3'	11	10	90.9
GLA-07	5'-GAAACGGGTG- 3'	13	10	76.9
GLA-09	5'-GGGTAACGCC-3'	11	10	90.9
GLA-14	5'-TCTGTGCTGG-3'	13	12	92.30
Total		106	93	87.73

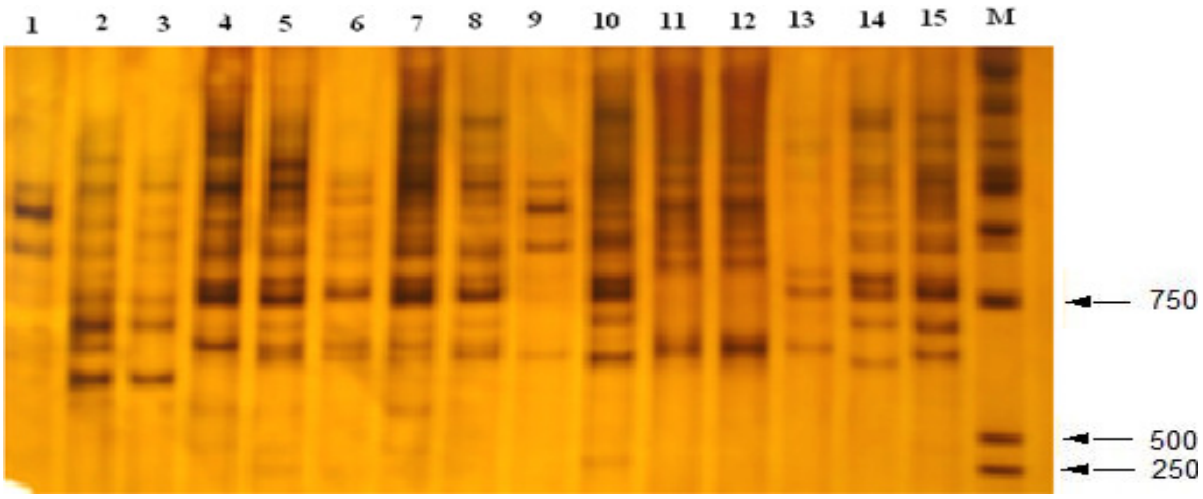

Figure 1. The silver stained banding pattern of amplified RAPD primer GLA-04 of all fifteen samples (Lanes 1 to 3: Gujranwala, lanes 4 to 7: Dera Gazi Khan, lanes 8 to 11: Khanewal and lanes 12 to 15 Jhelum).

Table 2. Genetic variation within population.

Population	N_p	p	H	S
Jhelum	78	74	0.205	0.297
Khanewaal	26	25	0.107	0.153
Gujranwala	26	25	0.098	0.144
Dera Gazi Khan	68	65	0.211	0.303
Overall	106	87.73	0.3696	0.5400

N_p = Number of polymorphic loci, P = percentage of polymorphic loci, H = Nei's (1973) genetic diversity, S = Shannon's information index Lewontin (1972).

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

Population	Jhelum	Khanewaal	Gujranwala
Jhelum (Azad Kashmir)	*****		
Khanewaal	0.167	*****	
Gujranwala	0.215	0.273	*****
Dera Gazi Khan	0.138	0.137	0.243

applying RAPD markers. The level of genetic diversity among the four populations ranged from H = 0.098, S = 0.144 (Table 2) in Gujranwala to H = 0.211, S = 0.303 (Table 2) in Dera Ghazi Khan. These levels of genetic diversity indicated that this species in Pakistan harbors a rich genetic resources and thus may withstand the climate change in future. The presence of this species in Punjab as well as in Himalaya is also an indication of adaptive qualities of this species under different habitat. High degree of genetic distances among the populations was observed. These levels are comparable with the light-footed clapper rail (*Rallus longirostris levipes*) (Nusser et al., 1996). The highest level of Nei's genetic distance (1978) was observed between Gujranwala and Khanewal (D = 0.273, Table 3) and the owest was between Dera Gazi Khan and Khanewal (D = 0.137,

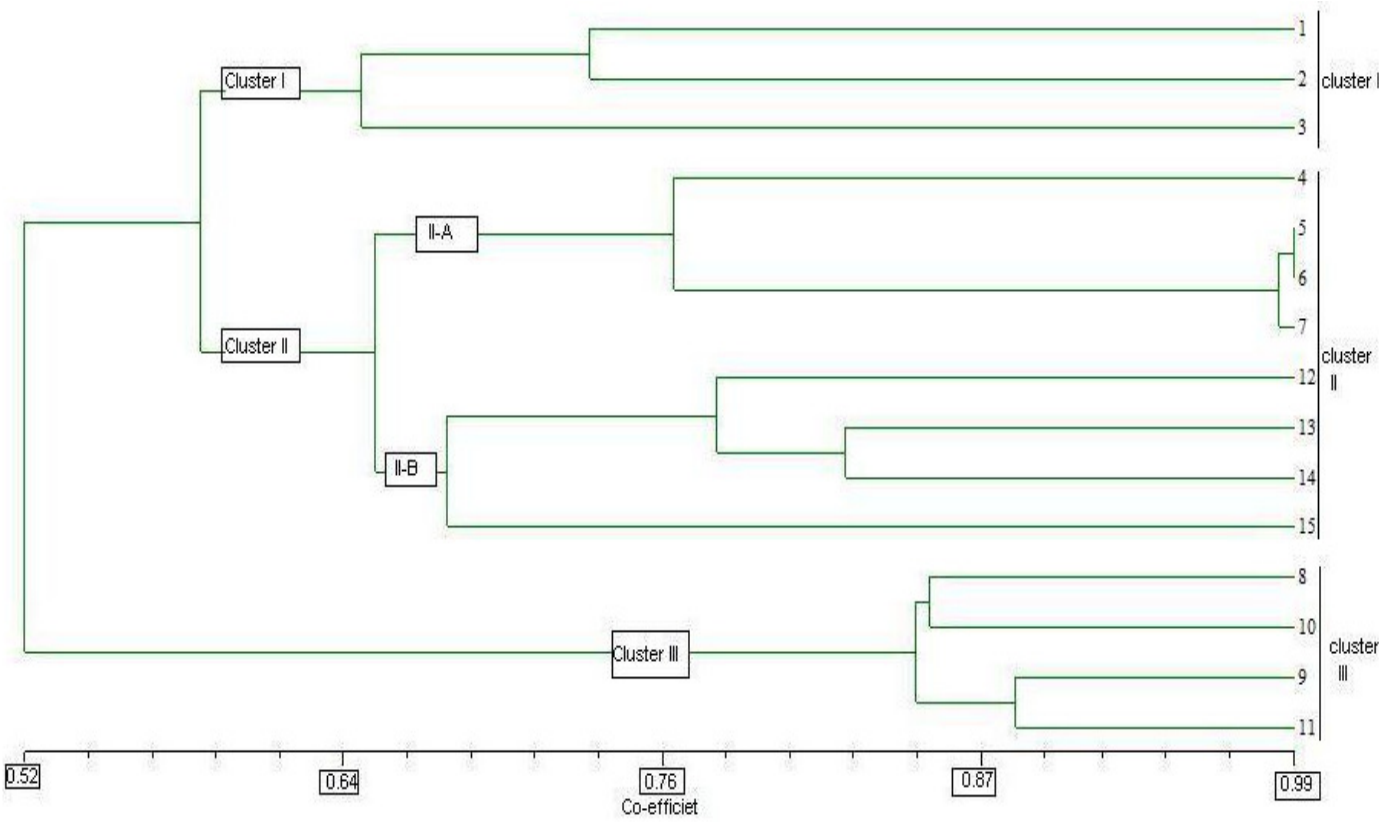

Figure 2. Genetic similarity dendrogram generated with the Jaccard coefficient and UPGMA algorithm.

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

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Table 3). Interestingly, the lowest geographic distance is also between these localities. The most isolated population is of Jhelum (Azad Kashmir) as this population is surrounded by the very high Himalayas Mountains and contact is only made through the roads but the low genetic distance was observed between Dera Ghazi Khan and Jhelum (D = 0.138, Table 3), which suggested that probably, the birds of Jhelum (Azad Kashmir) might have recently invaded this locality after the construction of roads and human settlements. Noticeably, this species in the rest of Pakistan and particularly in Punjab province usually remain around the roads in search of food (Khaliq et al., 2010). Dendrogram constructed for 96 loci of fifteen genotypes revealed four major groups (clusters, Figure 2) for four different populations of the common myna, further confirming the previously mentioned results. Cluster I comprised of three birds of Gujranwala, The cluster II has two sub clusters comprising of birds of two different populations, Dera Ghazi khan and Khanewal (Figure 2). The third larger cluster comprises of birds from Jhelum (Azad Kashmir) (Figure 2). Each population form a separate group is supported by the genetic distance between these populations.

The results concerning the genetic structure of the studied species' populations have implications for the conservation viewpoint. It is a well known fact that understanding the pattern of genetic variation is a critical step to assess species fitness and to plan a conservation strategy (Reed and Frankham, 2002). This study will help in the future conservation plans of this species in Pakistan.

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