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# Novel microsatellite based population genetic analysis of Pakistan endemic, jerdon's babbler (*chrysomma altirostre*: aves) from indus plains

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**Abstract**

Jerdon's babbler *Chrysoma altirostre* is a resident, sedentary endemic and globally endangered bird species inhibiting dense moist growth over the stretch of River Indus and its tributaries. The study was aimed at determining the genetic structure following microsatellite markers from *Passeridae* family, never used for Jerdon's babbler. The samples were procured over the stretch of River Indus from Chashma barrage (32°50'N, 71°20'E) in the north and Panjnad (29°20'47"N 71°1'14"E), as well as, Guddu barrages (28°26'N, 69°44'E) in the south. A sum of 25 microsatellite primers were used to study the genetic diversity among three distantly placed natural populations of the species in order to see the inbreeding effect? Out of 25, a total of 15 markers showed polymorphism.

During the analysis, a sum of 525 alleles were amplified from 22 samples. The number of polymorphic alleles ranged from 2-11. The observed and expected heterozygosities ranged from 0.212 - 0.354 and 0.715 - 0.528, respectively. Out of 15 polymorphic primers, 9 expressed the maximum heterozygosity. The phylogenetic analysis reflects a partial overlap of genetic material being exchanged among northern and southern populations, hence indicating dispersal and migratory-connectivity. Seemingly, the high levels of genetic diversity signifies a stronger gene pool, with least inbreeding effect that may withstand the environmental change.

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## Introduction

The Jerdon's babbler (*Chrysomma altirostre*), an endemic species inhabiting subcontinent listed as "vulnerable" (Collar *et al.,* 2001) and threatened (Bird Life International, 2003). It is sedentary in nature and found in tall grasses including *Phragmites karka* (reed beds), *Saccharum benghalense* (reed grasses) and *Typha angustata* (reed mace) in riverine tracts especially seen after annual monsoon flooding. Being restricted range species RRS, it prefers extensive thick reed-covered tall perennial coarse grasses rather semi-open habitats with short grasses and scattered bushes (Roberts, 1992).

In Pakistan, Jerdon's babbler straddles much of Punjab along the Indus, Jhelum, Chenab, Ravi and Sutlej rivers, and extending down the Indus into southern Sindh. In southeast Sindh it extends up to Sanghar and Tharparkar. In north and central Sindh, it is found in district Sukkur, Larkana, Khairpur, Shikarpur, Nawabshah and Dadu (Roberts, 1992). Most recent record comes from the site along side of Rohri canal (Showler and Davidson, 1999). There are also the records of its presence in southwest Punjab north to southern Kayber Pakhtunkhwa (Roberts, 1992). It has also been recorded from the Indus in central west Punjab (Showler and Davidson, 1999). There were the record of its population from the east (Ali and Ripley, 1987) but few recently published records also support this (Collar *et al.,* 2001), for which detailed taxonomic studies are required if same subspecies exists in the Indian territory? With the unpredictable change in global climatic pattern and loss of habitat structure, the extinction rate has risen considerably and is the major threat to RRS. Such species need instantaneous attention and suitable measures for conservation (Manuel, 2006).

In case of an endangered bird species, it is extremely important to spot the areas, which are valued for conservation. It is an established fact that different populations prefer to adapt distinctive environments mainly due to difference in their genetic makeup along with some specific ecological needs and RRS are ranked as the most responsive. In order to gauge population genetic structure of different populations, recent molecular approaches have helped the most (Haig *et al.,* 2006). The distribution of SSR genomics is non-random. An immense emphasis can be sited on biologically based differences among species populations by using microsatellites (Chun *et al.,* 2002). Therefore, in present study microsatellite markers were used to detect the polymorphism, based on comparison of spatially placed populations to identify the genetic diversity and to ascertain an inbreeding effect in order to prioritize the importance of the species' conservation future.

## Materials and methods

*Sampling*

The male birds exhibit territorial songs during early spring and post-monsoon and are easy to spot. Nevertheless, keeping the current conservational status in view, as being "vulnerable endemic", the sampling was restricted to only 22 samples, procured from three distantly located sites over the stretch of River Indus during February 2011 to March 2012. The birds were mist-netted from study area and a few rectrices (tail feathers) were plucked in order to obtain the genetic material without harming the birds. The sampling sites included Chashma barrage 32°50'N, 71°20'E, Panjnad barrage (29°20'47"N 71°1'14"E) and Guddu barrage 28°26'N, 69°44'E.

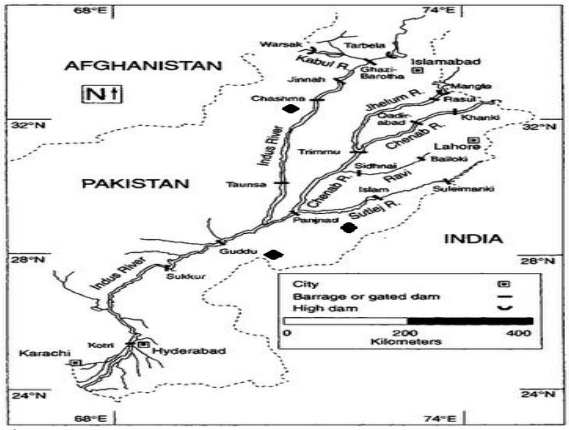


**Fig. 1.** Map of River Indus showing Chashma, Panjnad and Guddu as three sampling sites.

*DNA extraction and PCR*

A total of 22 samples from three geographic populations of Jerdon's babbler were collected (Fig. 1). The tail feathers were preserved in 95% ethanol.

Later they were stored at -50°C. Total genomic DNA from individual feathers was extracted following Bello *et al.,* (2001) from a fragment (0.5-1 cm long) derived from the base of quill. Some 500 µl of lysis buffer (50 mM Tris-HCl at pH 8, 20 mM EDTA at pH 8, 2% SDS) was added, followed by 10µl proteinase K (final concentration, 175 µg/ml). Each sample was incubated at 55°C overnight. Finally, phenol: chloroform protocol for DNA extraction was employed (Sambrook *et al.,* 1989). Total genomic DNA concentration was measured by nano-drop spectrophotometer (D11971, Barnstead, USA) at 260nm wavelength. Quality of DNA was checked by running 5µl of extracted DNA on 0.8% agarose gel prepared in 0.5X TBE buffer.

A sum of 25 microsatellite primers (Family: Passeridae) were used to study polymorphism and determination of heterozygosity (Table 1). The PCR reaction were performed with the following components: 1X buffer, 200 µM dNTPs, 1.5-4.5 mM $MgCl_2$, 0.25 µM primer, 1 units *Taq* polymerase and 100-200ng DNA. De-ionized water was added to the final volume of 15 µl. PCR reaction persisted in Gradient Master Cycler (*BioRad*). Cyclic protocol consisted of 5 minute denaturation at 95°C followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 52-63 °C depending on type of primer for 30 sec, extension at 72 °C for 45 sec and final extension at 72 °C for 5 min. Amplified products were electrophoresed on 8% polyacrylamide gel and stained by using silver nitrate. The gel was visualized under Gel Documentation System (*BioRad*). The observed number of alleles (No), effective number of alleles (Ne), observed heterozygosity (Ho) and expected heterozygosity (He) were estimated using GenAIEx software (Peakall and Smouse, 2006). Nei's genetic distances were calculated using DISPAN program and an UPGMA phylogenetic tree was constructed (Fig. 2).

**Results and discussion**

A total of 525 alleles were amplified by using 25 Passeridae specific microsatellite primers, applied upon 22 feather samples of Jerdon's babbler. Out of 25 primers, ten were monomorphic with unexpected size while fifteen produced polymorphic results (Table 1). The most plausible explanation for such unexpected size that the primer extends across a splice site or the introns in the corresponding sequence also responsible for this fragment or monomorphic bands (Varshney *et al.,* 2005). The number of polymorphic alleles ranged from 2-11. The observed and expected heterozygosities ranged from 0.212 - 0.354 and from 0.715 to 0.528 respectively, with the allele number two to eleven (Table 1).

A significant linkage disequilibrium was observed for primer (Sr. No. 1, 3, 4, 5, 8, 12 and 16). Two primers (Sr. No. 7 and 15) showed deviation from linkage disequilibrium. The linkage disequilibrium observed at different loci may due Wahlund's effect, caused by sub-structure of population (Wahlund, 1928). The same was not studied in the present study. Out of fifteen primers, the values for expected and observed heterozygosities showed that nine primers showed maximum heterozygosity. Resultantly, it can be assumed that microsatellite markers are independent and can be used for population genetic studies.

In phylogenetic analysis, two major groups were observed, representing the southern and northern populations. One sample from southern population was present in a separate cluster (Fig. 2). The first group contained most of the bird population sampled from Panjnad and Guddu barrages in south. Seemingly, two samples from northern population of Chashma, were forming cluster each with Guddu and Panjnad gene-pool. The second group contained most of the population sampled from Chashma barrage in north. Similarly, two samples from Guddu were forming a cluster with northern population, hence clearly depicting an exchange in genetic material from northern to southern population and vice versa. This further strengthens the population dispersal and existence of a partial local migratory connectivity among distantly placed populations over the stretch of River Indus.

The dissimilarity index among all the populations

indicated that they are so magnificently structured and do not exchange much genetic material, which could be observed in case of southern populations as they were not much diversified. The genetic distances among three groups showed that high level of dissimilarity was recorded between southern and northern populations (Fig. 2). However, partial mixing of the two populations as discussed above, at pars with the view of geographical distances have no great importance, in case of sedentary species (Bates, 2000, Francisco *et al.,* 2007).

The dendrogram clearly indicates of an overlap among northern and southern populations. The polymorphism might be the result of out-breeding and random genetic drift (Fig. 3). High genetic diversity and elevated level of polymorphism in populations may be considered as a good indicator of prosperous genetic resources of a species, as observed in *Francolinus* (Khaliq *et al.,* 2010, Forcina *et al.*, 2012) and *Prinia burnesii*, a sister endemic and parapatric species (Muhammad *et al.,* 2010).

**Table 1.** Microsatellite primers used and population analysis of Jerdon's babler.

| Sr. No. | Accession No. | Sequence 5'-3' | Size range (bp) | $T_A$ | N/ NA | $H_E$ | $H_O$ |
|---|---|---|---|---|---|---|---|
| 1 | AM158991 | CAGGCTGGTATTGTCTGGTTG<br>GATCAGACCCAGAGAGAGAGAGAG | 271-273 | 55 | 21/5 | 0.455 | 0.700 |
| 2 | AM158992 | GAGCTCACCAGTCTGCCCTTG<br>GATCACGTGGTCTGGGAGTCAG | 186-209 | 60 | M | - | - |
| 3 | AM158995 | GTGTATATGCAAATGACAAGACCAAAGC<br>TCACGCTGACCTAGATGCTATCAGAG | 282-297 | 60 | 21/6 | 0.608 | 0.000 |
| 4 | AM158996 | TGCCGTGAGGGATGTAAGTGA<br>CAGGCGACTTCAGCTTCTGC | 194-250 | 60 | 21/8 | 0.375 | 0.000 |
| 5 | AM158997 | ACAGAGCCCAGGAACCATCG<br>GGGCCATTTCATTCACATAGAAGG | 256-304 | 60 | 21/5 | 0.430 | 0.625 |
| 6 | AM158998 | TCAGAGAGGGCAGAAGGGATTTC<br>GCACCGCAGGAGAGCACTTT | 174-188 | 60 | M | - | - |
| 7 | AM158999 | GAGCACCCTCACACCCTCCTT<br>CTCACTTCATTCCTAAACAGCTTCTGG | 227-234 | 55 | 21/7 | 0.657 | 0.588 |
| 8 | AM159000 | TATGTCTGATAAACCAATCCCTGCAC<br>GATCTGTGGTAAATATGGTAATGGAGAGG | 340-560 | 60 | 21/5 | 0.095 | 0.000 |
| 9 | AM159001 | CATGGGCACAAGAAATGTGA<br>TCAAGAAGAAAATGGGTAATATACTGG | 100-130 | 60 | M | - | - |
| 10 | AM159002 | GATCCACAGAGCACTGACACTTTC<br>AATGCCTCTGACTGAATTAAGTTGC | 196-212 | 55 | M | - | - |
| 11 | AM159005 | TCAGTGGCTGCTCTCACAAG<br>GATCAAGGACAGGGCTTGG | 219-221 | 55 | M | - | - |
| 12 | AM159006 | TGGCAAGGAAGGAGGAATCG<br>AGCAATATAAGGCCCAGGTGCTC | 230-256 | 60 | 21/8 | 0.529 | 0.715 |
| 13 | AM159007 | GATCTTGTGTGTGTGTGTGC<br>AGGTTTGTTCTTGCAGCTC | 199-267 | 50 | 21/6 | 0.375 | 0.500 |
| 14 | AM159009 | CCTGCATGCAAGATTTAACACA<br>ACGGTCCATGTTGCCTAGC | 184-206 | 60 | 21/7 | 0.248 | 0.278 |
| 15 | AM159011 | CTCATTTGAACCACAGCCTGGA<br>TCTGGTTGAGAATCACAGCATGG | 216-241 | 60 | 21/8 | 0.622 | 0.582 |
| 16 | AM159012 | CCAGGGCTGGTCAGTGAAGG<br>GTGAAGTTATGGCTGTGCCTTGC | 225-267 | 60 | 21/10 | 0.900 | 0.000 |
| 17 | AM159013 | GATCAGATGCTTTGAATATATGAC<br>CTCCACAATGGACACAAAG | 198-202 | 50 | M | - | - |
| 18 | AM159014 | TGGATTCCATCTTCTGAACACACC<br>AATGCAAACGGGACCTCAGC | 258-267 | 60 | 21/2 | 0.212 | 0.354 |
| 19 | AM159015 | GCATTCAAAAATGGCAAGAGGA<br>GAGGCTACCCCTTTCCTGAACA | 183-221 | 60 | M | - | - |
| 20 | AM159016 | GGAGAATCACTGGAATCCACAGAGTAC<br>CAAATAGGACCCATATGCCAGTGAC | 178-230 | 60 | M | - | - |
| 21 | AM159020 | CCACACTCCACTGGGAACAT<br>GTGGAGCAGGGCAGAGATTA | 300-336 | 50 | 21/14 | 0.715 | 0.528 |
| 22 | AM159021 | ACAGCTGTCTAAAACACACACAC<br>CTTCAGATGACTGGGGATTC | 191-227 | 60 | M | - | - |
| 23 | AM159022 | TGCACAGACTCCTGTACCTGTCA<br>GCAAACACTCGACAGTGTGACC | 202-206 | 64 | M | - | - |
| 24 | AM159024 | CACCTGGACATATGAACCCCAAA<br>GGGGAGGGACAGGTGTCTGA | 215-258 | 60 | 21/15 | 0.812 | 0.410 |
| 25 | AM159029 | CTGCATGCCTTACACAGTGCATC<br>CTTTGCATCCCACAGCACACTC | 210-318 | 60 | 21/18 | 0.824 | 0.594 |

$T_A$= Annealing temp., N= No.of individuals scored, $N_A$= Number of allels, $H_E$= expected heterozygosity, $H_O$= observed heterozygosity; M= Monomorphic.

Genetic diversity plays a vital role in the survival and compliance of a species. Slight changes in ambient environment of a species are responsible for minor variations in genes and such changes are obligatory for survival. The loss of genetic variability may be a result of habitat disintegration, inbreeding, genetic drift, bottle-neck effect and stochastic effects (Nelson and Soule, 1987). The levels of genetic diversity indicated that this species in Pakistan harbors a strong gene pool and thus may withstand the imminent environmental change.

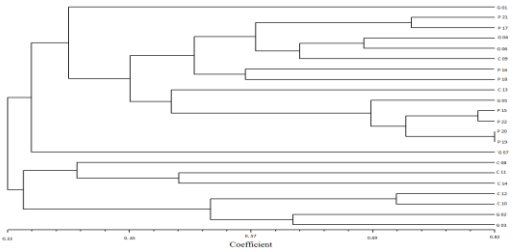


**Fig. 2.** Dendrogram revealing the genetic similarities among 22 *Chrysomma altirostre* genotypes based on amplification with 15 microsatellite primers. (C, Chashma; G, Guddu; P, Punjnad).

Recent reviews reported that UPGMA method works efficiently for high evolutionary rates and such readings were mostly expected with microsatellites (Huelsenbeck and Kirkpatrick, 1996). Among the closely related taxa, genetic relationships are of prime importance and are more appropriate for microsatellites. Hybridization tends to hold back the revival of true phylogenetic relationships in case of introgression occurs repeatedly, but it does not exclusive for microsatellites (Avise, 2004).

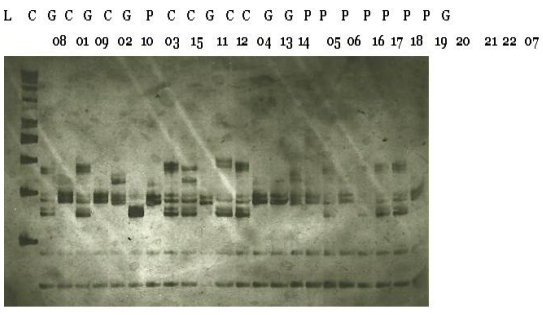


**Fig. 3.** Representative PAGE (Polyacrylamide gel electrophoresis) showing the silver stained banding pattern amplified by microsatellite primer AM159005 (L=ladder, C=chashma, G=guddu, P=panjnad, numbering 1-22 representing dendrogram populations).

It is suggested that the outcome of this research could help in future conservation management plans in case of reintroduction of the species, if needed. While deliberating upon the threats faced by such RRS, it is pertinent to cater the habitat requirements of Jerdon's babbler. Evidently, the widespread vegetation around barrages acts as important asylum for wetland avian fauna and proved to be critically important for their survival. The persistence of habitat loss is significant threat for Jerdon's babbler in Pakistan and elsewhere (Showler and Davidson, 1999). In such types of diversified habitats with more genetically differentiated vulnerable avian fauna are of great importance for the ecologists and conservationists and can be used as an ideal ecological unit.

## Conclusion

Jerdon's babbler (*Chrysomma altirostre*) is a restricted range species RRS and lives a sedentary mode of life at Indus plains. The RRS are the taxa at higher risk of extinction in the wake of global rise in temperatures and its drastic effects on sedentary habitats. We foremost account some novel microsatellites from *Passeridae* for Jerdon's babbler. The study indicates an out-breeding effect where dispersal and local migration among spatial populations is evident. Therefore, we assume vigorous population structure and sustainable future for conservation of the species in Pakistan.

*Contribution of Authors*

AAK conceived the overall research hypothesis and supervised field and ecological work. TR contributed upon the microsatellites lab work. TH, MA and MR also shared the microsatellite data processing and further analysis. All authors critically checked the manuscript for important intellectual contents and approved the final version.

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**References**

**Ali S, Ripley SD.** 1987. Compact handbook of the birds of India and Pakistan. 2nd ed. OU Press, Delhi, India.

**Avise JC.** 2004. Molecular markers, natural history and evolution. 2nd ed. Sinaeur Associates Inc. Sunderland, MA.

**Bates JM.** 2002. Allozymic genetic structure and natural habitat fragmentation: data for five species of Amazonian forest birds. Condor **102,** 770-783. http://dx.doi.org/10.1034/jc.1600048X.2002.330310.x

**Bird Life International.** 2003. Threatened birds of Asia. BirdLife International, Cambridge, UK.

**Collar NJ, Andreev AV, Chan S, Crosby MJ, Subramanya S, Tobias JS.** 2001. Chrysomma altirostre In: Threatened Birds of Asia: The BirdLife International Red Data Book: 2112-2119. BirdLife International.

**Forcina G, Panayides P, Guerrini M, Nardi F, Gupta BK, Mori E, Al-Sheikhly OF, Mansoori J, Khaliq I, Rank DN, Parasharya BM, Khan AA, Hadjigerou A, Barbanera F.** 2012. Molecular evolution of the Asian francolins (*Francolinus*, Galliformes): a modern reappraisal of a classic study in speciation. Molecular Phylogeny and Evolution **65,** 523–534. http://dx.doi.org/10.1016/j.ympev.2012.07.006.

**Francisco A, Pedro B, François F, Francesca R.** 2007. Recent Advances in Constraints, 11th Annual ERCIM International Workshop on Constraint Solving and Constraint Logic Programming, CSCLP 2006, Revised Selected and Invited Papers, p. 183, Springer LNAI 4651.

**Haig SM, Beever EA, Chambers SM, Draheim HM, Dugger BD, Dunham S, Smith E, Fontaine JB, Kesler DC, Knaus BJ, Lopes IF, Loschl P, Mullins TD, Sheffield LM.** 2006. Taxonomic considerations in listing subspecies under the U.S. Endangered Species. Conservation Biology **6,** 1584-1594. http://dx.doi.org/10.1111/j.1523-1739.2006.00530.x

**Huelsenbeck JP, Kirkpatrick M.** 1996. Do phylogenetic methods produce trees with biased shapes? Evolution **50,** 1418-1424. http://dx.doi.org/10.2307,1996.2410879

**Khaliq I, Babar M, Riaz M, Khan AA.** 2010. Genetic diversity in see-see partridge (*Ammoperdix griseogularis*, Galliformes) populations from sub-Himalayan Mountain ranges of Pakistan. Belgian Journal of Zoology **2,** 229-234. http://dx.doi.org/10.2010.66136117

**Chun Li-You, A Korol, T Fahima, A Beiles and E Nevo.** 2002. Microsatellites: genomic distribution, putative functions and mutual mechanism: a review. Molecular Ecology **11,** 2453-2465. www.ncbi.nlm.nih.gov/pubmed/12453231

**Manuel G.** 2006. Migratory Species and Climate Change: Impacts of a Changing Environment on Wild Animals UNEP/CMS Secretariat, Bonn, Germany.

**Muhammad S, Khan AA, Babar M, Riaz M, Akhtar N, Khaliq I.** 2010. Population genetic structure of Rofous vented prinia *Prinia burnesii* in Pakistan. African Journal of Biotechnology **9,** 9077-9081. http://dx.doi.org/10.5897/AJB10.1392

**Nelson K, Soule M.** 1987. Genetical conservation of exploited fishes. Pages 345-368 in Ryman, N. and F. Utter. Editors, Population genetics and fishery management. University of Washigton press, USA.

**Peakall R, Smouse PE.** 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes

**6,** 288-295. http://dx.doi.org/10.1111/j.1471-8286.2006.01155.x

**Roberts TJ.** 1992. The birds of Pakistan. Oxford University Press, Karachi, Pakistan.

**Sambrook J, Fritsch EF, Maniatis T.** 1989. Molecular cloning: a laboratory manual. CSHL Press, Cold Spring Harbor, New York. Journal of Veterinary Diagnostics Investigations **13,** 162-164. http://trove.nla.govau/work/13615226

**Showler DA, Davidson P.** 1999. Observations of Jerdon's Babbler *Chrysomma altirostre* and Rufous-vented Prinia *Prinia burnesii* in Punjab and North-West Frontier Provinces, Pakistan. Forktail **15,** 66-76. http://dx.doi.org/10.3126/on.v7i1.2554

**Varshney RK, Graner A, Sorrells ME.** 2005. Genic microsatellite markers in plants: features and applications. Trends in Biotechnology **23,** 48–55. http://dx.doi.org/10.1016/j.tibtech.2005.11.005

**Wahlund S.** 1928. Zusammensetzung von Population und Korrelationserscheinung vom Standpunkt der Vererbungslehre aus betrachtet. Hereditas **11,** 65–106. http://dx.doi.org/10.1111/j.16015223.1928.tb02483.x