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Genetic diversity within and among populations of the microchiropteran bat *Hipposideros speoris* based on a RAPD analysis

By K. E. RAJAN and G. MARIMUTHU

*Department of Animal Behaviour and Physiology, School of Biological Sciences,
Madurai Kamaraj University, Madurai, India*

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The microchiropteran bat *Hipposideros speoris* inhabits the southern and central parts of India. It prefers to roost in caves and old buildings (BROSSET 1962). It lives in colonies with 100–1000 individuals per colony. A colony consists of both sexes with mixed ages. Apparently *H. speoris* breeds throughout the year with maximum number of young between August and October (RADHAMANI 1996). Based on a survey, the Biodiversity Conservation Prioritisation Project, India, (1998) placed *H. speoris* in the category “Lower risk – near threatened”. In the present study we used RAPD – PCR to determine genetic variation within and between populations. The major objective is to provide first information on gene pool differentiation among regional populations in Southern India.

A total of 40 bats was captured using nylon mosquito net and mist net from five local populations ($n = 9, 6, 6, 11$, and 8 from the five populations, respectively; Fig. 1). Following the puncture of the median vein with a 27 – gauge needle we collected 0.25 ml blood. Blood samples thus collected were immediately mixed with anticoagulant (ACD) and stored in ice in the field and subsequently frozen at -70°C in the laboratory. Total genomic DNA was extracted from the blood and the DNA samples were diluted to 10 ng/ μl for experimental use.

The PCR reaction was standardized using a protocol modified from WILLIAMS et al. (1990). Total reaction volume of 20 μl was used with a final concentration of 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM MgCl_2 and 0.1% (w/v) gelatin; 2.5 mM each of dATP, dTTP, dCTP, and dGTP; 5 pM primers, 10 ng of template DNA; and 0.5 unit of Taq DNA polymerase (Pharmacia, Uppsala, Sweden). Amplification was done using a Perkin Elmer Gene Amp PCR system 2400. Amplified products were electrophoresed in 8% polyacrylamide gels. The gels were silver stained and photographed.

A total of 10 arbitrary primers (OPA 3, 4, 7, 8 and 10 and OPB 1, 3, 5, 6 and 7; Bangalore Genei Pvt. Ltd., India.) was tested for five populations. Among them OPA10 (5' GTGATCGCAG 3') was found to be ideal for producing RAPD polymorphism in *H. speoris*. The data were scored according to HAIG et al. (1994). Based on the known marker size, different polymorphic band sizes were calculated using Kodak Digital Science (ver 2.01. Kodak Scientific Imaging System, Eastman Kodak Company). TFGA (ver 1.3) (MARK 1997) was used to construct a Nei's (1978) distance matrix for UPGMA cluster analysis. In addition G^2 was estimated for RAPD data with Popgene (ver 1.21) (YANG and YEH 1993). Subsequently F_{st} was estimated along with contemporary estimates of gene flow (SLATKIN and BARTON 1989) using the formula $Nm = 0.5 (1 - F_{st})/F_{st}$. The dis-

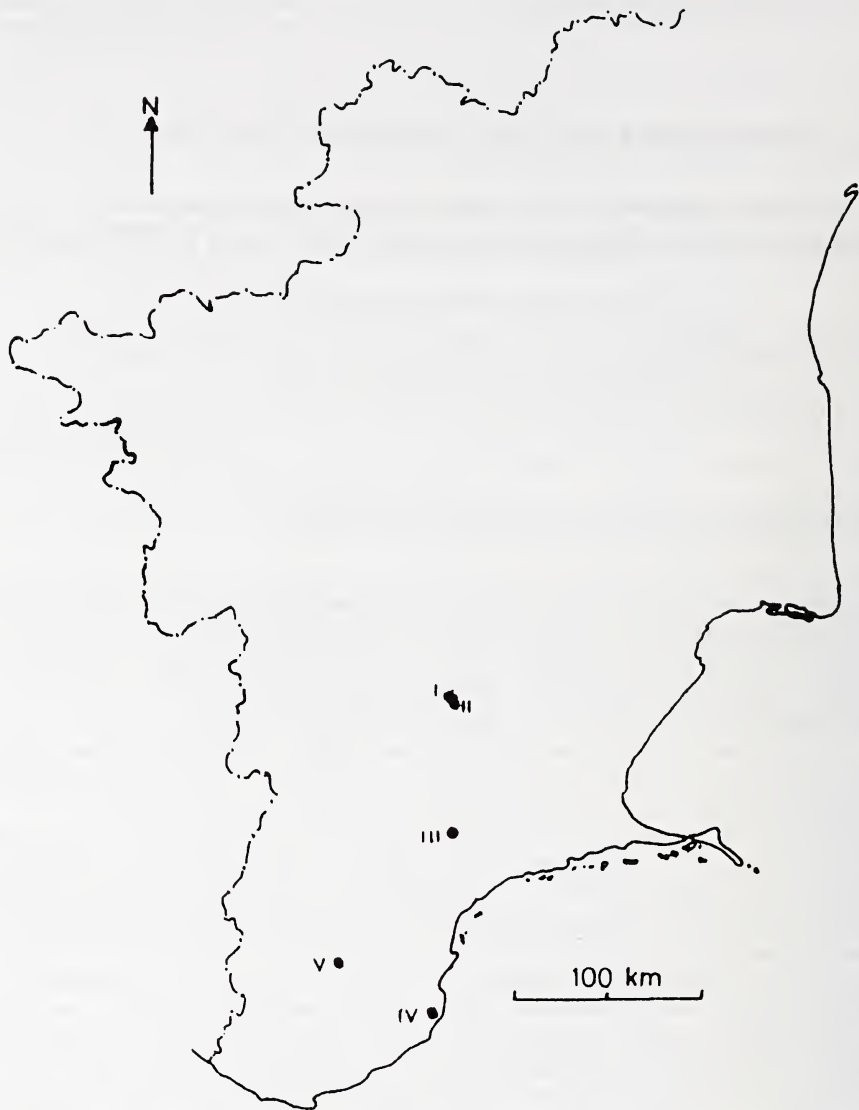


Fig. 1. Map showing the sample localities of the five populations (I to V) of *H. speoris* in South India.

tance matrices were analysed using WINAMOVA (ver 1.55) to estimate the variation among the local populations and individuals within the populations (EXCOFFIER et al. 1992).

RAPD band frequencies varied within and among all estimated populations. Figure 2 shows the representative RAPD pattern obtained with OPA 10 generated from samples of all five populations of *H. speoris*. Nei's (1978 unbiased) genetic index provides genetic diversity within the populations, as follows (Mean \pm SE): population I (0.23 ± 0.07), population II (0.21 ± 0.06), population III (0.16 ± 0.08), population IV (0.22 ± 0.09) and population V (0.15 ± 0.07).

In no case did any two individuals share all the scored bands; thus, individuals of 100% similarity were not found. Nei's (1978 unbiased) genetic distance provides a second

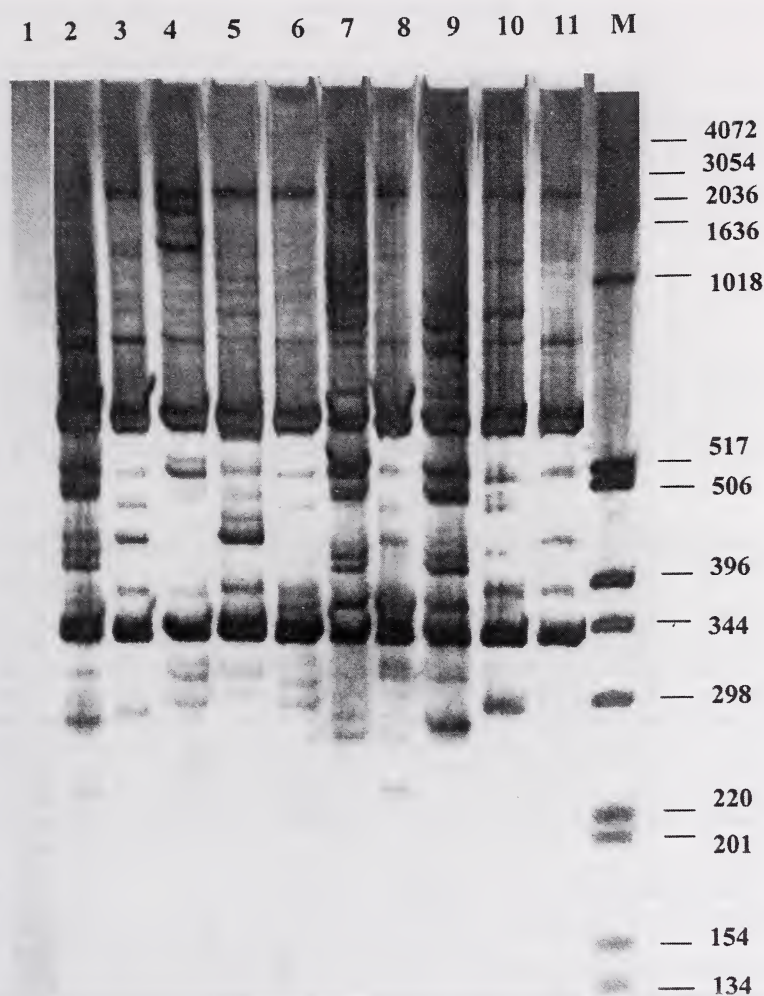


Fig. 2. RAPD amplifications with primer OPA 10 for a representative of five populations of *H. speoris*. Lane 1 is duplicate, M indicates marker, sizes in base pairs. Lanes 2 and 3 indicate population I, lanes 4 and 5 indicate population II, lanes 6 and 7 indicate population III, lanes 8 and 9 indicate population IV and lanes 10 and 11 indicate population V.

index of genetic differentiation between populations and the plotted dendrogram (Fig. 3) shows populations I and II (both were located within 3 km) clustered together as a group. Similarly, population IV and V (200 km apart from population I) clustered into another group. Population III deviated from these two groups.

An overall F_{st} value of 0.21 was obtained. N_m was 1.98, indicating a large amount of gene flow between populations. Similarly, gene frequencies vary significantly across the populations ($G^2 = 16.35$, $df = 4$, $P < 0.001$). The Analysis of Molecular Variance (AMOVA) permitted to partition the overall variations into two levels (Tab. 1). The individuals within populations accounted for about 76.7% of the total variance component and among populations for 23.3%. They were significantly greater than zero at the 0.05 level.

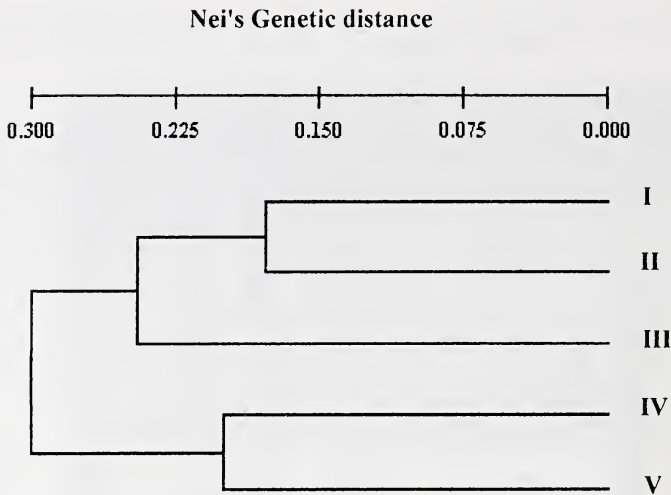


Fig. 3. UPGMA dendrogram constructed by using genetic distance (NEI's 1978) between the populations of *H. speoris*.

Table 1. Analysis of Molecular Variation (AMOVA) for 40 individuals of *H. speoris*. The total data set contains individuals from five populations. Statistics include sum of squared deviations (SSD), mean squared deviations (MSD), variance component estimates, the percentages of total variance contributed by each component and the probability (P) of obtaining a more extreme component estimate by chance alone.

Source of Variation	SSD	MSD	Variance Component	% Total variation	P*-value
Among Populations	152.89	38.22	1.37	23.3	<0.001
Individuals within Populations	536.37	17.23	4.54	76.7	

* After 1000 permutations.

RAPD is one of the methods in the study of population genetics to estimate genetic variability, relatedness, inbreeding level, species verification and pedigree analysis (JAYASANKAR and DHARMALINGAM 1997). It is used to measure the genetic diversity in population studies, based on which numerous conservation efforts have been undertaken in desert fishes (VIRJENHOEK 1995), sea turtles (BOWEN and AVISE 1995) and whales (BAKER and PALUMBI 1995).

Our results enable us to emphasize the genetic diversity in the local populations of *H. speoris*. Similarly, the genetic diversity within and between populations was observed in other bat species such as the little red flying-fox *Pteropus scapulatus* (SINCLAIR et al. 1996), *Tadarida brasiliensis* (McCRACKEN et al. 1994), Australian flying-fox *Pteropus* spp. (WEBB and TIDEMANN 1996) using conventional protein (allozymes) markers and *Myotis myotis* (PETRI et al. 1997) using DNA (mtDNA and simple sequence repeats) markers. SLATKIN (1985) suggested that significant differentiation between populations would be expected only when the N_m value was < 1 . The high value of N_m (1.98) in our study indicates a large amount of gene flow between all populations. Similarly, the N_m was low at

0.05 for the megachiropteran *Haplonycteris fischeri*, a species known to have low vagility compared with an Nm of 7.5 for another megachiropteran *Cynopterus brachyotis*, which is an effective seed disperser (PETERSON and HEANEY 1993).

In *H. speoris* the probability of outbreeding in a geographically closely located population is high. In this species, during the reproductive period freshly matured females move away from the native colony (RADHAMANI 1996). Such natal dispersal behaviour influences also the genetic variation in *Oryctolagus cuniculus* (WEBB et al. 1995) and generally avoids inbreeding (PUSEY and WOLF 1996). Our genetic analysis suggests that *H. speoris* should be treated as an interbreeding unit for management purposes.

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Authors' address: K. EMMANUVEL RAJAN and G. MARIMUTHU, Department of Animal Behaviour and Physiology, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India (e-mail: gmarimuthu@usa.net)

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