International Journal of Biology Research
ISSN: 2455-6548
Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 3; Issue 1; January 2018; Page No. 141-144

Evaluation of genetic diversity within the populations of the Indian false vampire bat, *Megaderma lyra* deduced by RAPD – PCR

Deep Narayan Prasad, Ram Kumar, * Dr. V Elangovan
Department of Applied Animal Sciences, School for Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow, Uttar Pradesh, India

Abstract
Megaderma lyra is a non-migratory carnivorous bat and maintains high level of roost fidelity and its dispersal ability is still obscure. The present work was conducted to examine genetic diversity within the populations of *M. lyra*. RAPD-PCR approach was used to assess the genetic diversity within five colonies located at Barabanki, Uttar Pradesh, India. The RAPD-PCR data illustrates quite a low level of diversity within five populations. A total of 59 bands were amplified with the 6 primers OPA-11, OPAA-09, OPW-02, OPB-18, OPR-08, and OPA-13 with an average of 9.8 bands per primer. The molecular weight ranged from 100-900 bp. The polymorphic loci within the populations of *M. lyra* varied over 46.5%. Among the primers used OPR-08 produced a maximum of 12 bands. The similarity coefficient values ranged from 0.432 to 1.00 while the polymorphic information content (PIC) ranged from 0.651 (OPAA-09) to 0.720 (OPA-11). The alleviated level of heterozygosity reveals that the population will be less resilient to threats and less adaptive.

Keywords: gene flow, genetic diversity, *Megaderma lyra*, polymorphism, RAPD-PCR

Introduction
Megadermatidae bats belong to a large family of carnivorous bats and restricted to the Old World tropics [1]. The Indian false vampire bat, *Megaderma lyra* is a heterogeneous group of echolocating bat. *Megaderma lyra* scattered in patches in different regions of Uttar Pradesh. Like many other tropical bats, *M. lyra* also show its roost fidelity for several years and exhibit no seasonal migration. The sexes of philopatric species exhibit differences in foraging, the male forage about 500 m form the roost while female makes a flight for about 5 km [2]. Female *M. lyra* gets an opportunity to acquire new roosting sites since their home range is relatively larger. It has specially become important for species with isolated distribution, small neighbouring colonies and fragmented populations are more prone to inbreeding, genetic drift, and ultimately leading to loss of genetic diversity [3]. Gene flow may aid adaptation to local conditions by the introduction of either maladaptive or advantageous alleles into a population [4]. Gene flow is often considered to be an important factor to bring genetic diversity within these populations. For instance, it counters drift and may prevent inbreeding depression in fragmented populations [5, 6]. Even within the populations, spatially restricted gene flow may lead to a genetic substructure and may allow selection to lead to micro-site adaptation [7]. Although, IUCN lists *M. lyra* as a least concerned (LC), but conserving this species is of significant importance. Genetic analyses have revealed the consequence of current behavioral and demographic status often supporting evidence for conservation. The physical integrity of *M. lyra* makes them a champion gleaners among bats and often predating insects, pests and even rodents that destroys paddy and crops plants. They are well adapted in caves, old buildings, thatched huts, old disused wells, temples, forts, tunnels, mines, cow sheds in caves, and artificial underground [8]. Several studies were carried out to investigate the behaviour and genetic diversity of *M. lyra* but the exact reasons are still not certain [9, 10]. Now molecular genetic techniques provide easy way to understand the complex behavior and genetic structure [11, 12]. Here, we tried to report the genetic diversity of *Megaderma lyra* via RAPD marker within the neighboring roosts to assess population variation, gene flow, and behavioral information to establish valuable guideline for conservation measures.

Materials and Methods
The tissue samples of bats were collected from the neighbouring colonies of Barabanki, Uttar Pradesh (latitude 26°53'18.72"N; longitude 81°33'24.84"E), India. The roost of *Megaderma lyra* were located at the basement of a storehouse, demolished temple, thatched hut, Pillar crevices and old disused well. They were trapped using a hoop net with an extensible aluminum pole. Bats were also trapped using mist nets. The tissues samples were collected from five neighboring populations located at Barabanki, i.e. FTP [1] (Fatepur Population – 01), RNR [2] (Ramnagar population – 02), NBJ [3] (Nawabganj population – 03), GSP [4] (Gauspur population – 04) and HDH [5] (Haidergarh population – 05). All the neighboring populations were located between 7 and 9 Km without any physical barriers (Fig. 1).

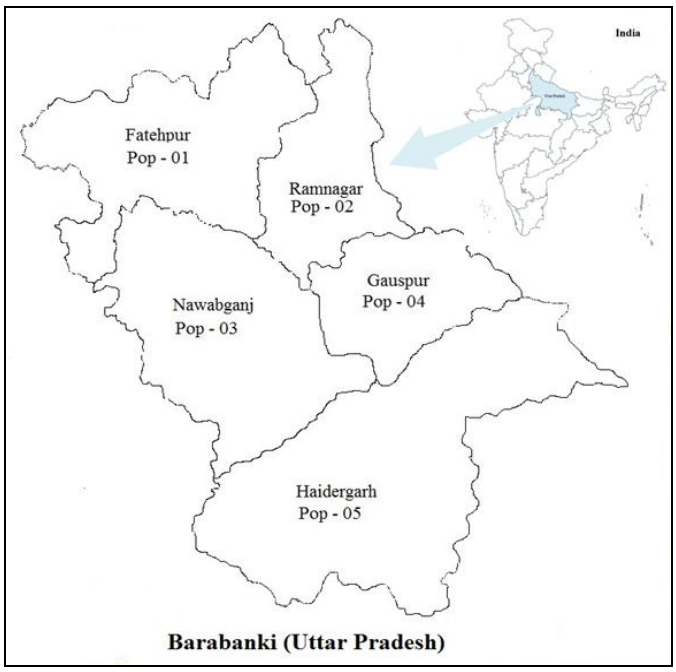

Fig 1: Represents the study sites in Barabanki district, Uttar Pradesh.

A small bit of patagium tissue (>3 sq. mm) from individual bat was collected using a sterile biopsy-punch and the sampled bats were collared for recognition. After collection of each sample, the punched patagium and the biopsy punch were sanitized with 70% ethanol. No biopsy mark was found during the subsequent observation made after a fortnight. The tissues were preserved at –20 °C in 25% DMSO for further DNA extraction. Bats were handled with extreme care and not scarified. Precautions were also taken in capturing them to minimize the possibility of stress and accidental fatalities.

DNA isolation screening

The tissue samples were homogenized using mortor and pistle containing 100 µl of lysis buffer 1% SDS, 10 mM Tris-HCl (pH 8.5), 125 mM NaCl, 5 mM EDTA and 0.5 mg/ml proteinase K (Sigma Aldrich). Followed by homogenization, the DNA extraction was carried out by following phenol:chloroform method [13]. Both the quality and quantity of extracted DNA were confirmed using 0.7% agarose gel electrophoresis and spectrophotometrically at A260 and A280 nm (Perkin-Elmer USA).

Polymerase chain reaction

RAPD-PCR was designed using a set of six primers such as OPA-11, OPAA-09, OPW-02, OPB-18, OPR-08 and OPA-13. Conditions for amplification were set by following [14]. Optimization was done by taking different concentrations of template, primer, $MgCl_2$, and *Taq* DNA polymerase in order to avoid primer dimer (Banglore Genei, India). Primer optimization was also done by calculating the (T_m) value and annealing temperature. The reactions were carried out in a total volume of 15 µl containing 25 ng of genomic DNA, 1.5 mM $MgCl_2$, 200 µM of each DNTP, 50 pmol of a single decamer primer (Banglore Genei, India), 0.5 µg/mL BSA, and 1 U *Taq* DNA polymerase. PCR was carried out in a thermocycler (Gene Amp PCR system 2400, Perkin Elmer) programmed at 94 ºC for 3 min followed by 43 cycles of 30 sec at 94 ºC, 1 min at 35 ºC, and two min at 72 ºC. Finally at last cycle, there was a 5-min of extension at 72 ºC. Initially investigation was carried out with all 13 primers using genomic DNA from five different locations. RAPD-PCR analysis was operated twice so that only primers that gave strong bands were used in the investigation of all the five locations. Gels with amplification fragments were visualized under ultraviolet trans-illuminator and photographed using Gel Doc (Bio Rad).

RAPD profile analysis

NTSYS-pc version 2.0 software was exploited to evaluate the RAPD data. All calculations related regarding genetic similarity (GS) were done with the help of Jaccard's coefficient of similarity GS(ij) = a/(a+b+c). GS(ij) signifies the amount of genetic similarity between the species i and j, b denotes the frequency of bands present in the i while absent in j, whereas c indicates the number of bands present in j while absent in i, respectively. Each RAPD patterns was carried as a single character and was scored as 1/0. This binary matrix was constructed for all the bands scored to generate similarity coefficients. Finally a dendrogram using UPGMA was created by data provided by Jaccard's coefficient. The Polymorphism information content (PIC) a measure of informativeness of these RAPD markers was calculated by following Botstein *et al*. (1980) [15].

Results

The sample collected from the Population site showed noticeable changes in genetic diversity from barabanki (Uttar Pradesh) (Fig. 1). Out of 10 primers screened, most polymorphisms were obtained with OPA-11, OPAA-09, OPW-02, OPB-18, OPR-08, and OPA-13 (Table 1).

Table 1: List of primers, decamer sequences and percent of G+C content.

S. No.	Primer	Sequence	% G+C content
1	OPA-11	CAATCGCCGT	60
2	OPAA-09	AGATGGGCAG	60
3	OPW-02	ACCCCGCCAA	70
4	OPB-18	CCACAGCAGT	60
5	OPR-08	CCCGTTGCCT	60
6	OPA-13	CAGCACCCAC	70

The RAPD fingerprinting data for the five populations of *Megaderma lyra* showed significant level of genetic variation (polymorphism) within the neighboring colonies (Fig. 2). All the primers used were polymorphic in nature and a total of 59 loci were obtained. Out of which 25 bands were polymorphic and 34 bands detected were monomorphic. The highest polymorphic loci was obtained with primer (OPA-11) and lowest with 2 (OPB-13) and (OPB-18) each. The amplification product size value ranged from 100 to 900 bp. The PIC value ranged from 0.656 (OPA-13) to 0.720 (OPA-11) with a mean PIC value of 0.64 the discrimination power of each locus was evaluated by the (polymorphic information content) value (Table 2).

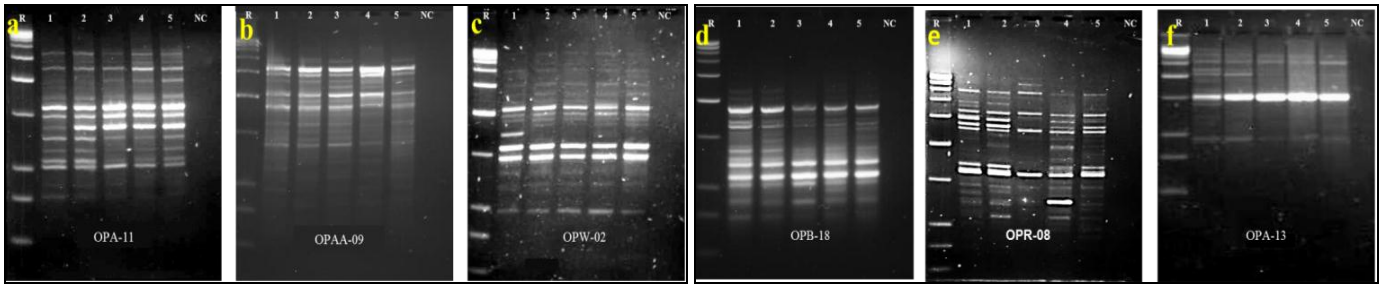

Fig 2: The five neighbouring populations of *M. lyra*: [1] FTP (Fatepur Population – 01), [2] RNR (Ramnagar population – 02), [3] NBJ (Nawabganj population – 03), [4] GSP (Gauspur population – 04) and [5] HDH (Haidergarh population – 05) with reference to primer OPA-11 (a), OPAA-09 (b), OPW-02 (c), OPB-18 (d), OPR-08 (e) and OPA-13 (f). R is a 1000bp DNA ladder. NC represents the negative control.

Table 2: The well amplified primers with number of monomorphic bands, polymorphic bands, and percent of polymorphism value.

S. No	Primer code	No. of bands	No. of monomorphic bands	Size of monomorphic bands (bp)	No. of polymorphic bands	Size of polymorphic bands (bp)	Polymorphism (%)	PIC
1	OPA-11	13	6	600-200	7	407-199	46.15	0.720
2	0PAA-09	10	6	700-200	4	562-144	60.00	0.651
3	OPW-02	10	5	900-300	5	912-223	50.00	0.660
4	OPB-18	8	6	900-250	2	771-251	25.00	0.568
5	OPR-08	12	7	750-300	5	800-300	58.33	0.585
6	OPA-13	6	4	100-230	2	300-550	40.00	0.656
	Total	59	34		25			

The genetic similarity values based on RAPD banding patterns were evaluated by using the method Jaccard's coefficient analysis (Table 3). The cluster constructed through NTSys (2.02 Pc) that is represented in the form of dendrogram (Fig. 3). The UPGMA clustering generated form the binary matrix of the studied population comprised of two groups (group A and group B). Group A is further separated into two sub groups representing three morphotypes (pop 05 and pop 03) in one group and (pop 01) as segregated genotype. Group B comprises of two morphotypes (pop 02 and pop 04) respectively. The % similarity within the populations was 72% between FTP [1] and RNR [2], 42% FTP [1] and NBJ [3], 54% FTP [1] and GSP [4], 74% FTP [1] and HDH [5], 62% RNR [2] and NBJ [3], 57% RNR [2] and GSP [4], 67% RNR [2] and HDH [5] and NBJ [3] and GSP [4] each, NBJ [3] and HDH [5], 66% GSP [4] and HDH [5] respectively.

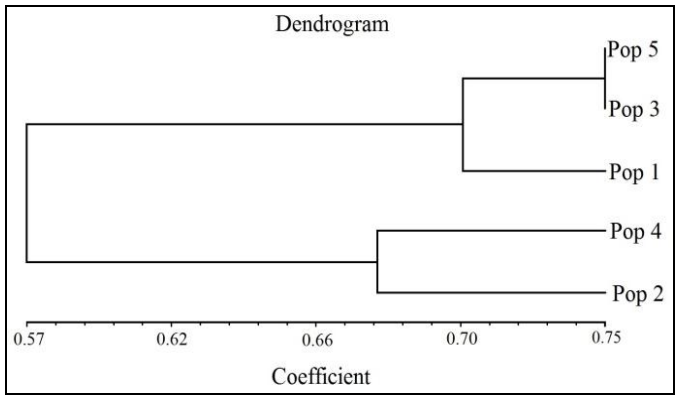

Fig 3: UPGMA based dendrogram showing the phylogenetic relationship within the populations of *Megaderma lyra*.

Table 3: Jaccard's coefficient among five populations of *Megaderma lyra*

	1	2	3	4	5
1	1.00				
2	0.728	1.00			
3	0.423	0.627	1.00		
4	0.543	0.576	0.677	1.00	
5	0.745	0.677	0.610	0.661	1.00

Discussion

The RAPD-fingerprinting showed a high percentage of genetic similarity within the populations of *Megaderma lyra*. Increase in similarity percentage also predicts that the populations maintain high roost fidelity because of long term intra population selection history. However, the low genetic variation limits the species ability to responds to threats in both long and short term [16]. Inbreeding leads to low level of heterozygosity. Heterozygosity may lead to greater probability of the two alleles at a loci being identical. The polymorphic information content (PIC) deduced from the RAPD – fingerprinting ranged from 0.651 to 0.720 suggests less heterogygosity. Decrease in heterozygosis or polymorphism within a population also causes higher mortality, lower fecundity, reduced mating ability, slower growth, more prone to disease, lower ability to withstand stress, leading to intra and inters specific competitive ability [17, 18, 19, 20]. *Mederma lyra*, a non-migratory insectivorous species maintains high degree of roost fidelity and rarely migrate, or disperse over longer distance [21]. It has been reported that the bats equipped with slow manoeuvrable flight and specialized gleaners are likely to make small colonies and maintains site fidelity compared to long range aerial dispersers [22].

It is evident that RAPD markers may also be useful for systematic investigations at the species level as well [23]. More advanced molecular techniques is needed to determine that under what conditions populations could be purged of the genetic loads. Genetic problems are adding to the decline and vulnerability. The contribution of genetic variability to population viability is basically an interaction with physiological, behavioral and ecological processes. For more productive management, options should involve actions to restore genetic variability by reestablishing gene flow by selective translocation.

Acknowledgements
We thank to the Archaeological Survey of India for permitting us to conduct field surveys at historical monuments of Uttar Pradesh. The financial assistance was provided by the University Grants Commission, New Delhi.

References

1. Bates PJ, Ratrimomanarivo FH, Harrison DL, Goodman SM. A description of a new species of *Pipistrellus* (Chiroptera: Vespertilionidae) from Madagascar with a review of related Vespertilioninae from the island. Acta Chiropterologica. 2006; 8(2):299-324.
2. Audet D, Krull D, Marimuthu G, Sumithran S, Singh JB. Foraging behavior of the Indian false vampire bat, *Megaderma lyra* (Chiroptera: Megadermatidae). Biotropica. 1991, 63-67.
3. Avise JC. Molecular Markers. Natural History and Evolution. Chapman and HALL: An International Thomson Publishing Company. 1994.
4. Slatkin M. Gene flow and the geographic structure of natural populations. Science (Washington). 1987; 236(4803):787-792.
5. Young A, Boyle T, Brown T. The population genetic consequences of habitat fragmentation for plants. Trends in Ecology and Evolution. 1996; 11:413-418.
6. Conner JK, Hartl DL. A primer of ecological genetics. Sunderland, MA: Sinauer Associates. 2004.
7. Prentice HC, Lonn M, Lefkovitch LP, Runyeon H. Associations between allele frequencies in *Festuca ovina* and habitat variation in the Alvar grasslands on the Baltic island of Oland. Journal of Ecology. 1995; 83:391-402.
8. Brosset A. The bats of central and western India. Journal of Bombay Natural History Society. 1962; 59:583-624.
9. Goymann W, Leippert D, Hofer H. Sexual segregation, roosting, and social behaviour in a free-ranging colony of Indian false vampires, *Megaderma lyra*. Zeitschrift fuer Saeugetierkunde. 2000; 65:138-148.
10. Balasingh J, Subbaraj R, Isaac SS. Sexual segregation in the Indian false vampire bat, *Megaderma lyra* (Microchiroptera). Bat Res. News. 1994; 35:7-9.
11. Sugg DW, Chesser RK, Dobson FS, Hoogland JL. Population genetics meets behavioral ecology. Trends in Ecology and Evolution. 1996; 11:338-342.
12. Hughes C. Integrating molecular techniques with field methods in studies of social behavior: a revolution results. Ecology. 1998; 79:383-399.
13. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual (No. Ed. 2). Cold spring harbor laboratory press. 1989.
14. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic acids research. 1990; 18(22):6531-6535.
15. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American journal of human genetics. 1980; 32(3):314.
16. Amos W, Harwood J. Factors affecting levels of genetic diversity in natural populations. Philosophical Transactions of the Royal Society B: Biological Sciences. 1998; 353(1366):177.
17. Allendorf FW, Leary RF. Heterozygosity and fitness in natural populations of animals. Conservation biology: the science of scarcity and diversity. 1986, 57-76.
18. Darwin CR. Variation of animals and plants under domestication. John Murray. Landon. United Kingdom. 1986; 2:1-495.
19. Falconer DS. Introduction to quantitative genetics, 3rd ed. Longman, London. 1989.
20. Ledig FT. Heterozygosity, heterosis, and fitness in outbreeding plants. 1986.
21. Advani R. Seasonal Fluctuations in the Feeding Ecology of the Indian False Vampire, *Megaderma lyra* (Chiroptera: Megadermatidae) in Rajasthan. Zeitschrift für Säugetierkunde. 1981; 46(2):90-93.
22. Entwistle AC, Racey PA, Speakman JR. Social and population structure of a gleaning bat, *Plecotus auritus*. Journal of Zoology. 2000; 252(1):11-17.
23. Dinesh KR, Lim TM, Chua KL, Chan WK, Phang VP. RAPD. 1993.