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*Full Length Research Paper*

# Genetic diversity and population structure of leaf-nosed bat *Hipposideros speoris* (Chiroptera: Hipposideridae) in Indian subcontinent

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**Genetic variation and population structure of the leaf-nosed bat *Hipposideros speoris* were estimated using 16S rRNA sequence and microsatellite analysis. Twenty seven distinct mitochondrial haplotypes were identified from 186 individuals, sampled from eleven populations. $F_{ST}$ test revealed significant variations between populations in the overall pairwise estimation ($F_{ST}$ = 0.710; p < 0.001). In addition, haplotype network and analysis of molecular variation analysis (AMOVA) consistently suggest the prevalence of genetic structure in the sampled populations. However, the mtDNA data was not significantly different in few closely located urban populations, but significant difference has been observed with the use of microsatellite data. The Bayesian clustering analysis identified eight clusters among the populations; the clustering pattern also corresponded to the haplotype networks. Overall, the present study suggests a "macrogeographic genetic isolation-by-distance" and possibility of gene flow among closely located populations.**

**Key words:** mtDNA, 16S rRNA, microsatellite, population structure, *Hipposideros speoris*.

## INTRODUCTION

The distribution pattern of natural population of an organism is mainly determined by geographical factors, which influence the gene flow either through isolation by distance or the existence of dispersal barriers. Particularly, the current genetic status of a species occurs mainly based on the interaction of historical events and geographical structure (Emerson et al., 2001). Variations in the geomorphological features constitute the major geographical barriers for gene flow in the main land system. Although, isolation of such barriers to gene flow may not always be spatially large, they still remain effective for long periods (Castella et al., 2000). The Schneider's leaf-nosed bat,*Hipposideros speoris* is distributed to central and southern part of India; it roosts in abandoned buildings, caves and underground tunnels. Its colony occurs as mixed-sex group with size ranging from a few individuals to hundreds. Females give birth to single young mostly during September to December (Habersetzer and Marimuthu, 1986). *H. speoris* exhibits a wide range of ecological specializations to forage and in almost all situations it emits calls (127 to 138 kHz) with CF-FM pattern (Habersetzer et al., 1984). Like other bat species, *H. speoris* also losing its suitable roosting and foraging habitats due to urbanization, human disturbances in semi-urban areas and agricultural practices.

Studies on the differences in allele frequency within and between populations facilitate assessing dispersal, immigration/emigration of nocturnal and highly mobile animals like bats. Nowadays, molecular markers such as mitochondrial DNA (mtDNA) and microsatellite are the valuable tools to study the phylogeographical trends at

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**Abbreviations: HWE,** Hardy-Weinberg equilibrium; **PCR,** polymerase chain reaction; **EDTA,** ethylene diamine tetraacetic cid; **SDS,** sodium dodecyl sulfate.

**Table 1.** Details of *H .speoris* bat samples collected from eleven sampling sites across different geographical regions in India.

| Populations | Longitude | Latitude | Number of individual | Catching site |
|---|---|---|---|---|
| 1 | 78°41'65'E | 10°45'85'N | 14 | Day roost/unused building |
| 2 | 78°48'62'E | 10°23'63'N | 16 | Day roost/unused building |
| 3 | 77°44'21'E | 08°43'53'N | 15 | Day roost/unused building |
| 4 | 77°46'62'E | 08°43'01'N | 15 | Day roost/unused building |
| 5 | 77°28'37'E | 10°01'59'N | 20 | Day roost/unused building |
| 6 | 78°26'55'E | 10°57'97'N | 12 | Day roost/unused building |
| 7 | 76°38'23'E | 10°46'58'N | 20 | Day roost/unused building |
| 8 | 76°12'74'E | 10°31'27'N | 20 | Day roost/unused building |
| 9 | 74°58'98'E | 13°04'92''N | 14 | Day roost/cave |
| 10 | 78°24'91'E | 17°23'04'N | 20 | Day roost/cave |
| 11 | 73°49'52'E | 18°32'96'N | 20 | Day roost/cave |

species level (Goldstein and Pollock, 1997) and the genetic status between populations (Jarne and Lagoda, 1996). In addition, application of nuclear and mtDNA genetic markers helps to understand the sex specific difference in behaviour from current genetic diversity (Rassmann et al., 1997). These molecular markers have been used to investigate the natal philopatry (Entwistle et al., 2000), male-biased dispersal (Castella et al., 2001; Petit et al., 2001; Kerth et al., 2002) and female-biased dispersal (Nagy et al., 2007). Indeed, both markers have been used to examine the mating system and social structure of the short-nosed fruit bat *Cynopterus sphinx* (Storz et al., 2001) and the colonization and dispersal pattern of Bechstein's bat *Myotis bechsteinii* (Kerth and Petit, 2005). According to the aforementioned studies, a fine scale approach with nuclear DNA marker and mtDNA sequence data provide reliable information about population structure and dispersal pattern. In the present study, we used mtDNA sequence and highly polymorphic microsatellite marker data to draw the outline of the genetic diversity and population structure of the leaf-nosed bat *H. speoris* populations across the entire known range in the Indian subcontinent.

## MATERIALS AND METHODS

### Collection of samples

A total of 186 (94 males, 92 females) adult *H. speoris* captured from 11 populations (pop 1 to 8 from urban region; 9 to 11 from forest region) across the species range in (southern and western regions) India were used for the study (Table 1 and Figure 1). From each individual, a 3 mm diameter wing membrane was taken with biopsy punch and preserved in TENS [Tris-Cl pH7.5, 10 mM ethylene diamine tetraacetic acid (EDTA), 125 mM NaCl, 1.0% sodium dodecyl sulfate (SDS)] DNA extraction buffer (Wilmer and Barratt, 1996). After collecting the samples, the bats were released immediately at their roosting sites. All procedures adopted in this study were approved by Bharathidasan University Wild Animals Ethical Committee (BUWAEC), Tiruchirappalli, India.

### Isolation of DNA and amplification of 16S rRNA

The total genomic DNA was extracted from TENS DNA extraction buffer-preserved tissue samples (Wilmer and Barratt, 1996). Approximately 600 bp of the mtDNA 16 S rRNA region was amplified with specific primer light chain (L): TTACCAAAAACATC ACCTCTAGC; heavy chain (H): CGGTCTGAACTCAGATCACGTA (Lin et al., 2002). The 16 S rRNA region was amplified in 50 µl reaction mixture containing 1 unit of *Pfu* DNA polymerase (Invitrogen Inc), 1.5 mM $Mg^{2+}$, 0.2 µM of primers, dNTP at 200 µM, and 20 ng template DNA. Amplifications were performed in a MJ-mini cycler (Bio-Rad Laboratories Inc), employing an initial denaturation (95°C, 1 min) followed by 25 cycles of denaturation (94°C, 1 min), annealing (56°C, 1.5 min), extension (72°C, 2 min) and a final extension at 72°C for 5 min. The polymerase chain reaction (PCR) products were excised from agarose gel, purified with spin column (RBC gel elution kit, Taiwan) and sequenced (Bangalore Genei Ltd, India) with L primer. The sequences were then aligned by using Clustal X software (Thompson et al., 1997). Identified haplotype samples were sequenced twice from independent PCR reactions to resolve PCR errors or any ambiguities present in sequencing passes.

### Mitochondrial DNA sequence analysis

The sequence diversity of mtDNA 16S rRNA region was estimated by calculating haplotype diversity ($h$), nucleotide diversity ($\pi$), polymorphic sites and pairwise population $F_{ST}$ (based on 1000 permutations) using Arliquin 3.11 (Excoffier et al., 2005).

### Analysis of population structure

Haplotype sequences were used to test the genetic relationship among the lineages. The relationship between unique haplotypes was established using median-joining (MJ) network method in NETWORK 4.1.1.2 (Bandelt et al., 1999). Based on the geographical region, the phylogenetic tree populations were categorized into two groups; group I (populations 1 to 8 located in urban within the range of 250 km) and group II (populations 9 to 11 located in forest, more than 300 km away from group I). Hierarchical analysis of molecular variance (AMOVA) was carried out in Arliquin 3.11 (Excoffier et al., 2005). Fixation indices were calculated in a conventional fashion and their significance was tested using a 1000 permutation approach described by Excoffier et al. (1992),

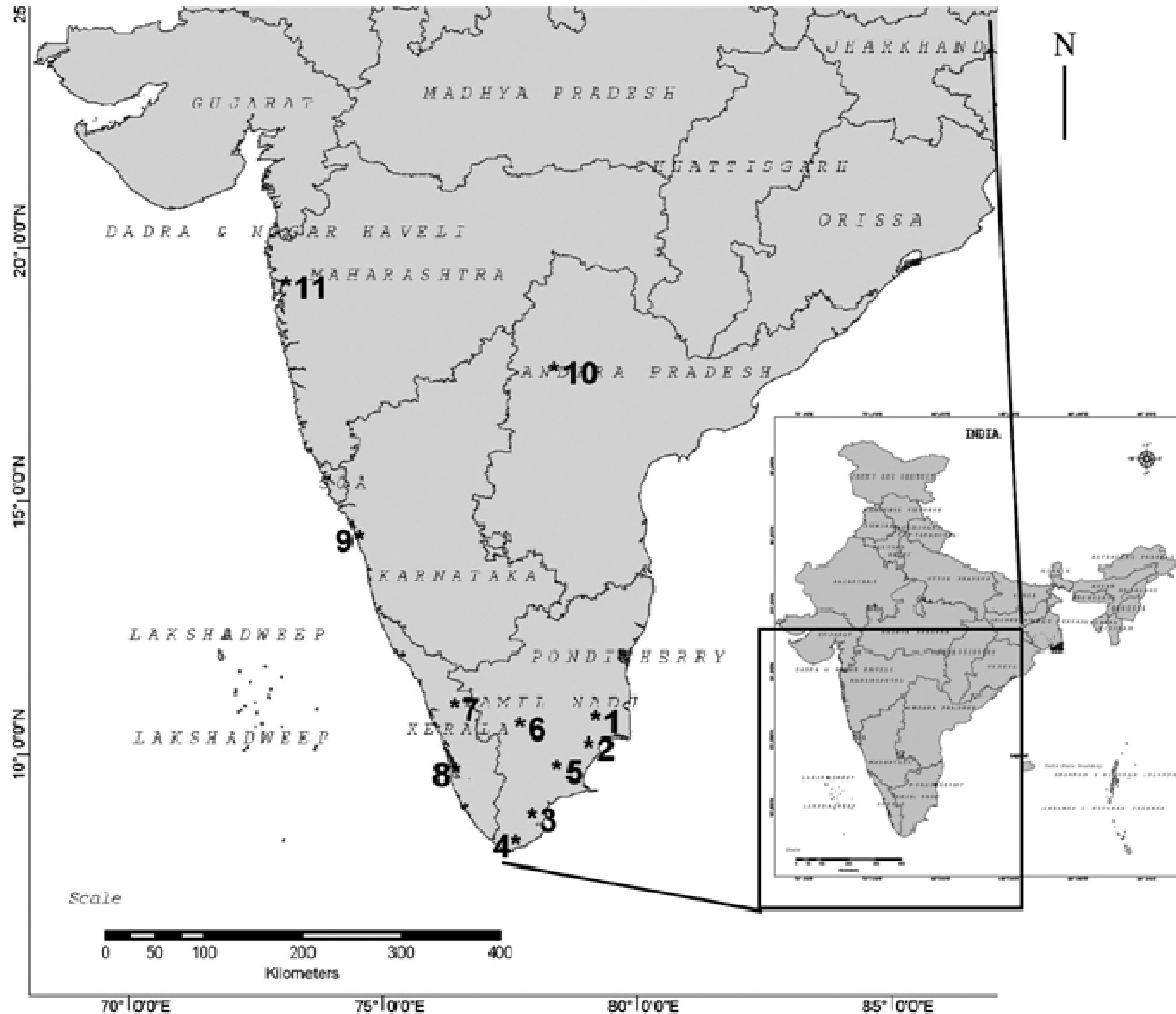


**Figure 1.** Geographic locations of the eleven sampled populations of *H. speoris.*

consisting of permuting haplotype, within population, among populations within and among groups.

**Microsatellite amplification**

Twelve microsatellite primer pairs (Rossiter et al.,1999; Sebastien et al., 2005), originally developed from super family Hipposideridae were chosen to examine the genetic diversity, gene flow and population structure of *H. speoris.* The optimal annealing temperature for each primer was standardized through a gradient PCR. Six primers (Rferr03, Rferr09, Rferr11, RHC3, RHD116 and RHD119) amplified reliably and were polymorphic in *H. speoris.* All the loci were amplified in a volume of 20 µl PCR mixture containing 1.5 mM $MgCl_2$, 1X *Taq* buffer, 0.2 mM dNTPs, 3 U *Taq* DNA polymerase, 0.2 pmol of each primer and 10 ng of DNA template. PCR cycles were as follows: 94°C for 1 min, followed by 30 cycles of 94°C for 1 min, primer specific annealing temperature for 1 min, 72°C for 1 min and a final extension for 7 min at 72°C (Table 2). The PCR products were resolved in 6.0% polyacrylamide gel and the resultant bands were visualized by silver staining (Neilan et al., 1994). Allele sizes were calculated by comparing to the molecular weight of 100 bp DNA ladder (RBC Bioscience, Taiwan) using the 'Quantity One' software (Bio-Rad, USA). Negative controls (only sterile water and PCR reagents) were included in PCR runs. Each allele was confirmed by at least two or three independent PCR reactions. We distinguished each allele by comparing them with the banding profile of known single alleles.

**Microsatellite analysis**

Microsatellite data was used to estimate the allelic diversity, heterozygosity and allelic richness. The genotypes were further tested for linkage equilibrium and significance of departure from Hardy-Weinberg equilibrium (HWE) based on expected and observed heterozygosity. In addition, the obtained microsatellite data was used to compare the pairwise population genetic structure

**Table 2.** Characteristics of six microsatellite primers tested in *H. speoris*. Primer sequences are given together with allele number and size ranges.

| Locus | Primer sequences(5'-3') | Repeat motif | T*a* (°C) | Allele size range (bp) | Number of alleles |
|---|---|---|---|---|---|
| Rferr03 | F:CTAACCTGGTGAACCTCCCATTC<br>R:CTATCACCTGCATCACCTGTGAAG | $(GT)_{18}$ | 57 | 211-241 | 11 |
| Rferr09 | F:GGTCCTGGAAAAACACACACACTG<br>R:TCCTCCCTTTGCGTTCTCTAGG | $(GT)_{18}$ | 65 | 122-140 | 9 |
| Rferr11 | F:AGGGGAGTGACAGAGAGGGAATC<br>R:TGGAGGTGGGAGGACAGAAACCTG | $(GT)_{21}$ | 57 | 182-204 | 10 |
| RHC3 | F:TTGATTGGCTGCTGGATA<br>R:CCGAGGAACTTACTGAGTCC | $(TAAA)_8$ | 52 | 186-194 | 9 |
| RHD116 | F:CCCTCTCCCTCTCTCTCTC<br>R:AGGCAGAAACACAGTCATTC | $(TATC)_{11}$ | 51 | 128-156 | 8 |
| RHD119 | F:CCTGCTTGCTCTGTCTGTTTA<br>R:CATCCACCATTTCACTGTGTC | $(TCTA)_2$<br>TTAATA $(TCTA)_{10}$ | 51 | 144-156 | 6 |

F = forward primer; R = reverse primer; T*a*, annealing temperature.

$F_{ST}$ in FSTAT version 2.9.3 (Goudet, 1995). Sequential Bonferroni corrections adjusted critical probability values for multiple tests to minimize type I errors (Rice, 1989) in $F_{ST}$ comparisons. The significance of genotypic disequilibrium between all pairs of loci in all populations was determined with Fisher's exact test in GenePop 4.0 using 5000 demorization, 500 batches and 5000 iterations per batch (Raymond and Rousset, 1995). Mantel test ($F_{ST}/(1-F_{ST})$) and the natural log of the geographical distance (Rousset, 1997). Geographic coordinates for each colony was determined and the geographical distance between each pair of colonies was calculated as the direct aerial distance between geographical co-ordinates. In all tests, the significance of the correlation was assessed using 10000 random permutations. Spatial genetic structure and individual coefficient of ancestry (q) of the sampled populations were investigated with Bayesian analysis implemented in Bayesian analysis population structure (BAPS) version 3.2 (Corander et al., 2003; Marttinen et al., 2006) based on MCMC. Comparison test showed that BAPS has comparable statistical power to structure and increased power over small geographical distances (Corander and Marttinen, 2006; Latch et al., 2006). The analysis identifies genetically distinct clusters (*K*) based on allele frequencies across the loci; we ran 5 replicates for every level of *K* (*K* is maximum number of clusters) to test population clusters. When estimating individual ancestry coefficients via admixture analysis, we tested 186 individuals and repeated the admixture analysis 50 times per individual.

## RESULTS

The mitochondrial DNA 16S rRNA region sequence was aligned and 478 bp data set was considered for analysis. We found that out of 478 sites, 444 were conserved and 34 were polymorphic and half of them were parsimony informative. We identified 27 distinct haplotypes and the haplotype sequences are available in GenBank. The haplotype diversity (*h*) ranged from 0.286 to 1.00 in closely located urban and from 0.810 to 0.905 in distantly located forest populations, revealing clear differences among populations. We found a similar pattern of nucleotide diversity (π), ranging from 0.001 to 0.0116 (Table 3).

Mitochondrial DNA 16S rRNA sequence data were used to test pairwise analysis. Pairwise analysis revealed genetic structure between populations with a significant difference in overall pairwise estimation ($F_{ST}$ = 0.710, $P < 0.001$) (Table 4). Nevertheless, only a few populations showed significant mtDNA pairwise differences within closely located urban populations, while the remaining did not vary significantly. When populations 9 to 11 were compared with populations 1 to 8, a significant genetic difference ($F_{ST}$) was observed (Table 4).

Mitochondrial diversity was not randomly distributed throughout the sampled populations. The genetic relationships between the individual haplotypes from the eleven populations are displayed as median-joining network. The median-joining network appears as two clusters, indicating a genetic structuring with reference to the geographical region (Figure 2). However, haplotype population 3 (P3d25) and 4 (P4e23) cluster within those from distantly located forest populations (9, 10 and 11) that are providing information about the distinctiveness and genetic relations of these haplotypes. Furthermore, occurrence of one haplotype from population 3 (P3c13) supports many haplotypes representing closely located different populations that suggests a single successful founder haplotype. The AMOVA revealed a significant genetic variation ($P < 0.01$, 1000 permutations) within and between regions and populations (among regions and populations, within regions and within populations). Although, the greatest variation (64.74%) was estimated among regions, the genetic variance is explained by the differences among groups relative to the whole data set. The variation among populations within groups was 6.34%, whereas, the variation within populations was 28.92%.

**Table 3.** Levels of genetic diversity estimated from mitochondrial 16s RNA (478 bp) regions of *H. speoris* populations; standard error values are given in parentheses. The summary of microsatellite analysis shows: A, mean number of alleles per locus; *HO*, observed heterozygosity; $H_E$, expected heterozygosity, $F_{IS}$, inbreeding coefficient.

| Populations | Nucleotide diversity (π) | Haplotype diversity (*h*) | GenBank accession no. | *A* | $H_O$ | $H_E$ | $F_{IS}$ |
|---|---|---|---|---|---|---|---|
| 1 | 0.007 (0.001) | 1.000 (0.126) | FJ825626-28 | 8.58 | 0.92 | 0.94 | -0.353 |
| 2 | 0.001 (0.000) | 0.762 (0.115) | FJ825629 | 8.94 | 0.92 | 0.94 | -0.295 |
| 3 | 0.0116 (0.002) | 0.933 (0.122) | FJ825637-38 | 9.47 | 0.90 | 0.94 | -0.322 |
| 4 | 0.005 (0.001) | 0.476 (0.171) | FJ825634-36 | 7.91 | 0.91 | 0.94 | -0.357 |
| 5 | 0.002 (0.000) | 0.667 (0.160) | FJ825642 | 8.13 | 0.87 | 0.94 | -0.313 |
| 6 | 0.001 (0.000) | 0.667 (0.160) | FJ825643-45 | 9.51 | 0.92 | 0.94 | -0.301 |
| 7 | 0.002 (0.000) | 0.524 (0.209) | FJ825639-40 | 10.65 | 0.94 | 0.94 | -0.275 |
| 8 | 0.001 (0.000) | 0.286 (0.190) | FJ825641 | 9.17 | 0.94 | 0.95 | -0.280 |
| 9 | 0.004 (0.000) | 0.905 (0.103) | FJ825647-50 | 8.98 | 0.88 | 0.94 | -0.342 |
| 10 | 0.006 (0.002) | 0.952 (0.096) | FJ825651-54 | 9.28 | 0.93 | 0.94 | -0.336 |
| 11 | 0.006 (0.003) | 0.810 (0.130) | FJ825655-57 | 8.29 | 0.93 | 0.94 | -0.315 |

No., Number.

Based on genotyping, 186 individual *H. speoris* representing eleven populations for six microsatellite loci, no pairs of loci showed linkage disequilibrium and no locus showed significant deviations from Hardy-Weinberg equilibrium (HWE). The mean number of alleles per locus was between 7.91 and 10.65, with expected heterozygosities ($H_E$) ranging from 0.94 to 0.95. With the average $H_E$ and $H_O$ values 0.94 and 0.91, respectively, we further obtained an average $F_{IS}$ value of -0.315. $F_{IS}$ value was not significant at the population level indicating a low level of inbreeding (Table 3).

We used the whole microsatellite data set to assess the population genetic structure and pairwise comparisons. In contrast to the mtDNA data, the microsatellite data significantly differed in majority of pairwise comparisons within the closely located populations with the exception of a few populations, which possess a low but significant $F_{ST}$ value (Table 4). Similarly, the analysis revealed a significant genetic structure among populations (over all $F_{ST}$ = 0.651, $P < 0.001$), but it is notable that a few comparisons (populations: 4 versus 7, 6 versus 8 and 7 versus 9) showed a lack of significant difference. The estimated pairwise genetic distance among all populations was plotted against geographical distance. The strong positive linear relationship obtained with significant ($r = 0.916$. $P < 0.01$), indicating that gene flow in this species is restricted over distance (Figure 3).

The Bayesian clustering analysis of multilocus genotypes using BAPS resolved for the eleven populations (Figure 4). The analysis identified eight population clusters (*K*=8). Among them populations 4, 7 and 9 are grouped as one cluster, populations 6 and 8 as another cluster and the remaining populations (1, 2, 3, 5, 10 and 11) stay independently. The observed population cluster pattern further supports the estimated pairwise distance between the populations (Table 4). This reveals that populations in these clusters have a similar genetic signature and thus, did not differ significantly.

## DISCUSSION

Phylogeographical studies on bats of Indian subcontinent are limited. Actually, our study presents a first glimpse of the pattern of genetic variation within *H. speoris* through mtDNA haplotype and microsatellite data analysis. The observed genetic diversity was not uniform, which shows the current pattern of distribution of *H. speoris* at this region. Usually, non-migratory bats exhibit a distinct genetic population structure (Ngamprasertwong et al., 2008), the leaf-nosed bat *H. speoris* being no exception to the rule. The estimated pairwise comparisons demonstrate the genetic differentiation among *H. speoris* colonies and exhibit a pattern of isolation by distance. The pattern of genetic differentiation reflects the common characteristics of small mammals including non-migratory bats (Chen et al., 2006; Ngamprasertwong et al., 2008). Interestingly, mtDNA and microsatellite data provide different pattern of genetic structure within closely located populations that are within the range of 250 km. Maternally inherited mtDNA data showed more than 60% of the pairwise comparisons which were not significantly different, whereas, the remaining showed low but significant partitioning of genetic variation among closely located populations. The estimated variations are possibly due to recent population fragmentation (Daniels and Walters, 2000) or low level of mtDNA evolutionary rate (Mayer and Kerth, 2005) or female-biased dispersal (Clutton-Brock, 1989; Nagy et al., 2007). In addition, mtDNA evolutionary rate may be influenced by the reproductive biology, metabolic rate and biogeographic history of *H. speoris* (Rand, 1994; Caccone et al., 2004). The observed

**Table 4.** Pairwise differences between mtDNA FST values are given below the diagonal and microsatellite FST values are given above the diagonal.

| Population | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | 0.1202** | 0.0978** | 0.0702** | 0.0848*** | 0.0880*** | 0.0970*** | 0.0804*** | 0.1116*** | 0.1022*** | 0.0932*** |
| 2 | 0.1548* | - | 0.0079* | 0.0456** | 0.0974*** | 0.0457*** | 0.0894*** | 0.0510*** | 0.0757*** | 0.1182*** | 0.1318*** |
| 3 | 0.0666$^{NS}$ | 0.1153$^{NS}$ | - | 0.0246** | 0.0738** | 0.0278*** | 0.0705*** | 0.0289*** | 0.0573*** | 0.0921*** | 0.1071*** |
| 4 | 0.2084* | 0.1083$^{NS}$ | 0.1782** | - | 0.0359** | 0.0221*** | 0.0502$^{NS}$ | 0.0176*** | 0.0484*** | 0.0608* | 0.0788*** |
| 5 | 0.2488* | 0.1442$^{NS}$ | 0.1732* | -0.0036$^{NS}$ | - | 0.0251** | 0.0234*** | 0.0406*** | 0.0373*** | 0.0232*** | 0.0336*** |
| 6 | 0.1110* | 0.0794$^{NS}$ | 0.1062* | 0.1018* | 0.1331** | - | 0.0265$^{NS}$ | 0.0230$^{NS}$ | 0.0113* | 0.0344** | 0.0416*** |
| 7 | 0.0175* | 0.1025$^{NS}$ | -0.0243$^{NS}$ | 0.1748* | 0.1588$^{NS}$ | 0.1014$^{NS}$ | - | 0.0470*** | 0.0178$^{NS}$ | 0.0149*** | 0.0254*** |
| 8 | 0.3137** | 0.1702* | 0.2338$^{NS}$ | 0.1361* | 0.0152$^{NS}$ | 0.1260** | 0.2222$^{NS}$ | - | 0.0447** | 0.0601*** | 0.0777*** |
| 9 | 0.5333** | 0.2719** | 0.4074* | 0.2005*** | 0.1033$^{NS}$ | 0.1563** | 0.41667** | 0.0000$^{NS}$ | - | 0.0284*** | 0.0336*** |
| 10 | 0.7989*** | 0.7075*** | 0.7891*** | 0.4922*** | 0.6892*** | 0.4273*** | 0.80142*** | 0.7896*** | 0.8364*** | - | 0.0256*** |
| 11 | 0.7246*** | 0.6272*** | 0.7128*** | 0.4269*** | 0.6204*** | 0.3888*** | 0.72464*** | 0.7200** | 0.7692*** | 0.3256*** | - |

Significant values, * P < 0.05, **P < 0.01, and ***P < 0.001.

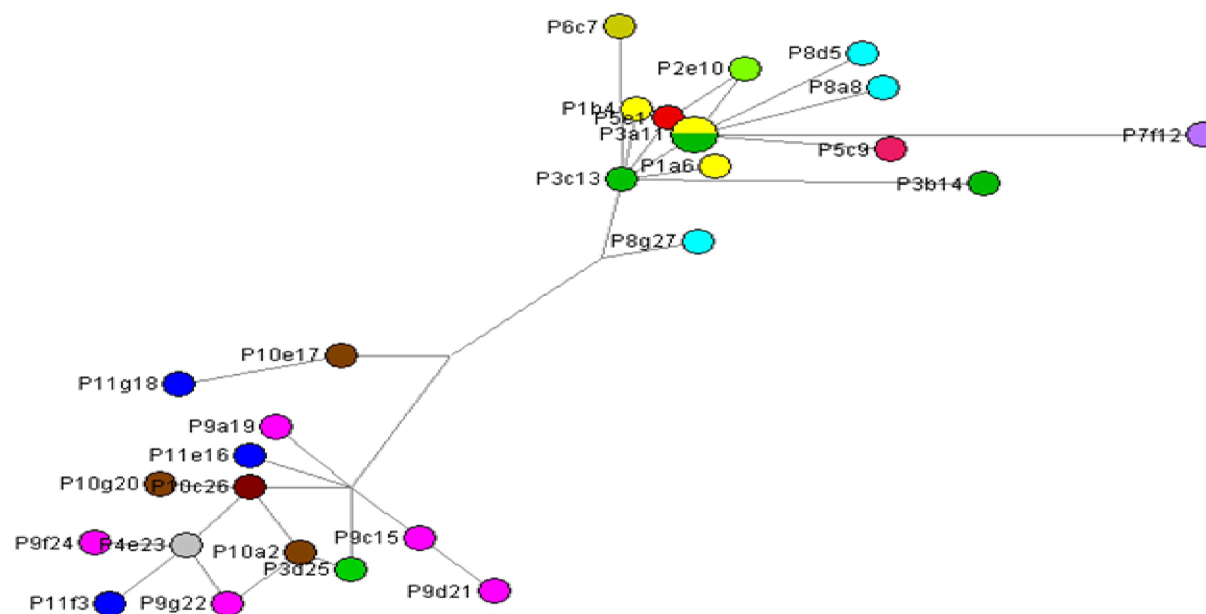


**Figure 2.** Median joining network of 28 mtDNA 16S rRNA haplotypes obtained from eleven populations. Each circle indicates one haplotype, and the size of the circle is proportional to its frequency; the length of the branch connecting two haplotypes is proportional to the number of mutations separating them.

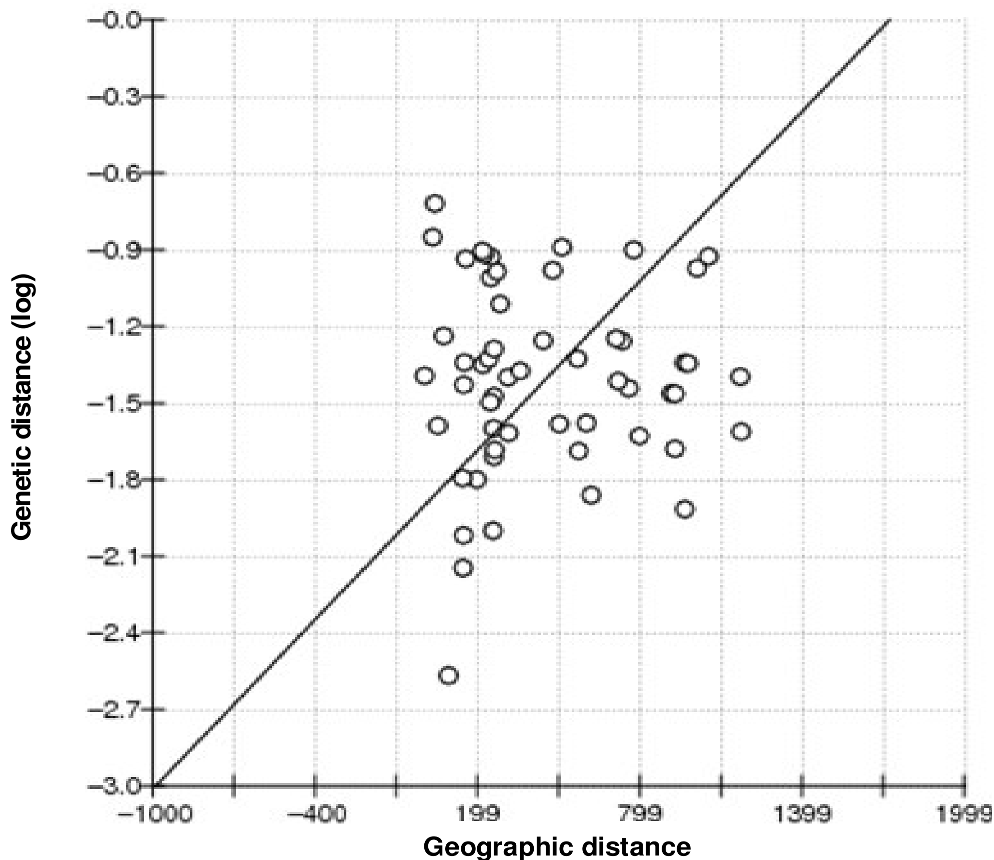


**Figure 3.** Scatter plot showing the relationship between $F_{ST}/(1 - F_{ST})$ and the natural logarithm of geographical distances between populations of *H. speoris*.

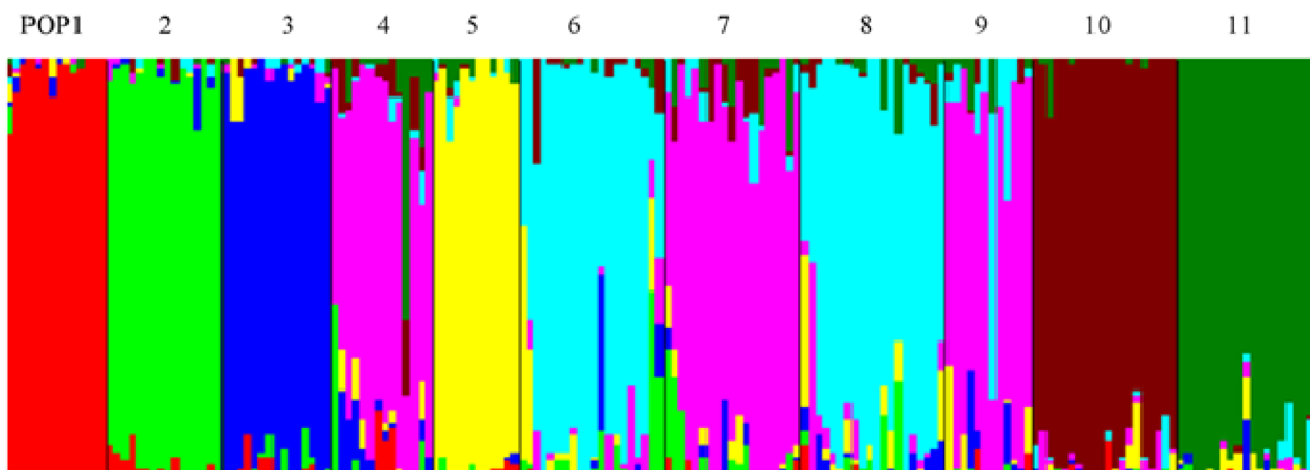


**Figure 4**. Assignment of individual *H. speoris* using BAPS based on population and *K* = 8. Each individual is depicted by a horizontal line that is partitioned into K coloured sections, with the length of each section proportional to the estimated membership coefficient (Q) of the bat to each cluster.

significant difference among the distantly located populations possibly due to lower value of aspect ratio (short, broad wings) of *H. speoris* (Ahmad, 1984). This limits move-ment is only to short distance and adds further evidence for restricted gene flow imposed by geographical distances.

We predicted that gene flow among colonies would be limited due to geographic isolation. However, the median

joining network unique haplotypes exhibit a broad geographic distribution. The two haplotypes from population 3 (P3b) and 4 (P4e) networked with distantly located populations (9, 10 and 11) reflecting the haplotype representation. The internal placement in the haplotype network suggests that, haplotypes are shared between geographically isolated individuals that are otherwise characterized as ancestral sequences or colonization pattern, whereas, all other relations of haplotypes are restricted to their geographically closely located populations. In terms of geographic region, AMOVA indicates a significant genetic break among populations. The highest percentage of genetic variation between the regions suggests that, the gene flow is restricted by geographic distance. Isolation by distance has been noted at microgeographical (Burland et al., 1999) and macrogeographical level in the present study region (Storz, 2002). Furthermore, AMOVA indicates a low percentage of variation within the region and comparatively a higher variation within populations, which support the hypothesis of inter-colony gene flow and existence of out breeding in *H. speoris*.

Population structure has been examined with microsatellite data, the negative $F_{IS}$ value and heterozygosity in all tested populations (even in the closely located urban populations) indicates as an out breeding unit. Heterozygosity is usually above 0.7 in outbred populations (Goldstein and Schlötterer, 1999), estimated heterozygosity value (Table 3) provide additional support to explain *H. speoris* as an out breeding unit. Population differentiation values ($F_{ST}$) can be used to determine the degree of genetic differentiation among populations (Wright, 1978). $F_{ST}$ values of microsatellite data between many population pair were significant, that indicates heterogeneity in the populations. The relationships between the populations showed a broad-scale genetic structure and thereby, suggesting past colonization. Isolation by distance in *H. speoris* might also reflect a mode of stepwise colonization event in the past.

We detected different pattern of genetic structure for microsatellite and mtDNA data. The mtDNA data are not significantly different in most of the closely located urban populations. Whereas, the microsatellite data infer that these populations are significantly different and they are not an inbreeding unit. These differences are best explained by differences in mutation rates or female biased dispersal. Unfortunately, spatial pattern of dispersal or the mating system of *H. speoris* is unknown. The marker that is likely to infer the dispersal rate for females has low evolutionary rate (Goldstein and Schlötterer, 1999). Population cluster analysis further support the obtained genetic structure based on the microsatellite data.

Our study explains the genetic structure of *H. speoris* based mainly on ‘isolation-by-distance’ on populations and availability of foraging habitats and mating sites may facilitate gene flow. Taken together, the results of this study contribute to knowledge of the diversity of *H. speoris* in this biogeographic region, where prior studies in bats have been limited. Our study highlights the importance of further studies of this species and of other endemic bats in India.

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