Octa Journal of Biosciences
International peer-reviewed journal
Octa. J. Biosci. Vol. 3(1):13-17

ISSN 2321 – 3663
Jun. 2015

Octa Journal of Biosciences

journal homepage: www.sciencebeingjournal.com

Population Genetic Structure of Golden Jackal, *Canis aureus* in Gujarat, India

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ARTICLE INFO

Received 29 May 2015
Revised 5 June 2015
Accepted 15 June 2015
Available online 30 June 2015

Keywords: Genetic Diversity, Genotyping, Golden Jackal, Microsatellite Markers

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ABSTRACT

Genetic diversity of Golden Jackal, *Canis aureus* was estimated to understand the role of Rann of Kachchh in their movement between Kachchh region and the mainland of Gujarat, a western state in India. A total of 30 samples were collected and genotyped with 10 polymorphic microsatellite loci. The analysis was done within and between the Golden Jackal populations in Bhal and Kachchh region of the state. Altogether, 78 distinct alleles were found with mean allelic number of 8.8 (±2.33). Out of 10 microsatellite loci used, 9 loci showed PIC value higher than 0.5 and considered informative for population genetic studies. Mean observed heterozygosity (Ho) was found to be 0.812 (±0.233) while mean expected heterozygosity (He) was 0.815 (±0.083). No evidence of linkage disequilibrium was observed among pair of loci. Mean Fis value approaching zero (0.018±0.235) was found for this population. Pairwise Fst-Rst values of 0.0182-0.026 indicate little genetic differentiation between Golden Jackal populations. Further, the structure showed only one cluster of Golden Jackal population. The study revealed that Rann of Kachchh is not a barrier for the movement of Golden Jackal and the population across the region of Kachchh and the mainland of Gujarat is continuous.

INTRODUCTION

The Golden Jackal (*Canis aureus*, Linneaus 1758) is a medium-sized canid with a wide range of distribution. Their presence have been found in northern and eastern Africa, south eastern Europe and the Middle East which then extends eastwards into central, southern and south east Asia and up to Thailand. In India they occur in all types of habitat ranging from sea level to low altitudes in the Himalaya (Jhala and Moehlman, 2008). The species is fairly common throughout its range with high densities observed in areas with abundant food and cover. A minimum population estimate of over 80,000 is estimated for the Indian sub-continent (Jhala and Moehlman, 2008). Having included in CITES Appendix II and featured on Schedule III of Wildlife Protection Act 1972, Golden Jackals are still afforded the least legal protection.

In pastoral areas of the country such as Gujarat, Maharashtra, Rajasthan and Haryana, jackal populations achieve high densities. Gujarat is the extreme west state of India, and geographically divided into three regions: Saurashtra Peninsula, a rocky region interspersed with low lying mountains; Kachchh (also known as Kutch), a semi-arid region located in the north east; and mainland, which is a fertile plain. A large part of north and east Kachchh is known as Rann of Kachchh, which is a shallow wetland. This region has a rare and unique type of ecosystem because sea water inundates this for most of the year imparting a high residual salinity level. In the present work we have undertaken a detailed population genetic study of the Golden Jackal to understand whether the hostile Rann of Kachchh has been a barrier for the movement of Golden Jackal between Kachchh and the mainland of Gujarat state.

MATERIALS AND METHODS

Sampling and DNA Extraction

Tissue samples of 30 Jackals from Kachchh and Bhal regions of Gujarat were collected (Fig 1). Blood samples from 10 Jackals captured for a rediotelemetry study in the Bhal were used while the remaining samples were obtained opportunistically from road kills. Genomic DNA was isolated from these samples using traditional Phenol-Chloroform extraction method (Sambrook et al., 2001) and QIA-quick DNeasy Blood/Tissue kit (Qiagen, Germany) as per manufacturer's protocol.

Microsatellite loci and PCR Amplifications

A battery of 10 microsatellite markers (Table 1) selected from the International Society for Animal Genetics Domestic Dog (Canis familiaris) panel, were utilized. Each forward primer was tagged on the 5'end with fluorescent dye FAM. Multiplexing was done with the caution of size range. Each PCR reaction was carried out using 50-80ng of genomic DNA in a 25µl volume including 0.2 units of Ampli Taq Gold (Applied Biosystems), 25mM MgCl2, 10x reaction buffer, 1x BSA, 2µm each dNTP, and 10µm each primer. Amplifications were performed in a PTC-200 (MJ Research) and Gene Amp PCR system 2700 (Applied Biosystems). The amplification conditions used were: 10 min initial denaturation at 94°C, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1.30 min and extension at 72°C for 1.30 min with a final extension at 72°C for 7 min. 5 µl of PCR products were mixed with 1 µl of loading buffer and then loaded onto a 2% agarose gel, electrophoresed and visualized over UV light after ethidium bromide staining to detect amplification. PCR products were denatured at 95°C for 5 min before genotyping.

Microsatellite genotyping was carried out using ABI 3130 Genetic Analyzer (Applied Biosystems) with Gene Scan-500 LIZ as the internal lane size standard. Data were collected and analyzed using Gene Mapper software (version 3.7, Applied Biosystems).

Genetic Diversity Analysis

Genetic diversity analysis was done within and between the populations. The estimates of genetic diversity i.e., observed number of alleles (N), effective number of alleles (Ne), observed (Ho) and expected (He) heterozygosities were carried out using GeneAlEx 6.0 (Peakall and Smouse, 2006). Using allelic frequencies, polymorphic information content (PIC), a measure of marker's informativeness was also calculated with Cervus version 3.0.3(Marshall et al., 1998). Deviation from Hardy-Weinberg equilibrium was tested using probability test of Genepop version 4.1 (Raymond and Rousset, 1995b; Rousset, 2008). Heterozygote deficiencies were estimated as Fis = (Ho-He)/He, where Ho and He are the observed and expected frequencies of heterozygotes, respectively. Linkage disequilibrium (LD) test between loci was performed in Genepop (version 4.1). To analyze differentiation within and between population structures, Fst values (Weir and Cockerham, 1984) and pairwise Fst and Rst (estimates of genetic differentiation) were also calculated using Genepop (version 4.1).

Genetic Structure

The Bayesian clustering procedure implemented in computer program STRUCTURE version 2.3.3 (Pritchard et al., 2000, Falush et al., 2003, 2007) was used to simultaneously infer the number of distinct genetic clusters within golden jackal population and likewise probabilistically assign each analyzed individual to one of the inferred clusters. Admixture model was assumed and analysis was performed considering correlated allele frequency models. STRUCTURE analyses were performed using values of K (the assumed number of clusters) ranging from 1 to 2, an initial burn-in of 4×104 steps, followed by 1.0×105 Markov-Chain Monte Carlo (MCMC) analysis sweeps. Number of iteration was set to 10.

RESULTS AND DISCUSSION

All Golden Jackal samples were successfully amplified and 78 distinct alleles were distinguished over the 10 microsatellite loci used (Table 2). Linkage disequilibrium was not detected between the

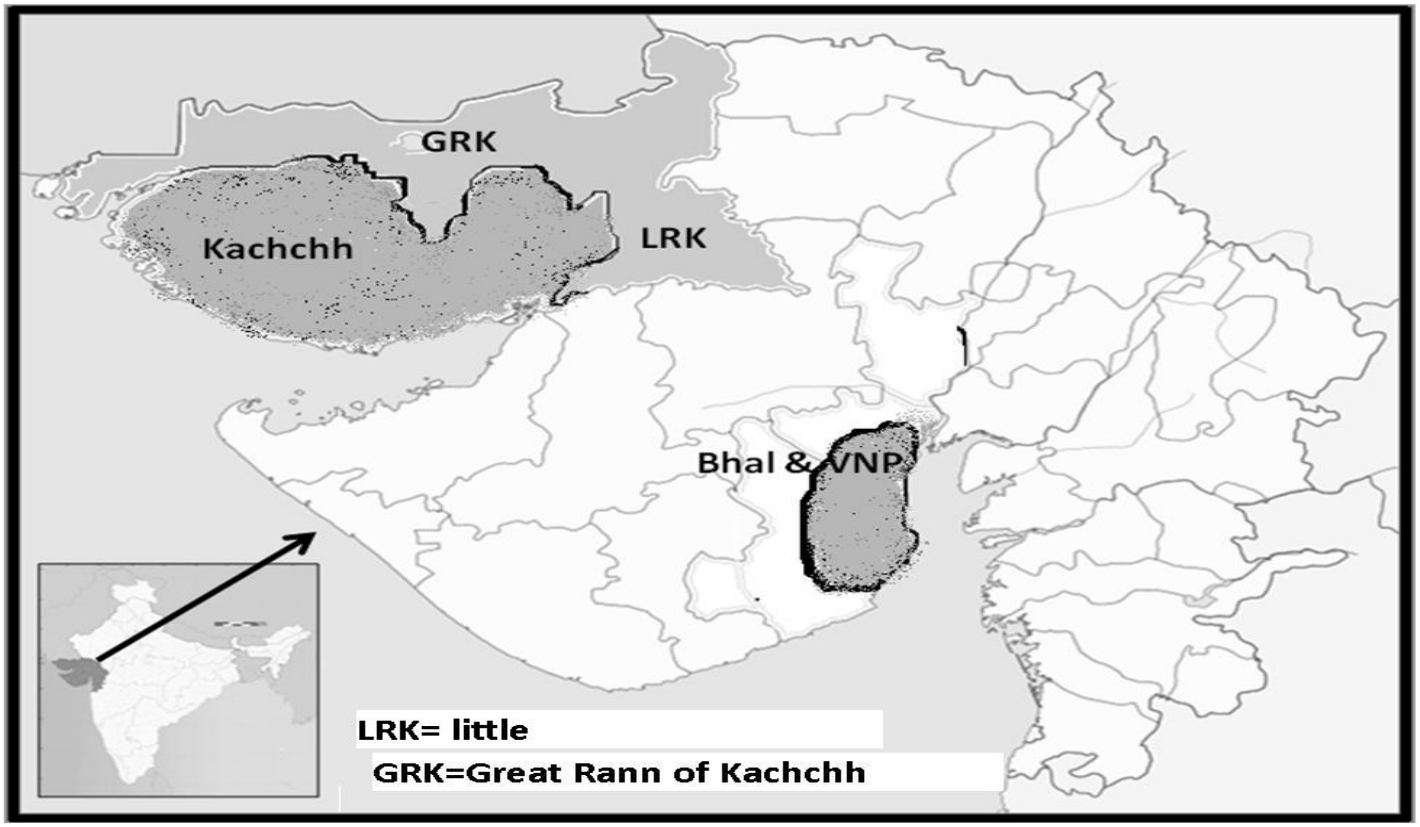

Fig 1. Map showing Golden Jackal sampling location in Western India (Gujarat).

Table 1. Description of Microsatellite loci used

Locus	Fluorescent Label	5'-3' Sequence		Size range
		Forward	Reverse	
INRA21	FAM	ATGTAGTTGAGATTTCTCCTACGG	TAATGGCTGATTTATTTGGTGG	87-111
AHTk253	FAM	ACATTTGTGGGCATTGGGGCTG	TGCACATGGAGGACAAGCACGC	277-297
FH2328	FAM	ACCAGGTAGTTTTCAGAAATGC	AGTTATGGGACTTGAGGCTG	171-213
REN54P11	FAM	GGGGGAATTAACAAAGCCTGAG	TGCAAATTCTGAGCCCCACTG	224-242
REN105L03	FAM	GGAATCAAAAGCTGGCTCTCT	GAGATTGCTGCCCTTTTTACC	231-249
INU030	FAM	GGCTCCATGCTCAAGTCTGT	CATTGAAAGGGAATGCTGGT	143-157
INU055	FAM	CCAGGCGTCCCTATCCATCT	GCACCACTTTGGGCTCCTTC	204-220
LEI004	FAM	CATCATGCATCAAGCAGAGC	TCATGTAAGCAGAGACTGAC	86-112
FH2412	FAM	GCTGGGGATTTATTCTGACC	AAATTAACCAAATGTTTGCAACA	162-186
FH2079	FAM	CAGCCGAGCACATGGTTT	ATTGATTCTGATATGCCCAGC	266-286

investigated loci. Therefore, all the loci were retained for the further analysis. The number of observed alleles ranged from 5 for locus FH2412 to 13 for locus FH2328, with an overall mean of 8.8(±2.33). The effective number of alleles ranged from 2.49 for locus FH2412 to 8.93 for locus FH2328, with an overall mean of 6.15(±1.91). The observed number of alleles for all the 10 loci exceeded the effective number of alleles. All the microsatellite loci showed PIC values higher than 0.5 except FH2412 (PIC-0.370) which is normally considered as informative in population genetic analyses. The mean PIC in the present study was found to be 0.798. Observed heterozygosity (Ho) over 10 loci was ranged between 0.250 (locus FH2412) and 1.0 (loci INRA21, INU055, LEI004) with a mean of 0.812±0.233. Values for expected heterozygosity (He) ranged from 0.599 (locus FH2412) to 0.888 (locus FH2328) with a mean of 0.815±0.083. Fis values ranged between -0.308 for locus INU005 to 0.563 for locus FH2328. The mean Fis estimate was found to be 0.0184±0.238. Calculated Fst values ranged from 0.001 for locus INU005 to 0.046 for locus FH2412 with a mean of 0.021±0.0125 (Table 2).

Both Bhal and Kachchh populations showed almost same allelic variability with an average of 7.6 alleles per locus for Bhal while 7.8 for Kachchh (Table 3). The locus with the highest number of alleles in the Bhal population was FH2328 with 11 alleles while in Kachchh it was FH2328 and LEI004 with 10 alleles each. The value for observed heterozygosity (Ho) for each locus ranged from 0.417 to 1.0 with a mean of 0.799 (±0.059) for Bhal population and 0.58 to 1.0 with a mean of 0.768 (±0.085) for Kachchh region population. The expected heterozygosity (He) ranged 0.681 to 0.868 with mean 0.807 (±0.020) for Bhal population while 0.462 to 0.885 with a mean of 0.789 (±0.038) for Kachchh population of Golden Jackal. Mean Fis value for Bhal population was found to be 0.014 (±0.066) while it was 0.009 (±0.055) in Kachchh population. Pairwise Fst and Rst values for Bhal and Kachchh populations were found to be 0.0182 and 0.026, respectively (Table 4).

The result of STRUCTURE showed no strong pattern of population differentiation and only one population cluster was identified (Figure 2). Using a Bayesian MCMC approach and considering a range of one to two potential populations, the probability value (mean value of ln likelihood) obtained for two populations (K=2) was -879.7±31.3 while for one population (K=1) it was found little higher -878.8±30.9

Table 2. Genetic Diversity Statistics of Golden Jackal in Western India

Locus	Number of alleles (Na)	Effective Number of alleles (Ne)	Polymorphic Information content (PIC)	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Fis*	Fst**
INRA21	8.000	6.545	0.829	1.000	0.847	-0.180	0.018
AHTk253	10.000	7.024	0.842	0.875	0.858	-0.020	0.022
FH2328	13.000	8.930	0.925	0.833	0.888	0.062	0.015
REN54P11	7.000	5.908	0.808	0.875	0.831	-0.053	0.005
REN105L03	10.000	6.621	0.832	0.917	0.849	-0.080	0.027
INU030	10.000	6.545	0.831	0.708	0.847	0.164	0.027
INU055	7.000	4.251	0.73	1.000	0.765	-0.308	0.001
LEI004	11.000	8.662	0.874	1.000	0.885	-0.131	0.015
FH2412	5.000	2.494	0.37	0.250	0.599	0.583	0.046
FH2079	7.000	4.608	0.505	0.667	0.783	0.149	0.035
Mean	8.800	6.1587	0.7976	0.8125	0.8151	0.0184	0.021
SE***	2.3305	1.9125	0.1722	0.2231	0.0831	0.2389	0.0125

* Heterozygote deficiency; ** F statistics; *** Standard error

Table 3. Measures of Genetic Variability for each Locus analyzed for Golden Jackal in Bhal and Kachchh population

Locus	Population							
	Bhal				Kachchh			
	Na	Ho	He	Fis	Na	Ho	He	Fis
INRA21	7	1.0	0.799	-0.252	8	0.855	0.865	-0.157
AHTk253	9	0.833	0.868	0.040	9	0.917	0.809	0.023
FH2328	11	0.750	0.868	0.136	10	0.86	0.882	0.120
REN54P11	7	0.750	0.813	0.077	7	0.785	0.840	0.043
REN105L03	8	0.833	0.840	0.008	9	0.856	0.813	0.005
INU030	9	0.833	0.858	0.028	9	0.583	0.792	0.026
INU055	6	1.0	0.764	-0.309	6	1.0	0.764	-0.309
LEI004	9	1.0	0.858	-0.166	10	1.0	0.885	-0.129
FH2412	5	0.417	0.681	0.388	4	0.083	0.462	0.320
FH2079	5	0.583	0.726	0.196	6	0.750	0.785	0.154
Mean	7.6	0.799	0.807	0.014	7.8	0.768	0.789	0.0096
SE	0.618	0.059	0.020	0.066	0.628	0.085	0.038	0.055

Table 4. Pairwise Fst (below diagonal) and Rst (above diagonal) values among two sampling locations of Golden Jackal based on ten microsatellite loci

Pairwise Fst, Rst	Bhal	Kachchh
Bhal	-	0.026
Kachchh	0.0182	—

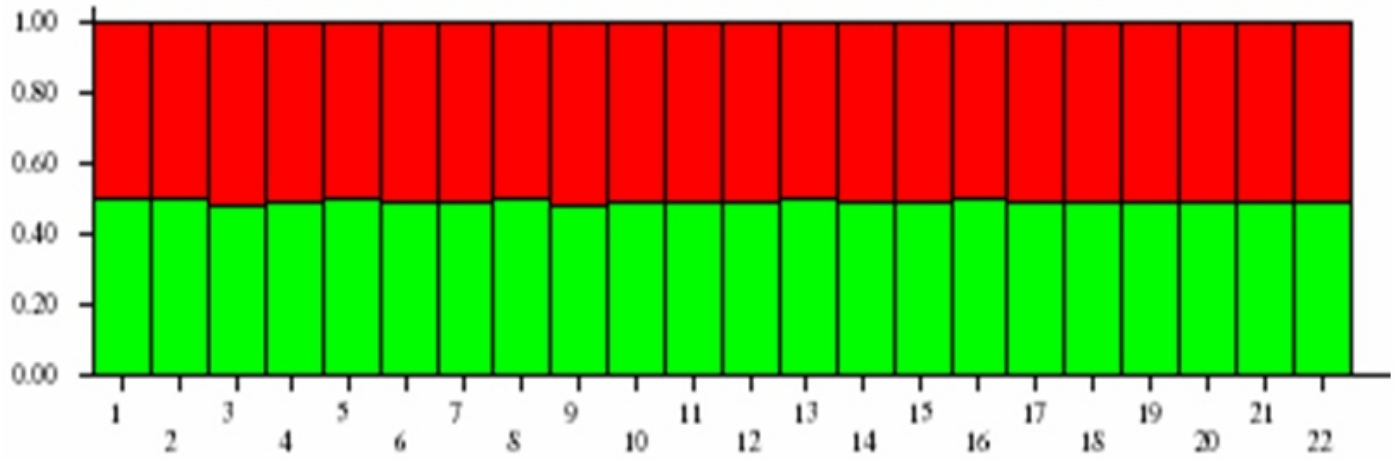

Fig. 2. STRUCTURE analysis of microsatellite data showing a single clustered population of golden jackal, Canis aureus in Gujarat, Western India.

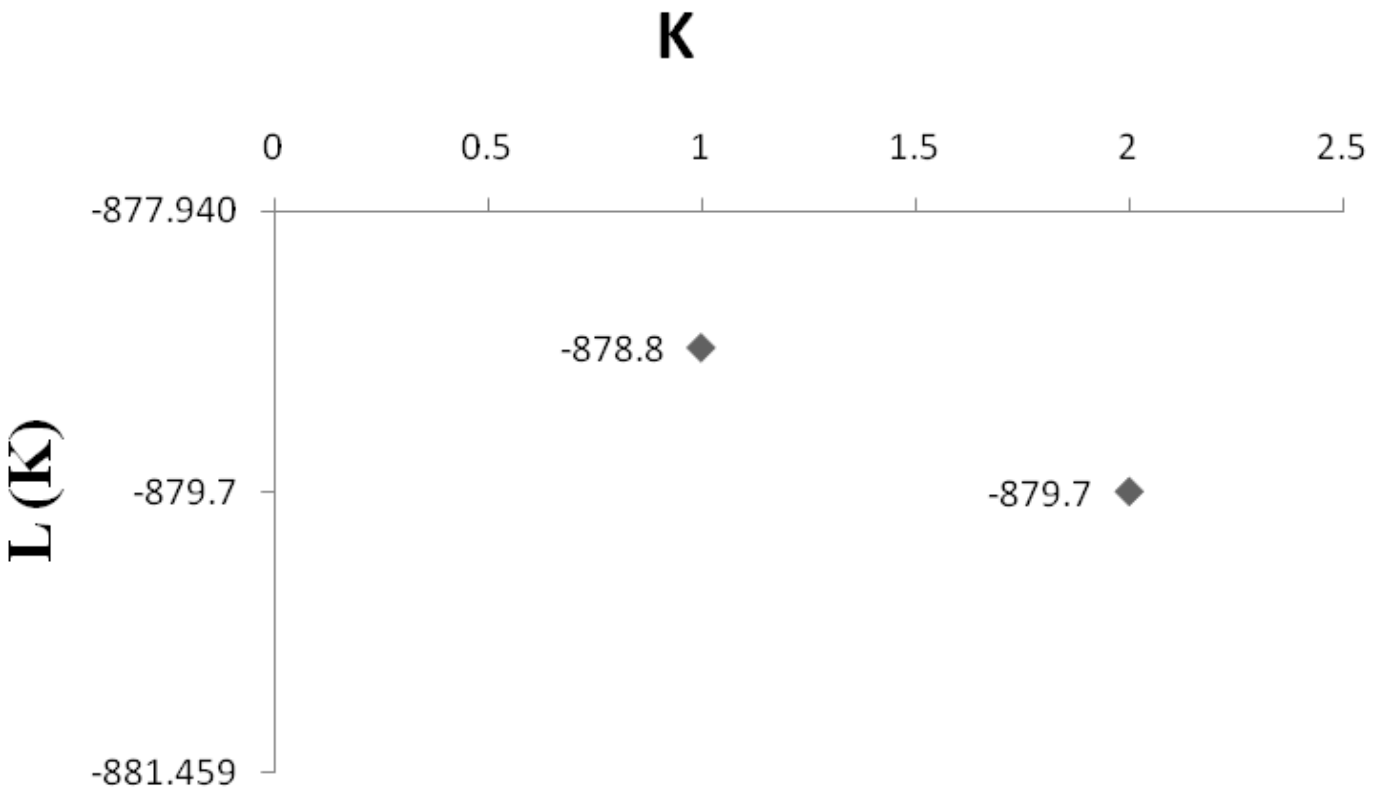

Fig. 3. Log probability of data [L(K)] as a function of K averaged over 10 independent runs for golden jackal, Canis aureus derived using a Bayesian clustering algorithm implemented in STRUCTURE

Golden Jackal in Gujarat has substantial genetic variation based on their gene diversities and average number of alleles per locus. To our knowledge few population genetic studies have been done on Golden Jackal worldwide. Roy et al., (1994) genotyped 18 Golden Jackals from Kenya at 10 microsatellite loci and found a moderate genetic diversity (Ho 0.41, He 0.52). Zachos et al., (2009) analyzed 121 individuals from Serbia using eight loci, finding a very low genetic diversity (Ho 0.29, He 0.34). On the other hand Cohen et al., (2013) analyzed 88 jackals from Israel using 14 STRs and found comparably high level of genetic diversity (Ho 0.67, He 0.67) and no evidence of genetic bottleneck. Recently, Fabbri et al., (2014) genotyped 120 jackals from north-western Europe and found moderate level of genetic diversity (Ho 0.47, He 0.51) which is close to Kenyan Golden Jackal. Thus, compared to their conspecifics, higher values for Indian jackal make them highly diverse with high genetic variability even with limited samples (30 jackals) within a single state of India.

Overall positive and relatively small mean Fis value (0.018) approaches zero, which in natural population is indicative of random mating. The Fis estimates also conclude very less or no inbreeding and an indication of no barrier to gene flow between the Kachchh and Bhal populations. Thus, our study suggests that the Jackal population of Kachchh and Bhal does not suffer from inbreeding issues and the effect of genetic isolation and subsequent differentiation due to genetic drift does not observed. Additionally the pairwise Fst-Rst values (0.018 and 0.026, respectively) in this study fall within the low range of differentiation of 0-0.05, suggesting no evidence of significant difference between the Bhal and Kachchh region population of Golden Jackal in Gujarat.

No genetic structure was detected for golden jackal in Western India as there was no evidence for unique genetic clusters within these two populations. A high rate of dispersal appears a likely explanation for the pattern of population structuring. The golden jackal is one of the most mobile terrestrial mammal that disperse rapidly over a large distance comparable to wolves that can disperses upto 900 km (Fritts, 1983; Mech and Boitani, 2003). Linear movement of golden jackal of >40 Km was recorded by Jhala (Pers. Comm.). Long-distance dispersal capabilities combined with the ability to occupy a variety of habitat imply high rates of gene flow that reduces genetic differentiation among local populations.

The geological data have shown that the landscape of Kachchh has evolved as a result of several phases of tectonic movement since the Late Jurassic (Biswas, 1987, 2005). Based on known data, there were two main areas of quaternary sedimentation in Kachchh.

Among them the first one was vast saline tract of the Little Rann of Kachchh and Great Rann of Kachchh which was a product of drying up of a pre-existing shallow sea and the Banni plain which forms a sort of transition between the Kachchh mainland and the Ranns. It has also been suggested that the Ranns were submerged under sea-water until ~2ka which correlates with the termination of lacustrine deposition in Nal and Narmada valleys resulting in drying up of Ranns providing a unique and rare ecosystem to Golden Jackal. Thus, taken together, the geologic and genomic results indicate that the Little Rann of Kachchh is not a barrier for the movement of Golden Jackal and the population across Kachchh and Saurashtra is a single and continuous population in the state of Gujarat.

CONCLUSION

This is the first genetic study on Golden Jackal and among the few genetic studies published so far on this species. Applying the molecular tolls even with limited sample size has made it possible to gain greater knowledge of the current status of Golden Jackal in Gujarat, a western part of India. However, more data from across India and eastern part of the Golden Jackal range is needed to gather better insights of genetic characteristics of this species. Higher heterozygosity values make Indian Jackal highly diverse and harbor high genetic variability than Kenyan, Serbian, Israelian and European Jackal. Moreover, though being separated by a hostile Rann habitat, very low differentiation between the Bhal and Kachchh population and presence of one genetic cluster is suggestive of no genetic structure and good gene flow between these populations. This finding implies that Golden Jackals are good dispersers and landscape and habitat feature like those of the Rann have a minimal effect on dispersal of jackal making it genetically homogeneous in western part of India.

ACKNOWLEDGMENT

We thank the Chief Wildlife Warden of Gujarat for giving permissions to capture and collaring Golden Jackals. Funding for the study was provided by the Wildlife Institute of India. We also thank Dr R.C. Fleischer and Dr J.E. Maldonado of Centre for Conservation and Evolutionary Genetics, Smithsonian Institution, USA who provided advanced training in molecular techniques to Tripti Negi during her short term stay at Smithsonian Institution and also helped in data analysis. Bibek Yumnam is thanked for assistance in data interpretation.

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