GENETIC DIVERSITY ANALYSIS IN HOUSE SPARROW (*PASSER DOMESTICU*S) USING MICROSATELLITE MARKERS IN PUNJAB, PAKISTAN

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ABSTRACT

The sparrow is a thriving ubiquitous species around the world, predominantly considered a serious bird pest in cultivations of variety of habitats.Genetic diversity one of key evolutionary variable and necessary for persistence and viability of such species. Present studies therefore, explored genetic variations in house sparrow using microsatellite markers. In all, 229 sparrows were captured with mist nets from Faisalabad and Rawalpindi while blood was taken from 193 samples and 16 microsatellite markers were incorporated to evaluate genetic relationships. Average allele numbers (Na) for all loci populations in Faisalabad were 4.10, and for Rawalpindi were 2.81, and corresponding heterozygosity (H_o) for the respective habitats were 0.51 and 0.38. Present studies, served apparent indicators for the genetic diversity analysis viz. allelic richness, heterozygosity, and also occurrence of intra and inter populationgenetic differentiation, and phylogenetic tree with atypical results for the sparrow populations.

Key words: Genetic diversity, population structure, microsatellite markers, *Passer domesticus.*

INTRODUCTION

The house sparrow (*Passer domesticus*) is among communal species of birds round the world, prefers to inhabit the light timbered vegetation of both the rural and urban ecosystems and also has symbiotic association with man (Ali and Ripley, 1983; 1987; Anderson, 2006; Ghosh *et al*., 2010). Sparrows are largely considered to maintain equilibrium in the ecosystems being member of various food chains and food webs (Rajashekaraand Venkatesha, 2008). According to Anderson (2006), they are predominantly omnivorous, dimorphic and adapted to sedentary life. Once, considered as thriving ubiquitous species, decline in sparrow population has been reported during the last decades possibly due to changing environment (Hole, 2002; Ringsby *et al*., 2006; DeLaet and Smith, 2007). It is a critical need for species to adapt these environmental changes (Frankham 1999). For population to cope these environmental changes genetic diversity is required. Genetic diversity is variation in genotype and alleles in species or population (Frankham *et al*., 2002).

Genetic diversity addresses important ecological issues to formulate the relationship for the animal fitness, allowing its persistence and adaptation in the vulnerable habitats (Fisher, 1930; Lenormand, 2002; Bazin *et al.,* 2006; Garant *et al.,* 2007; Hughes *et al.,* 2008). Genetic variations have numerous correlations with fitness traits such as growth, survival and disease resistance (Falconer and Mackay, 1996; Reed and Frankham, 2003) therefore, conservation biologists are very apprehensive in preserving genetic variation in species. Importance of genetic diversity among evolutionary variables, that it interacts with population size to determine viability and continuity of species (Liu *et al*., 2013). For the variety of species, reviewing genetic diversity and population structure report on the mechanism of genetic differentiation and the colonization (Sakai *et al.,* 2001; Lee *et al*., 2002).

Population genetic structures have been widely studied with genetic markers which provide detailed population analysis instead continuous field visitations in various habitats and are proven to be more reliable and restrict repeated field visitations (Dawson *et al*., 2012). Present study was therefore, aimed to investigate the possible genetic differentiation and variation for the house sparrow using microsatellite markers as the genetic resources of this species (*Passer domesticus*) had not been studied in this region (Pakistan) before.

MATERIALS AND METHODS

a. Selection of Study Sites: Two major habitats (Faisalabad 31º.15N, 73º.03E) and (Rawalpindi 33º.6N, 73º.05E) were selected for the present study. Both the sites were separated by about 600 km with varied climatic conditions. Of these, sub-habitatswere randomly selected four from Faisalabad and four from Rawalpindi. All the sub-habitats viz. University of Agriculture Faisalabad, PARS, Jinnah Garden and Wildlife Park, Gatwala, while Ayyub Park, Safari Park, Nawaz Sharif Park and PMAS ARID AGRI University comprised roosts of house sparrow. They were regularly surveyed during each month to estimate population abundance and

roost characteristics. Sparrows were captured from both the designated habitat from April-August (2016) out of breeding season.The breeding season of the sparrow occurs during the spring (February-April) every year (Kumar *et al.*, 2015). For this, fine mist nets installed and captured sparrows were maintained in existing aviary of the department. In all, 229 sparrows were captured from Faisalabad and Rawalpindi while blood was taken from 193 samples.

b. **Blood sample collection, DNA extraction and quantification:** Blood samples (10-20 μL) were extracted from brachial vein and were place in vacutainerscontainingethylene diamine tetra-acetic acid (EDTA) and were stored at -20ºCfor further DNA extraction. DNA isolation was obtained from blood samples following phenol/chloroform standard protocol as proposed by (Sambrookand Russell, 2001).The isolated DNA was quantified spectrophotometrically, and later with the agarose gel electrophoresis for final dilution mixture and working solution. The markers used in the present findings were Pdoμ1, Pdoμ3 (Neumann and Wetton, 1996), Pdoμ4, Pdoμ5, Pdoμ6 (Griffith *et al.,* 1999), Pdo9, Pdo10 (Griffith *et al.*, 2007) and finally, Pdo16, Pdo17, Pdo19, Pdo22, Pdo27, Pdo32, Pdo36, Pdo44 and Pdo47 (Dawson *et al.*, 2012). Selection of the present loci was attributed to their sufficient power to detect population structure among the introduced and native sparrow populations (Schrey *et al.,* 2011). Significantly, the loci Pdoμ6 and Pdo10 remained on the same chromosome (Griffith *et al.,* 2007) but were not statistically correlated (Schrey *et al.,* 2011). Implication of the annealing temperature in polymerase chain reactions was adjusted on the software for obtain of high proportions of amplified DNA products. PCR was structured into four multiplex sets and compatibility between primer sets was checked by AutoDimer (Vallone and Butler, 2004). In first multiplex Pdoμ1, Pdoμ4,Pdo32 and Pdo47, the second continued Pdoμ3, Pdoμ5, Pdo6 and Pdo36, while the third multiplex reaction contained Pdo10, Pdo16, Pdo19 and Pdo22, the last set comprised of Pdo9, Pdo17, Pdo27 and Pdo44. The reaction was conducted using 10μl reaction volume containing the 5-10ng template DNA, 1 μM of each primer, 4 μl dNTPs, 0.2U Taq DNA polymerase; 1.5-2 mMmagnesium chloride and 2 μl ten times (10 X) buffer solution. The temperature profile was of 3-minute preliminary denaturation (95ºC), subsequently 35 cycles of 30s at 94 ºC,90s at 57 ºC and 60s at 72 ºC. Finally, 5 min at 72ºC of annealing step was included. The resulting products were later differentiated using polyacrylamide gel electrophoresis (PAGE) in the ratio (19:1 acrylamide: bis-acrylamide), as only available method. Electrophoresis, using the sequencing gel system, was run for period of 30 minutes as (pre-run), and finally at (60 W), till the loading dye reached the bottom of the

plate. The fragments (bands) of DNA were stained through the silver-staining (Bassam *et al.,* 1991).

c. **Data Analysis:** The analysis of the data was made to measure the genetic diversity among the captured house sparrows from both the major sites. The analysis of population differentiation, heterozygosity, allele frequency, linkage disequilibrium, deviation from Hardy-Weinberg and inbreeding coefficient were calculated using different softwares viz., POPGENE (Yeh *et al.,* 1997), FSTAT (Goudet, 2002) and STRUCTURE (Hubisz *et al.,* 2009). For scoring of the genotyping error MICRO CHECKER (Van Oosterhout *et al.*, 2004) was used.The genetic diversification among two populations of house sparrow was determined by using Nei's (1987) standard genetic distances (D_S) through MOLKIN 2.0 (Gutierrez and Goyache, 2005). Similarly, the phylogenetic relationship between two populations was determined from Nei's standard genetic distances (D_S) by using MEGA 3.0 (Kumar *et al.,* 2004) software.

RESULTS

Sixteen microsatellite loci were amplified for the 193 sparrow samples and produced definite banding patterns.Moreover, all amplified loci were polymorphic.

Genetic variability with in populations: Genetic diversity analysis indicated that all populations were similar with respect to intrapopulation level of genetic variations. The mean number of alleles (Na) observed for all loci for sparrows in both major habitats, ranged from 2.81 for the sub-site III in Rawalpindi, while it remained 4.10 in the sub-site III for Faisalabad region. The level of observed heterozygosity was moderate and varied from 0.36 (sub-site-III) in Faisalabad up to a maximum of 0.51 (sub-site-IV) in Rawalpindi respectively across all the sub-site populations of house sparrow. However, the highest average level of expected heterozygosity was found for sub-site IV Rawalpindi (0.69) to lowest for sub-site I Faisalabad (0.58) populations (Table 1). The level of observed heterozygosity was lower than the corresponding expected heterozygosity indicating that all the sub-site populations were deficient in heterozygotes.

Population differentiation: Apparently, FST (variation due to population differences) and FIS (variation within in a population) were categorized for relative abundance of each genetic marker for two habitats, conforming to the allele frequency variations (Tables 2, 3). All loci contributed significantly to the overall differentiation among populations across sub-habitats of district Faisalabad and district Rawalpindi. A deficiency of heterozygotes (positive FIS values) was observed in both house sparrow populations across all loci. The mean overall FIS values ranged from 0.10 (10%) for sub-habitat IV of Rawalpindi population to 0.22 (22%) for

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sub-habitat II and III of Faisalabad population across 16 loci (Table 1). Significantly, pair-wise FST estimates ranged -0.0016-0.0382 strongly established level of population differentiationamong populations. No evidence on genotyping error of stuttering and large allele dropout was found as checked with MICRO CHECKER. All markers had significant variation from HW equilibrium across two populations of house sparrow indicating that no population was at equilibrium at these loci. However, out of 16 total loci there was one (Pdo6) that deviated from HW equilibrium, it has estimated null allele frequency of 0.008.

Phylogenetic relationships: The unweighted pair group method of arithmetic means (UPGMA) was used to obtain the phylogenetic tree having two main clusters denoted by "a" and "b" (Fig. I). The topology of UPGMA tree agreed well to the history and geographic distribution of house sparrow populations with few atypical results. The cluster "a" contained house sparrow populations from sub-habitat I, II and III of Faisalabad and Sub-habitat III of Rawalpindi population. The second cluster "b" possessed house sparrow populations from sub-habitat IV of Faisalabad and Sub-habitat I, II and IV of Rawalpindi population. The topology of the phylogenetic trees depicted grouping of two populations in one cluster, indicating their common origin.
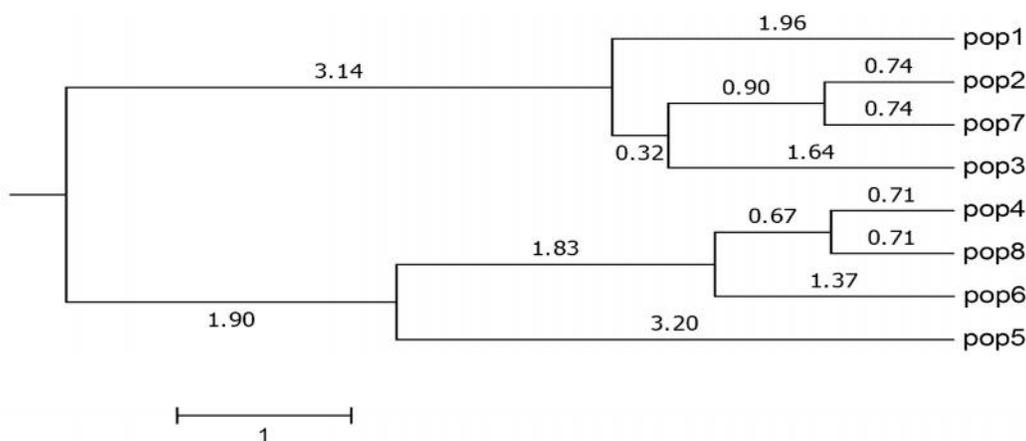

Fig. 1. UPGMA dendrogram showing the genetic relationship among eight population sites in the two major sites.
Faisalabad: sub-site I = Pop 1, sub-site II = Pop 2, sub-site III = Pop 3, sub-site IV = Pop 4
Rawalpindi: sub-site I = Pop 5, sub-site II = Pop 6, sub-site III = Pop 7, sub-site IV = Pop 8

Table1. Genetic diversity estimates based at 16 microsatellite loci for house sparrow from four sub-habitats of the Faisalabad and Rawalpindi.

Populations		Na	Ar	Ho	He	FIS
Faisalabad	Sub-site I	3.12	3.03	0.38	0.58	0.19
	Sub-site II	3.25	3.15	0.41	0.62	0.22
	Sub-site II	4.10	3.91	0.36	0.61	0.22
	Sub-site IV	3.56	3.47	0.42	0.61	0.20
Rawalpindi	Sub-site I	3.12	2.99	0.44	0.64	0.17
	Sub-site II	2.93	2.94	0.44	0.62	0.20
	Sub-site III	2.81	2.71	0.44	0.63	0.17
	Sub-site IV	3.62	3.51	0.51	0.69	0.10

Na (number of alleles), Ar (allelic richness), Ho (observed heterozygosity), He (expected heterozygosity) and F_{IS} (inbreeding coefficient)

Table2. Pair-wise population differentiation (*FST*) between four populations of house sparrow for (16) microsatellite loci from Faisalabad.

Population	I	II	III	IV
I	-----			
II	-0.0016[**]	------		
III	0.0149[**]	0.000[***]	------	
IV	0.0133[*]	0.0086[**]	0.0049[**]	-----

Table 3. Pair-wise occurrence of population differentiation (*FST*) for *Passer domesticus* for the designated (16) loci from Rawalpindi.

Population	I	II	III	IV
I	-------			
II	0.0083**	-------		
III	0.0205*	0.0148*	-------	
IV	0.0382*	0.0508*	0.0387*	------

Faisalabad: sub-site I = University Agriculture Faisalabad, sub-site II = PARS, sub-site III = Jinnah Garden, sub-site IV = GatWala Park

Rawalpindi: sub-site I = Ayyube Park, sub-site II = Nawaz Sharif Park, sub-site III = Safari Park, sub-site IV = PMAS Arid Agriculture University.

DISCUSSION

Population genetic structural analysis and the genetic differentiation have inference for assessing population dynamics and evolution. Today, habitat fragmentation for the faunal distribution appears to be serious threat and therefore, to inhibit biodiversity. In the present study attempt was made to determine the genetic diversity of house sparrow (*Passer domesticus*) for two widely diverse habitats. It indicated the categorization of the population of house sparrow of the smaller sub-habitats in the two regions. Of 193 genotypes individuals corresponding to 54 alleles showed amplification at 16 loci. Mean number of alleles is an appropriate measure of genetic variation in comparison with heterozygosity because it is expected to be larger when the extent of polymorphism is higher, while the heterozygosity is hardly affected by the low-frequency alleles (Nei, 1987). The present findings are in agreement with those of Dawson *et al*., 2012. There is a rich allelic variation in the house sparrow for 16 loci. The mean observed number of alleles (N_a) demonstrated that almost all the microsatellite loci utilized in the present study are sufficiently polymorphic. High N_a (allelic diversity) per locus displayed high genetic variation which indicates that the population is under mutation drift equilibrium. This pattern of allelic diversity among house sparrow populations is more likely to reflect their distinctive evolutionary histories. The observed numbers of alleles in this study are less (2.81 and 4.10) than those presented (Mean numbers of alleles per locus ranged between 4.8 and 7.9) by Vangestel *et al*. (2012). According to selective standard of microsatellite DNA loci, it ought to have at least four alleles to be considered useful for the evaluation of genetic diversity in order to reduce the standard error of distance estimates. The average number of effective alleles found across 16 loci, considering all populations, was close to this value (2-8) and thus there were certain loci which had higher values thus can be considered useful for the evaluation of genetic diversity in *Passer domesticus* (Vangestel *et al*., 2011 a, b)

Both observed (Ho) and expected heterozygosity (He) may represent significant genetic variations among the populations with respect to certain locus (Table 1), which leads to genotypic disequilibrium of the species (Tambasco *et al*., 2003). For this study for 16 microsatellites used for house sparrow in two different habitats, showed significant heterozygosity with mean value (0.38-0.51), while Takezaki and Nei (1996) had also reported comparable value (0.3-0.8) for a population to describe effectiveness of genetic variations for the populations; the average (Ho) estimation remained of lower proportions as obtained and described by Vangestel *et al*. (2012).

Magnitude of FST populations among majority of animals relies on overall genetic differentiation, and for the present work, mean FST value depicted less total genetic variations owing to significant distance between both the habitats (Faisalabad and Rawalpindi). Moreover, the mean population estimates (mean number of alleles ranged 2.81-4.10, allelic richness ranged 2.71-3.91, expected heterozygosity ranged between 0.58 and 0.69) was considered to be identical (mean numbers of alleles per locus ranged between 4.8 and 7.9, allelic richness ranged between 5.86 and 9.62 and expected heterozygosity ranged between 0.60 and 0.71) with (Vangestel *et al.*, 2012), who had also reported similar genetic based differentiation. Concomitantly, the FIS values showed the frequency of occurrence for heterozygosity alongside the inbreeding impacts on house sparrow in the two locations. As reported from (Table 1) deficient heterozygotes appears to impact strong response to progressive inbreeding, genetic hitch-hiking, the presence of null alleles and population sub-structures seemed to enforced lowered heterozygous conditions (Nei, 1987).

From the phylogenetic aspect, both types of sparrow populations did not depict significant variations with least heredity differences. It was important that in lack of heterozygosity observed, mainly the results were explained according to the distance co-efficient factors, while construction of dendrogram were largely based on (Nei's, 1987) standard genetic distances (DS) matrices and the un-weighted pair group method of arithmetic means (UPGMA) for the twin sites for sparrows. Analytically, two main clusters denoted (a, b) were

apparent and were reported to be closely linked with past evolutionary history and geographic distribution patterns with some by "a" and "b". The topology of UPGMA tree agreed well to the history and geographic distribution of two populations of house sparrow with few nonconforming results. Cluster a represented sub-habitats (I, II, II) sparrow populations of Faisalabad and Rawalpindi, and that of"b" displayed sub-habitats (IV), of Faisalabad region and (I, II and IV) of Rawalpindi, but of the similar origin.

Conclusions: Result of the present study indicated that population genetics of house sparrow was largely affected by reduced gene flow as observed by positive FIS values. Nonetheless, it would be beneficial to evaluate population structure of this bird in other locations. Moreover, deficiency of heterozygotes also predicted the incidence of inbreeding and reduced sparrow population size.Therefore, this empirical change for genetic differentiation may serve as an indicator of loss of genetic diversity. The phylogenetic aspect, both types of sparrow populations did not portray significant variations with least heredity differences.

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