

Genetic relationship among duck populations of India

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ABSTRACT

Microsatellite data were generated on 24 microsatellite loci in 4 indigenous and 1 commercial ducks populations to study genetic relationship. All the markers were highly polymorphic in ducks. The genetic distances and relationship among 5 populations were estimated based on allele frequencies and number of repeat units. Nei's D_A and Chord distances were utilized for construction of phylogenetic tree using Neighbour Joining algorithm. All the genetic distances point to large differentiation between the coastal ducks of India and the land ducks of India. The homogeneity of the coastal ducks especially of Orissa and Tamil Nadu reveal free gene flow among the populations and very little to no structuring of the duck populations. The genetic differentiation between the land and coastal duck populations point to their being separate entities and may be treated differently for development of breeding plans for improvement.

Key words: Ducks, Genetic distances, Microsatellite, Phylogenetic tree

The total duck population of India is 10 million and it ranks second in the world after Indonesia. In India, the ducks are mainly concentrated along eastern and southern coastline. The ducks produce 5.3% of the total eggs produced in India. Of the total duck eggs, indigenous ducks contribute 91% while 9% are produced by improved varieties. Indigenous ducks produce about 130–140 eggs per annum, and they are a good dietary source of proteins. The characterization and conservation of ducks assume prime importance as they provide food security to the rural folk. These locally adapted ducks are important biodiversity resources as they are important genetic reservoirs and are essential for facing the future challenges of disease resistance and better quality of meat. There has been no systematic study of ducks in India however few reports relate to ducks of China (Maak *et al.* 2000, Stai and Hughes 2003, Genet *et al.* 2003, Xiao *et al.* 2004, Huang *et al.* 2005, Tang *et al.* 2007, Li *et al.* 2007 and Su *et al.* 2007).

In this study an attempt has been made to estimate genetic distances among the 4 indigenous duck populations and one commercial duck population using allele frequency and data on number of repeats of 24 microsatellite loci.

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MATERIALS AND METHODS

The blood samples (240) were collected from the breeding tract of 4 duck populations (West Bengal, Orissa, Tamil Nadu and Jharkhand). Khaki Campbell was taken as an out-group. The birds were selected at random ensuring that as far as possible these were unrelated and the samples represent the breeds/populations. 0.5 ml of whole blood was collected from wing vein in heparin coated vacutainer tubes and transported to laboratory at 0–5°C. DNA was extracted from whole blood using the standard protocol (Sambrook *et al.* 1989).

A total of 24 microsatellite loci were chosen for the study. These loci were Caud010, Caud011, Caud013, Caud016, Caud017, Caud019, Caud022, Caud023, Caud024, Caud025, Caud026, Caud027, Caud031, Caud032, Caud033, Caud035, APH01, APH03, APH07, APH09, APH10, Caud001, Caud004 and MCW328. The criterion for selection of the microsatellite loci was based on their polymorphism in ducks, PIC value and number of alleles. The 5' end of the forward primers was labeled with any of the FAM, VIC, NED or PET dyes. The 24 loci were located on 9 different linkage groups of duck chromosomes. The genotyping was carried out on ABI-3100 AVANT automated DNA sequencer with LIZ 500 as internal lane standard (size standard). The post PCR multiplexing was used to simultaneously genotype 3 or 4 loci depending upon the size and dye label of the PCR product. The sizing and allele calling was performed using Genotyper ver. 2.0 software. The allele data thus generated

was used for further statistical analysis. The allele frequencies for all the alleles at the 24 microsatellite loci were estimated using Popgene (Yeh *et al.* 1999). Genetic distances were estimated on the bases of allele frequencies obtained. D_s Nei's Standard genetic distance and Nei's minimum genetic distance (1972, 1973), Reynold *et al.* (1983), Cavalli Sorza and Edward genetic distance (1967), Nei's D_A (1983), shared allele distance (Chakraborty and Jain 1993), were calculated using allele frequency data. Goldstein (μ^2) and Sheiver's D_{SW} genetic distances were calculated using number of repeats of each allele at a locus. The bootstrapping over loci was carried out using 1000 replicates and trees were constructed using Neighbor Joining (NJ) algorithm (Saitou and Nei 1987). The consensus tree from 1000 genetic trees was formed using consensus of the Phylip package (Felsenstein 1985)

RESULTS AND DISCUSSION

The relationship among the population can be examined using the genetic distances. The genetic distances can be estimated based on the allele frequency data and number of repeats. Both the methods are in vogue for studying the evolutionary relationships of closely related populations. The separation of populations and subsequently the changes in the allele frequency are based on reproductive isolation, drift leading to fixation of alleles. Another reason for the change in allele frequency is attributable to inbreeding among the individuals of the same population. The most common genetic distances based on the allele frequency data on Nei's Standard genetic distances, D_s (Nei 1972), chord distance D_c (Cavalli Sforza and Edwards 1967), Nei's D_A (Nei 1983) and Reynolds distance (Reynold *et al.* 1983). These genetic distances are useful for finding out the genetic relationship among the populations for allozyme analysis and other classical markers where each mutation gives rise to a new allele, IAM (Kimura and Crow 1964). This model seems to apply approximately to classical genetic markers such as protein and blood group polymorphism (Nei 1987). In this present paper we have utilized the microsatellite data for finding out the relationship among the duck populations/breeds of India. These microsatellite loci are used for the

Table 1. Genetic Distances between duck populations based on allele frequencies

	West Bengal	Jharkhand	Khaki Campbell	Orissa	Tamil Nadu
West Bengal	0.00	0.51	0.78	0.22	0.36
Jharkhand	0.22	0.00	0.39	0.37	0.44
Khaki Campbell	0.30	0.21	0.00	0.51	0.53
Orissa	0.12	0.19	0.25	0.00	0.07
Tamil Nadu	0.19	0.22	0.26	0.05	0.00

Nei's D_s (Above diagonal) and Reynolds distance (Below diagonal)

Table 2. Genetic distances between duck populations based on mathematical and geometric parameters

	West Bengal	Jharkhand	Khaki Campbell	Orissa	Tamil Nadu
West Bengal	0	0.27	0.40	0.19	0.25
Jharkhand	0.42	0	0.25	0.22	0.27
Khaki Campbell	0.55	0.41	0	0.30	0.36
Orissa	0.36	0.37	0.45	0	0.08
Tamil Nadu	0.42	0.43	0.50	0.24	0

Nei's D_A (above diagonal) and chord distance D_c (below diagonal).

phylogenetic analysis (Bowcock *et al.* 1994, Roy *et al.* 1994, Deka *et al.* 1995) and these loci seem to follow a mutational pattern that roughly follows Ohta and Kimura (1973) step-wise mutational model (SMM).

The Nei's standard genetic distance (D_s) is basically a correlation among the allele frequencies between the populations. The Nei's standard genetic distance is given in Table 1 (above diagonal). The maximum distance value was between West-Bengal and Khaki Campbell ducks (0.78) while the minimum genetic distance was found between Tamil Nadu and Orissa ducks (0.07). The Reynolds's genetic distance which provides a measure of population divergence by drift only and provides co-ancestry coefficient (Reynold *et al.* 1983) have been shown in Table 1 (below diagonal). The least values were between Orissa and Tamil Nadu ducks (0.05) while the maximum was between West Bengal and Khaki Campbell ducks (0.30).

The values of Nei's D_A genetic distance are given in Table 2. The highest values were between West Bengal and Khaki Campbell ducks (0.40) while the value was least between Tamil Nadu and Orissa ducks (0.08). The Cavalli-Sforza Edward Chord distance is a distance based on geometric multidimensional hyper sphere and is independent of any mutational model (Nei 1987). The various genetic distance

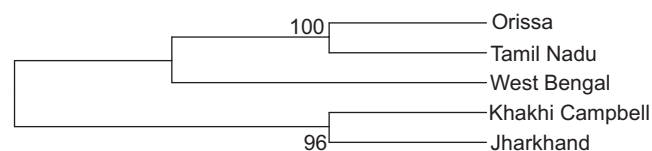


Fig. 1. Phylogenetic tree based on chord genetic distance

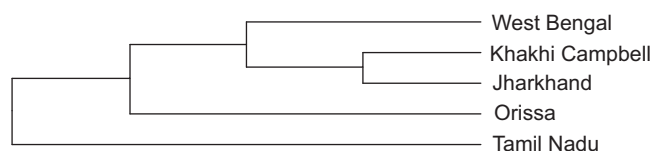


Fig. 2. Phylogenetic tree based on Nei's D_A genetic distance O Orissa ducks; WB, West Bengal ducks; KC, Khaki Campbell ducks; JD, Jharkhand ducks; and TN, Tamil Nadu ducks.

values are given in Table 2. The maximum distance was found to be 0.55 between WestBengal and Khaki Campbell ducks, while the lowest value of 0.24 was found between Orissa and Tamil Nadu ducks. The West Bengal and Orissa ducks were also very close (0.36).

The value of genetic distances based on step-wise mutational model are presented in Table 3 with Shriver *et al.* (1995) genetic distance above diagonal and Goldstein *et al.* (1995b) (d_{μ})² below the diagonal. The maximum values in Shriver's genetic distance were obtained between Tamil Nadu and Khaki Campbell ducks and the minimum between Jharkhand and WestBengal ducks. Similar lowest distance were obtained in Goldstein's measures. The highest genetic distance was obtained between Tamil Nadu and West Bengal ducks.

The Rogers genetic distance for the 5 duck populations were also calculated (Table 4). The allele sharing distances and Rogers genetic distance are given in Table 4. The Rogers genetic distance was maximum between Khaki Campbell and West Bengal ducks (0.46), while it was minimum between Orissa and Tamil Nadu ducks (0.16).

The efficiency of constructing the phylogenetic trees by means of genetic distances depends on the linear relationships with time (Nei *et al.* 1983, Tajima and Takezaki 1994). The computer simulation studies (Takezaki and Nei 1996) revealed that D_A and D_C are the most efficient in obtaining the correct tree topology in several conditions they examined. This has been revealed despite the fact that D_A and D_C are based on geometric distances of population represented on a multidimensional hyper sphere and are independent of the mutational models (Nei 1987). The results obtained in this study were very similar to those obtained by Nei *et al.* (1983) and suggest that different distance measures should be used for the reconstruction of a topology and estimating the Branch lengths. In general D_A and D_C are superior for constructing a topology (Vijh *et al.* 2005a, b). NJ method was used for the construction of the phylogenetic tree. The tree generated using chord genetic distances and Nei's D_A (Figs 1 and 2) were similar to one another. The interindividual NJ tree was

constructed using the allele sharing distances. The relationship among 5 duck populations based on Rogers genetic distance was also calculated and an NJ algorithm based radiation tree was constructed (Fig. 3).

The various genetic distances utilized in the present study revealed very similar pattern forming two distinctive groups. The Jharkhand ducks and the outgroup club together being land birds while the ducks of coastal areas of West Bengal, Orissa and Tamil Nadu join together. The relationship is evident in all the genetic distances and trees constructed using the genetic distances. More so the bootstrapping over loci confirms the relationship on this pattern, the bootstrap values being as high as 96 and 100%. The topology of the coastal area is almost very similar and the rearing of these ducks assumes commercial dimensions as we move south to Tamil Nadu. The inter-individual distances also reveal similar pattern. Jharkhand and Khaki Campbell ducks join each other. The West Bengal ducks form a separate cluster. Orissa and

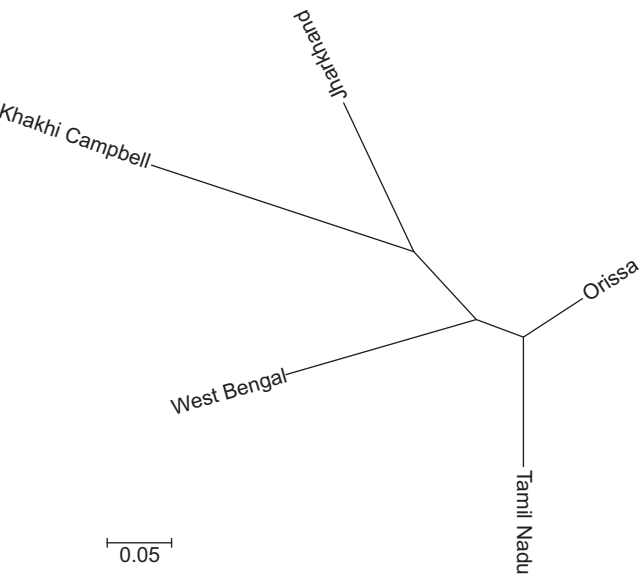


Fig 3. Relationship among duck populations based on Rogers genetic distance.

Table 3. Genetic distances between duck populations based on number of repeat units

	West Bengal	Jharkhand	Khaki Campbell	Orissa	Tamil Nadu
West Bengal	0.00	1.52	3.44	1.48	2.00
Jharkhand	38.27	0.00	1.91	1.59	2.23
Khaki Campbell	75.73	28.01	0.00	2.94	3.53
Orissa	84.52	48.40	56.29	0.00	0.40
Tamil Nadu	148.44	103.12	116.11	28.44	0.00

Shriver *et al.* genetic distance (1995) D_{SW} (above diagonal) and Goldstein *et al.* (1995) (d_{μ})² (below diagonal)

Table 4. Rogers genetic distances and allele sharing distance between populations

	West Bengal	Jharkhand	Khaki Campbell	Orissa	Tamil Nadu
WestBengal	0.00	0.45	0.61	0.35	0.43
Jharkhand	0.34	0.00	0.46	0.41	0.47
Khaki Campbell	0.46	0.34	0.00	0.52	0.55
Orissa	0.24	0.30	0.39	0.00	0.22
TamilNadu	0.30	0.34	0.40	0.16	0.00

Allele sharing distances (above diagonal), Rogers genetic distance (below diagonal).

Tamil Nadu ducks form 3 separate clusters and consist of birds from both the states. This shows the homogeneity of ducks of both the states. The similarity of these ducks is more to West Bengal ducks while these ducks are distinctive from the land ducks of Jharkhand and the out group.

The genetic distance studies based on 24 microsatellite loci reveal that ample diversity exists among the duck populations of the country. The clear cut differentiation between the land and coastal area ducks reflect the need to consider the 2 as distinctive populations and breeding plans need to be devised accordingly. The free gene flow among the coastal birds reveals homogeneity among the populations. The free gene flow also reflects lack of genetic structure among the coastal ducks and this can be attributed to negligible selection or breeding for specific purposes like meat and egg production.

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