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RESEARCH ARTICLE

Genetic diversity and relationship of Indian Muscovy duck populations

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ABSTRACT

Assessment of genetic diversity within and between populations is a prerequisite for sustainable utilization of domestic species. The domestic Muscovy duck (*Cairina moschata*) is an economically important species around the world for its unique meat taste and low-caloric content. It is one of the important domestic species in India as it ensures food security to the rural sectors. In this study, we have analyzed the genetic diversity and relationship of four Muscovy duck populations collected from different states (Assam, Mizoram, Odisha and Kerala) of India using mtDNA cytochrome b and nuclear DNA CYP2U1 genes. The results showed low genetic diversity among populations for both the genes. Kerala population showed significant genetic differences from the other three populations. The median joining network of cytochrome *b* gene suggested that the domestic Muscovy ducks present in India are the product of a single domestication event and probably introduced to India several years ago, as reported elsewhere. This study has also showed the suitability of nuclear DNA CYP2U1 gene in genetic diversity analysis.

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KEYWORDS
Muscovy; duck; cytochrome b; CYP2U1; domestication

Introduction

Muscovy is one of the most popular types of duck in India due to its unique meat quality and taste. They are reared mainly for their meat and found in good numbers at West Bengal, Odisha, Kerala, Assam and Mizoram. They are called by different names such as Moti, Kadna, Kadua Hans and China han in Odisha (Padhi 2014) and Chinae haras, Cina hanh, Bor China and Bhatt China in Northeast India (Alyethodi et al. 2010; Banerjee 2013). It is believed that, Muscovy duck was domesticated in South America and was introduced to India during sixteenth century (Donkin 1989; Peter et al. 2006). However, the genetic evidence for the domestication of Muscovy duck is not conclusive.

Recent advances in the field of molecular genetics have opened up new avenues to study the genetic diversity. Over the last three decades, different types of molecular markers have been developed to study the genetic diversity within and between populations. The mitochondrial cytochrome b gene and CYP2U1 gene are highly conserved in avian species (Nelson et al. 2004; Devos et al. 2010; Sun et al. 2012). The protein-coding cytochrome b gene contains both slowly and rapidly evolving codon positions, as well as more conservative and more variable regions. It has been widely used in avian species to study inter and intra species variation (He et al. 2008a; Mwacharo et al., 2011; Thomson et al. 2014; Award et al. 2015). However, little is known about the genetic structure and domestication history of Muscovy duck (Alyethodi et al. 2010; Veeramani et al. 2014). Therefore, we have analyzed the genetic diversity and relationship of different Muscovy duck populations of India using mtDNA cytochrome b and nuclear DNA CYP2U1 genes.

Materials and methods

Sample collection

Muscovy duck samples were collected from four different states of India, Assam, Mizoram, Odisha and Kerala, that are well known for duck farming. The blood samples were collected with due care to ensure that the samples collected were random and from unrelated ducks. Total genomic DNA was extracted from ethanol preserved blood samples using a standard phenol-chloroform method (Sambrook & Russell 2001). Quality and quantity of the genomic DNA was checked by agarose gel electrophoresis and Nano drop respectively.

Polymerase chain reaction and DNA sequencing

The 940 bp mitochondrial cytochrome b gene fragment was amplified using the following two different sets primers: DUKCYB1L (5′-ATCTTTCGCCCTATCCATCC-3′) and DUKCYB2R (5'-TTTGGTTTACAAGACCAATGTTTT-3'), or P105 (5′-GCCTCCTG CTAGCCATACAC-3′) and P106 (5′-TACGGCGGGAAAGAGAA ATA-3′). The 747 bp nuclear DNA CYP2U1 gene fragment was amplified using the following primers P103 (5′-GTTATTTG GTTATGCATATCGTG-3′) and P104 (5′-GAGACGGTTGGCGTATA TGG-3′). PCR amplification was carried out on a thermocycler with 25 µl volume reaction mixture, which included 50 ng of

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Supplemental data for this article can be accessed here.

genomic DNA, 12.5 µl master mix (Promega, Fitchburg, WI) and 2 µl (10 pmol) of each primers. The PCR was performed at 95 °C for 5 min, followed by 30 cycles of 30 s at 94 °C, 1 min at respective annealing temperature (cytochrome b: 54 °C and CYP2U1: 45 °C) and 1 min at 72 °C and a final extension step of 5 min at 72 °C. The amplified PCR products were sequenced using Big Dye terminator Cycle sequencing kit (Applied Biosystems, Foster City, CA) on ABI 3500 Genetic Analyzer.

Data analysis

The cytochrome b and CYP2U1 gene sequences were manually edited and aligned using Clustal W implemented in MEGA (Tamura et al. 2013). Genetic diversity indices such as number of haplotypes, haplotype diversity, polymorphic sites, pairwise F_{ST} values and AMOVA were calculated using ARLEQUIN (Excoffier et al. 2005). Multidimensional scaling plot was drawn using pairwise F_{ST} values by PSYCH in R package (www.r-project.org). The relationship between geographic distance and genetic distance was analyzed using IBD (Jensen et al. 2005). Bayesian phylogenetic tree was constructed for cytochrome *b* and CYP2U1 genes using MrBayes (Ronquist & Huelsenbeck 2003). MCMC chains were run for 3,000,000 cycles and the trees were sampled every 250 generations. All the sampled trees preceding stationarity were discarded (burnin = 3000) and the remaining tree samples were utilized to construct the 50% majority rule consensus tree. The median joining network was drawn using NETWORK program (Bandelt et al. 1999).

Results

Cytochrome b gene

The 940 bp cytochrome *b* gene sequences of 78 Muscovy ducks (KX985658–KX985735) revealed 11 unique haplotypes, which resulted from 10 polymorphic sites. Six haplotypes were observed in Assam population with the diversity value 0.7190 ± 0.0933, whereas 3 were observed in Mizoram population with diversity value 0.4394 ± 0.1581 (Table 1). There were no much differences among populations with respect to nucleotide compositions. However, high C (34%) and low G (16%) content and almost equal amounts of T and A (24 and 25) were noticed. AMOVA test showed 61% and 39% variation between populations and within population, respectively. Further, it showed maximum variation (71%) when it was calculated for Kerala population versus others. Among the four populations, high genetic distance (F_{ST}) was found between Kerala and Odisha populations (Table 2), whereas the low was found between Assam and Mizoram populations. The pairwise F_{ST} values were further analyzed through multidimensional scaling plot in order to find out the genetic relationship of four Muscovy populations (Figure 1(A)). Muscovy ducks sampled from East and Northeast regions of India scattered on the right side of the plot, whereas Kerala population was found on the other side of the plot. A significant positive correlation was obtained between geographic distance and genetic distance ($r = 0.6390$, $p < 0.08$), which confirmed that these four populations are in isolation by distance (Figure 2(A)).

Bayesian phylogentic tree was constructed using 81 Muscovy sequences. Of the 81 sequences, 3 were retrieved from GenBank (L08385, AF059098 and EU755254) and probably belong to USA and China. A chicken sequence was used to give root to the phylogenetic tree. The phylogenetic tree showed two major clades, clade 1 was encompassed with ducks from all the populations whereas clade 2 was exclusive to Kerala. The USA and Chinese Muscovy also appeared along with Indian Muscovy in the clade 1. A few minor clades were also noticed within clade 1 (Figure S1A). A median joining network was constructed to understand the phylogenetic relationship among four Muscovy populations (Figure 3(A)). There were 11 different haplotypes in the network in which a few were singleton. Of the 11 haplotypes, Hap1 showed high frequency (38 on 78) and was present in all the 4 populations. The next prominent haplotype, Hap2 was present only in Kerala population (18 on 25), which differed from the most common haplotype (Hap1) with 6 mutational steps. Out of 11 haplotypes, only Hap1 showed star-like appearance indicating population expansion. The haplotype sharing was found higher between Assam, Mizoram and Odisha as compared to Kerala population.

CYP2U1 gene

The 747 bp CYP2U1 gene sequences of 80 Muscovy ducks (KX985578–KX985657) revealed 9 unique haplotypes, which resulted from 17 polymorphic sites. Six haplotypes were found in Kerala population with the diversity value 0.7692 ± 0.0515, whereas 3 were found in Mizoram and Odisha populations with diversity value 0.3182 ± 0.1637 and 0.1775 ± 0.1062, respectively (Table 1). There were no much differences among populations with respect to nucleotide

Table 2. Pair wise F_{ST} values of Indian Muscovy populations.

	Assam	Mizorom	Odisha	Kerala
Assam	0.00000	−0.06432	0.05290	0.38807
Mizorom	0.02228	0.00000	0.03272	0.39125
Odisha	0.12085	0.06431	0.00000	0.51228
Kerala	0.66954	0.66571	0.67625	0.00000

Above the diagonal FST values of CYP2U1; below the diagonal FST values of cytochrome b.

Table 1. Genetic diversity estimates of Indian Muscovy populations.

	No. Samples		No. Haplotypes		Haplotype diversity	
Population	Cytochrome b	CYP2U1	Cytochrome b	CYP2U1	Cytochrome b	CYP2U1
Assam	18	20	6	5	0.7190 ± 0.0933	0.3684 ± 0.1351
Mizoram	12	12	3	3	0.4394 ± 0.1581	0.3182 ± 0.1637
Kerala	25	26	4	6	0.4567 ± 0.1046	0.7692 ± 0.0515
Odisha	23	22	5	3	0.5652 ± 0.1123	0.1775 ± 0.1062

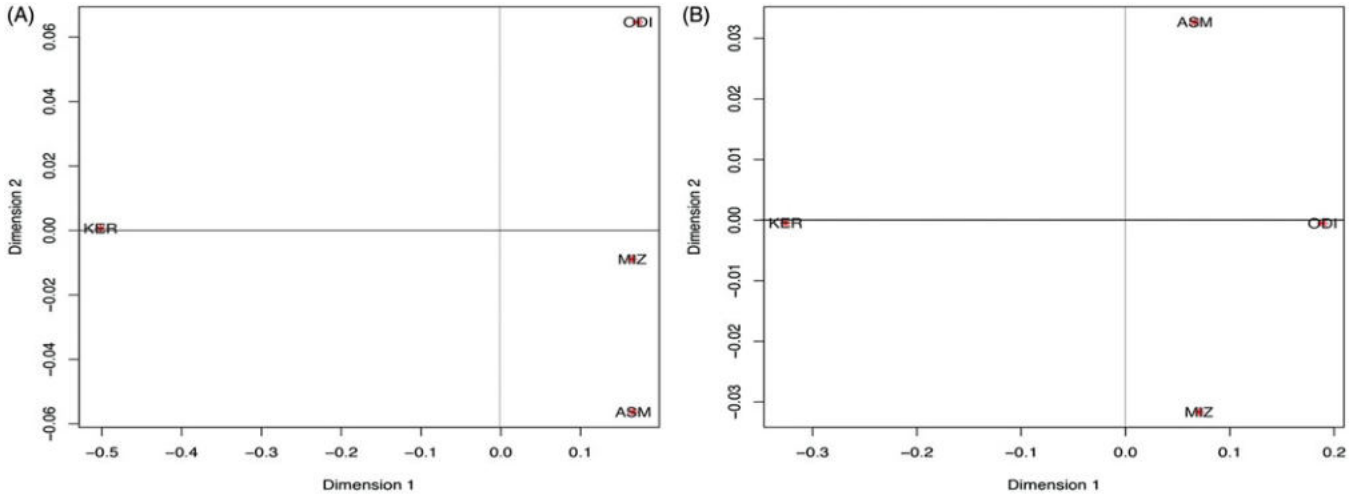

Figure 1. Multidimensional Scaling plot of Indian Muscovy populations. (A) Cytochrome b gene; (B) CYP2U1 gene.

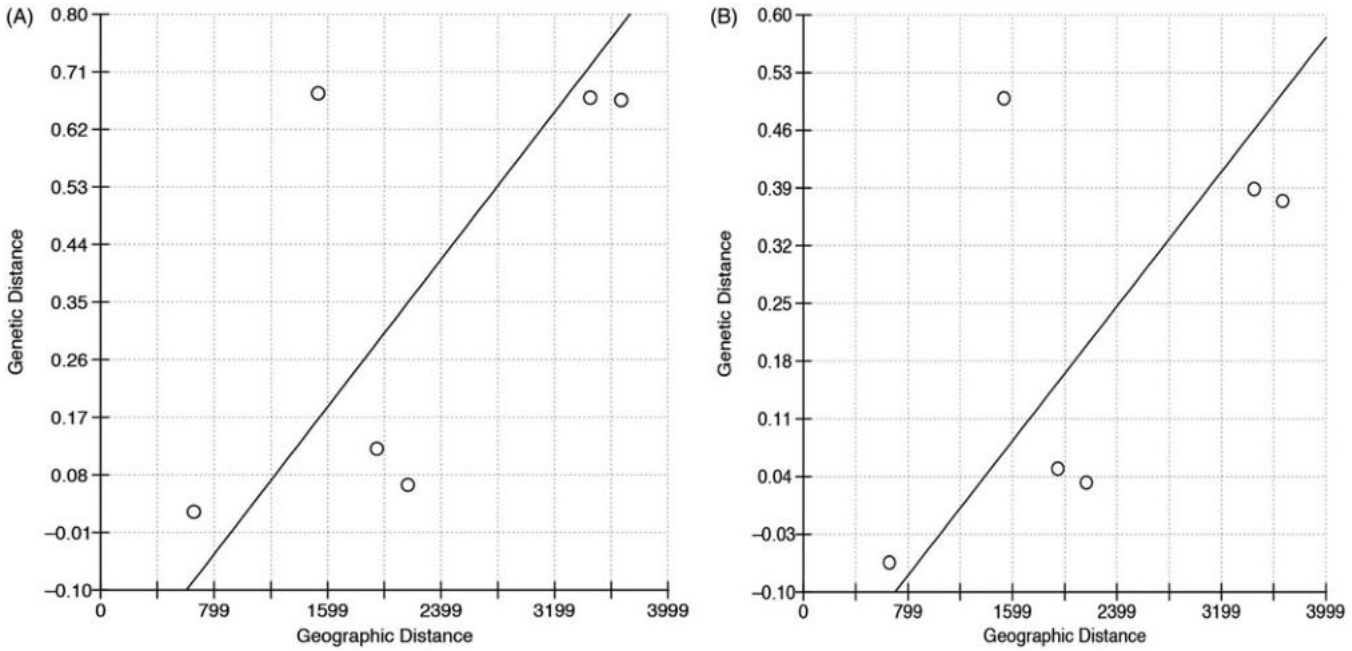

Figure 2. Isolation by distance of four Muscovy populations. Each data point represents a pair-wise comparison between two populations. Mantel test ($p < 0{\cdot}001$) indicates a significant correlation between geographic and genetic distances. (A) Cytochrome b gene; (B) CYP2U1 gene.

composition. However, high A and T (33% and 31%) and low G and C (17% and 18%) content were noticed. In contrast to cytochrome b gene, the AMOVA analysis showed high variation (63.85%) within population. Further, AMOVA test explained maximum variation (48.29%) for Kerala versus others while within population variation was slightly higher (51.46%) than between populations. Among the four populations, the high genetic distance (F_{ST}) was found between Kerala and Odisha populations (Table 2), whereas low was found between Assam and Mizoram populations. The MDS plot of pairwise F_{ST} values was similar to that of cytochrome b gene (Figure 1(B)). The IBD ($r = 0.5506$, $p < 0.23$) also showed consistency with cytochrome b gene (Figure 2(B)).

Bayesian phylogenetic tree was constructed using 80 Muscovy sequences. A chicken sequence was used to give root to the phylogenetic tree. The phylogenetic tree showed at least four distinct clades, clade 1 was mostly occupied by Kerala population along with a few ducks from Assam and Mizoram populations. Clade 3 was encompassed with ducks from all the 4 populations. Clade 2 had a few individuals of Kerala and Assam population whereas clade 4 was specific to Odisha population (Figure S1B). A median joining network was constructed using 80 sequences representing 4 Muscovy populations (Figure 3(B)). Of the 9 haplotypes, only Hap1 was found in all the 4 populations with high frequency (49 on 80). Haplotype sharing was higher among Assam, Mizoram and Kerala population as compared to Odisha population. A few singletons were noticed in Odisha population with 6–8 mutational steps from the most common haplotype (Hap1).

Discussion

In recent years, molecular techniques have contributed immensely to understand the evolutionary history of domestic

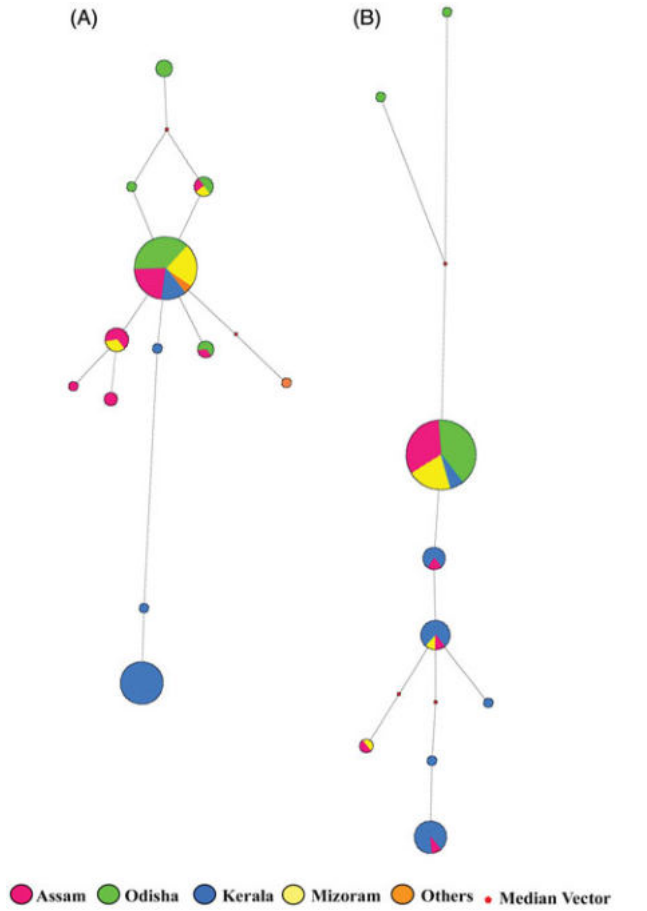

Figure 3. Median joining phylogenetic networks of four Muscovy populations. The populations are presented in different colors. The circle represents haplotypes and area of the circle is proportional to its frequency. The length of the branch is proportional to number of mutations. (A) Cytochrome b gene; (B) CYP2U1 gene.

species. In this study, we have utilized cytochrome P450 family CYP2U1 gene apart from the commonly used mtDNA cytochrome b gene to understand the demographic backgrounds of Indian domestic Muscovy ducks. The overall genetic diversity was low among Indian Muscovy duck populations for both the genes. A total of 11 and 9 haplotypes were found in cytochrome b and CYP2U1 genes, respectively. Similar, low genetic diversity has been reported in Chinese Muscovy ducks for mtDNA cytochrome b gene, d-loop and microsatellite markers. Sun et al. (2012) recorded 6 haplotypes from 31 cytochrome b sequences of two Chinese Muscovy duck populations. He et al. (2008b) reported 8 haplotypes from 30 d-loop sequences of two Muscovy duck populations. Chen et al. (2009) inferred the genetic diversity of Chinese Muscovy duck using d-loop region and observed only 4 haplotypes out of 70 Muscovy samples. The present study reconfirms the low genetic diversity exists among Muscovy duck populations. The AMOVA test showed high genetic variation (61.01%) between populations for cytochrome b gene, whereas high genetic variation (63.85%) was obtained within population for CYP2U1 gene. The high genetic variability in within populations as compared to between populations for CYP2U1 gene reflects the paternal DNA admixture among Muscovy populations.

In general, pairwise F_{ST} value is known to be correlated with geographical distance between populations. As expected, the geographically closer populations, namely Assam, Mizoram and Odisha were genetically more similar to each other than to more distant Kerala population. Further, the negative F_{ST} value observed between Assam and Mizoram populations for CYP2U1 gene showed close relationship of these two populations. A prominent haplotype (Hap1) was observed in the network for both the genes. The haplotype sharing was higher between Assam, Mizoram and Odisha as compared to Kerala population, which indicates the substantial gene flow between these regions. In order to understand the genetic relationship of Indian Muscovy duck with other Muscovy ducks, three cytochrome *b* gene sequences retrieved from GenBank were also included in the network (Figure 3(A)). Out of the three sequences, 2 (USA and China) shared their haplotye with haplotype 1 (the most comon haplotype), which suggested that the Muscovy duck present in India are descended from a single domestication event and probably introduced to India several years ago as reported elsewhere.

Conclusions

Genetic characterization is a prerequisite for sustainable utilization and conservation of Muscovy duck genetic resources. In the present investigation, we have studied the genetic diversity and relationship of four Muscovy duck populations of India using mtDNA and nuclear DNA markers. Among the four Muscovy populations, Kerala population showed significant genetic differences from other three populations. Cytochrome b gene showed that the present day Indian Muscovy duck are descended from a single domestication event. Further, this study has showed the suitability of CYP2U1 gene in population genetics.

Disclosure statement

The authors declare that there are no competing interests. The authors alone are responsible for the content and writing of the paper.

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