

A preliminary examination of genetic diversity in the Indian false vampire bat *Megaderma lyra*

K. Emmanuvel Rajan & G. Marimuthu

Emmanuvel Rajan, K. & Marimuthu, G., 2006. A Preliminary examination of genetic diversity in the Indian false vampire bat *Megaderma lyra*. *Animal Biodiversity and Conservation*, 29.2: 109–115.

Abstract

A preliminary examination of genetic diversity in the Indian false vampire bat Megaderma lyra.—Habitat loss and fragmentation have serious consequences for species extinction as well as genetic diversity within a species. Random Amplified Polymorphic DNA (RAPD) analysis was employed to assess the genetic diversity within and between four natural populations of *M. lyra*. Our results suggest that the genetic diversity varied from 0.21 to 0.26 with a mean of 0.11 to 0.13 (\pm SD). The mean G_{st} value of 0.15 was obtained from all four populations and estimated average Nm (1.41) showing gene flow between the populations. AMOVA analysis showed 88.96% within and 11.04% among the studied populations. Cluster analyses of RAPD phenotypes showed that specimens were not grouped by geographical origin. The genetic diversity found in the *M. lyra* population may be explained by its breeding behaviors. Though preliminary, the results indicate that all four populations should be considered to maintain the genetic diversity.

Key words: Genetic diversity, Genetic structure, *Megaderma lyra*, Microchiroptera, PCR–RAPD.

Resumen

Examen preliminar de la diversidad genética del falso vampiro mayor Megaderma lyra.—La fragmentación y la pérdida del hábitat tienen graves consecuencias para la extinción de las especies y su diversidad genética. Se empleó el análisis del DNA polimórfico amplificado aleatorio (RADP) para evaluar la diversidad genética dentro de cada población y entre poblaciones naturales de *M. lyra*. Nuestros resultados sugieren que la diversidad genética varía de 0,21 a 0,26, con una media de 0,11 a 0,13 (\pm DE). El valor G_{st} medio de 0,15 se obtuvo de las cuatro poblaciones y el promedio estimado Nm (1,41) indicador del flujo genético entre las poblaciones. El análisis AMOVA dio como resultados un 88,96% dentro de las poblaciones, y un 11,04% entre las poblaciones estudiadas. Los análisis de grupos de los fenotipos RAPD pusieron de manifiesto que los especímenes no estaban agrupados según su origen geográfico. La diversidad genética hallada en la población de *M. lyra* puede explicarse por sus conductas reproductoras. Aunque preliminares, los resultados indican que debería considerarse que las cuatro poblaciones mantienen su diversidad genética.

Palabras clave: Diversidad genética, Estructura genética, *Megaderma lyra*, Microchiroptera, RAPD–PCR.

(Received: 29 XI 05; Conditional acceptance: 2 II 06; Final acceptance: 1 VI 06)

K. Emmanuvel Rajan & G. Marimuthu, Dept. of Animal Behaviour and Physiology, School of Biological Sciences, Madurai Kamaraj Univ., Madurai–625 021, India.

Corresponding author: K. Emmanuvel Rajan, Dept. of Animal Science, School of Life Sciences, Bharathidasan Univ., Tiruchirappalli–620 024, India. E-mail: emmanuvel1972@yahoo.com

Introduction

Molecular genetic techniques nowadays are valuable tools in the studies on fields of population, behavioural and evolutionary biology where the use of traditional methods such as direct observation of individuals or a population is greatly restricted (Burland & Wilmer, 2001). Most of the temperate bats move between roosts over the course of a year and the sexes often use different roosts. In many species, females raise their offspring in maternal colonies and occasionally share their roost with males (Burland et al., 2001). In this context, the application of molecular genetic techniques extracts valuable biological and behavioural information to document population dynamics of the species.

Studies on different bat species using the molecular genetic approach have shown genetic diversity among distant populations (Mahardtunkamsi et al., 2000; Rossiter et al., 2000; Emmanuvel Rajan & Marimuthu, 2000), paternity (Petri et al., 1997), extra harem paternity (Heckel & Helversen, 2003), sex-biased dispersal (Dallimer et al., 2002) and suckling behaviour (Watt & Fenton, 1995).

The Indian false vampire bat *Megaderma lyra* lives in small colonies (30–50 bats) comprising both males and females. It roosts in temples, old buildings, caves and artificial underground tunnels (Brosset, 1962). It belongs to a heterogeneous group of echolocating bats called gleaners. Gleaning bats prefer to capture prey from ground and water surfaces. They consume large arthropods and small vertebrates such as frogs, geckoes, lizards, fish, mice, birds and even smaller bat species (Advani, 1981). The roosting ecology of *M. lyra* provides a unique opportunity to analyse the genetic structure of the natural population.

Like many tropical bats, individuals of *M. lyra* use their roost constantly for several years and exhibit no seasonal migration or hibernation. A recent study on *M. lyra* suggests that its reproductive success as well as the population size gradually decreased from 1995 to 2003 (Sripathi et al., 2004).

It is important to understand the genetic structure of declining population of *M. lyra* to identify priorities for its conservation. We chose Randomly Amplified Polymorphic DNA (RAPD) markers to estimate the genetic diversity, because (a) it reveals even minimal genetic differences by means of assessing polymorphism from large part of nuclear genome (Borowsky, 2000) and (b) RAPD-PCR is effectively employed to detect genetic variation in species listed as endangered, with scarce genetic variability or population sizes as a limiting factor (Haig et al., 1996; Kimberling et al., 1996; Kim et al., 1998; Vandwoestijne & Baguette, 2004). So far, very little is known about the mating behaviour and population dynamics and this is the first report of the genetic diversity in the Indian false vampire bat *M. lyra*.

Material and methods

Bats were captured by mist net from four different populations (I to IV) of *M. lyra* at four different locations, Population I (n = 9) from Pannian cave 15 km away from Madurai Kamaraj University campus, Madurai (9° 58' N, 78° 10' E); Population II (n = 11), 180 km away from population I towards the south, near Tirunelveli (8° 44' N, 77° 42' E); Population III (n = 15) 20 km away from population II towards the southeast; Population IV (n = 15) 60 km away from population II towards the southwest (fig. 1). We collected 0.25 ml of blood samples from each individual. Samples were stored (Emmanuvel Rajan & Marimuthu, 2000) until the genomic DNA was isolated. Along with blood sampling, the sex, reproductive status and body mass of each individual were recorded.

Total genomic DNA was extracted from the blood by Sambrook et al's method (1989). The concentration of DNA samples was quantified spectrophotometrically and diluted to 10 ng/μl for further experimental use. The PCR reaction was carried out using a modified protocol of Williams et al. (1990). Each RAPD reaction (20 μl) contained 10 x buffer; 2.5 mM each of dNTP; 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. The samples were subjected to the following PCR profiles: 5 minutes denaturation at 94°C followed by 40 cycles of 90 sec denaturation at 94°C, 90 sec annealing at 32°C, 2 min extension at 72°C and an additional one cycle for 7 min at 72°C as the final extension. Negative reactions with no DNA template were used to check for contamination.

The following primers were chosen based on their reproducibility, banding pattern and polymorphism OPA3–5'AGTCGCCACT3', OPA4–5'AATCGGGCTG3', OPA10–5'GTGATCGCAG3', OPB1–5'GTTTCGCTG3', OPB3–5'CATCCCCCTG 3'and OPB5–5'TGCGCCCTTC3' (Bangalore Genei Pvt. Ltd., India). The consistency of the RAPD reaction was examined through a series of repeatability test for each individual from all four populations tested within and between PCR runs. Finally, successfully repeatable samples only were included for subsequent genetic analysis and considered as a sample size for each population.

For analysis, the amplified products were electrophoresed in 6.0% polyacrylamide gel, at 100 V for 12 h in 1 x TAE buffer. Gels were silverstained (Sanguinetti et al., 1994) and photographed for subsequent analysis. Based on the known marker size (mixture of 1018 bp fragment and its multimer with pBR322 fragment), different polymorphic band sizes were calculated using Kodak Digital Science (ver 2.01, Kodak Scientific Imaging System, Eastman Kodak Company). Amplified polymorphic bands were scored as dicerte, binary data was created for each individual's presence (1) / absence (0) and analyzed as allelic variants hav-

ing a dominant inheritance. One individual variable was chosen as the minimum criterion for band polymorphism (Haig et al., 1994).

A data matrix of the individual X marker containing the band scoring information was transformed to allele frequencies under the assumption that each amplified band corresponds to a different RAPD locus. This data set was used to calculate Nei's genetic diversity (h) (Nei, 1973) and Shannon's Index (S) (Allnutt et al., 1999). The gene flow between the populations was estimated from the G_{st} for each locus:

$$Nm = 0.25 (1 - G_{st}) / G_{st}$$

(Slatkin & Barton, 1989). In addition, gene frequencies across the population were tested by the homogeneity test (G -square statistics) using Popgene version 1.31. AMOVA-PREP version 1.01 (Miller, 1998) was used to construct a data matrix for AMOVA version 1.55 (Excoffier et al., 1992), which is used to examine genetic difference among populations. An analysis of molecular variation (AMOVA) was performed on total molecular variation divided into two separate components (i) inter-individual difference within each population and (ii) inter-population difference by means of the Best Cut Test on 1000 permutation. An Unweighted Pair Group Method with Arithmetic Average (UPGMA) dendrogram was constructed using genetic distance based on Sneath & Sokal (1973).

Results

A total of 14 markers generated from the primer were used in this study. RAPD band frequencies varied within and among all estimated populations, and the PCR fragment sizes ranged from 154 bp to 1636 bp (fig. 2). In no case did any two individuals share all the scored bands, and therefore no individuals of 100% similarity were found. The lowest genetic diversity estimated by Nei's genetic diversity index and Shannon's index was observed in population IV ($h = 0.21 \pm 0.11$; $S = 0.22 \pm 0.14$), while the highest value was observed in population I ($h = 0.26 \pm 0.13$; $S = 0.28 \pm 0.16$). Pairwise G_{st} value was calculated against each of them, and showed that population IV contributed less than other populations (excluding population IV the G_{st} value 0.11) and population I contributed more than other populations (excluding population I the G_{st} value 0.27). The mean G_{st} value of 0.15 was obtained. This resulted in an average estimate Nm of 1.41 indicating a large amount of gene flow between populations. Gene frequencies among different populations from different geographical regions were compared using the G^2 test. All population pairs were significantly heterogeneous ($G^2 = 10.85$, $df = 3$, $P < 0.001$). The pairwise value derived from AMOVA indicated a large number of significant differences between populations ($P < 0.05$) when population IV was excluded from the analysis. The result showed a

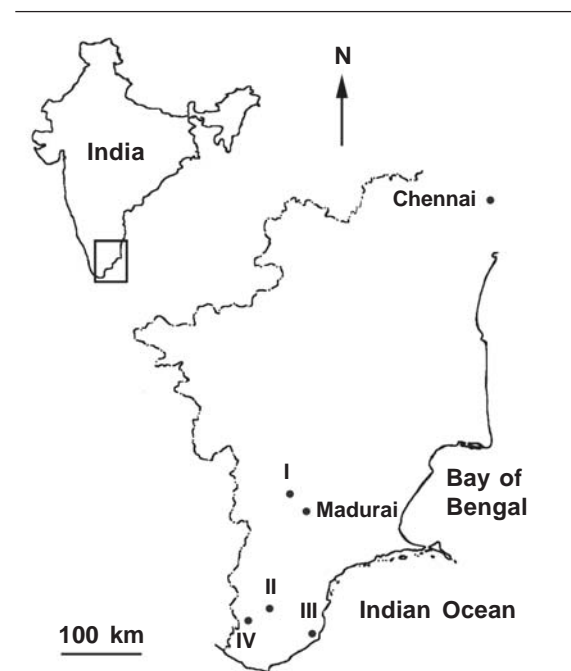


Fig. 1. Locations of four populations (I to IV) of *M. lyra* in and around Madurai in Southern India.

Fig. 1. Localización de las cuatro poblaciones (I a IV) de *M. lyra* en y alrededor de Madurai, en el sur de la India.

genetic variation at 0.05% significant level of 88.96 % within populations and 11.04 % between populations (table 1).

When genetic distances between the populations were used to construct a cluster diagram in order to examine the relationship between the populations, no consistent geographical structure of the RAPD variation in *M. lyra* was compatible with restricted gene flow and long-term isolation (fig. 3).

Discussion

A recent study on population I of *M. lyra* at Madurai (South India) indicates that the size of the population is dwindling (Sripathi et al., 2004). A decrease in population size is usually due to (a) habitat destruction (b) human poaching or (c) loss of genetic variation (i.e. only a small number of individuals that actually contribute to a gene pool; this may increase the probability of population extinction through a decline in fecundity and viability) (Pusey & Wolf, 1996). The development of RAPD (Welsh & McClelland, 1990) has been useful to demonstrate the genetic status of endangered species and the technique is also useful in

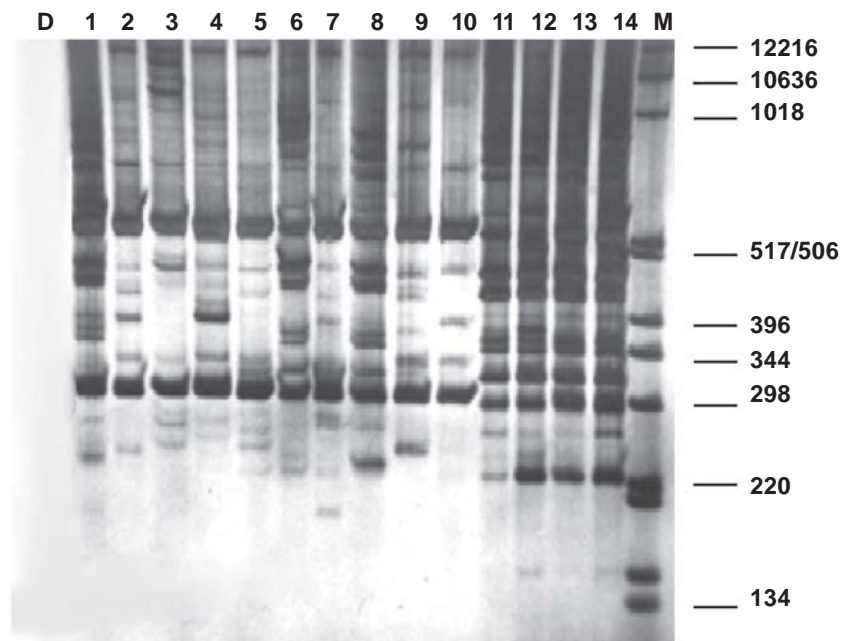


Fig. 2. Examples of RAPD amplifications with primer OPB 5. Lane 2–3, 4–5, 6–7, 8–9, 10–11 are mother–young pairs of *M. lyra* and lane 1, 12, 13, 14 are male samples: D. Negative control without template DNA; M. Indicated marker.

Fig. 2. Ejemplos de amplificaciones RAPD con el cebador genético OPB 5. Las pistas 2–3, 4–5, 6–7, 8–9, 10–11 son parejas madre–hijo de *M. lyra*, y las pistas 1, 12, 13, 14 proceden de muestras de machos: D. Control negativo sin plantilla de DNA; M. Marcador indicado.

conservation efforts in desert fishes (Vrijenhoek, 1995), sea turtles (Bowen & Avise, 1995) and Blanding's turtle (Rubin et al., 2001).

In the present study, we used three different approaches for data analysis, genetic diversity, het-

erozygosity and genetic differentiation. The analysis of genetic diversity in *M. lyra* shows that no population has a higher degree of diversity. The genetic diversity found in this study is related to other bat species such as *Tadarida brasiliensis*

Table 1. Analysis of Molecular Variation (AMOVA) for 35 individuals of *M. lyra*. The total data set contains individuals from three populations. Statistics include the sum of squared deviations (SSD), mean squared deviations (MSD), variance component estimates (VCE), the percentages of total variance contributed by each component (%TV) and the probability (P) of obtaining a more extreme component estimated by chance alone: *** After 1,000 permutation.

Tabla 1. Análisis de variación molecular (AMOVA) para 35 individuos de *M. lyra*. La serie completa de datos contiene individuos procedentes de tres poblaciones. Las estadísticas incluyen la suma de las desviaciones elevadas al cuadrado (SSD), las desviaciones medias elevadas al cuadrado (MSD), las estimaciones de los componentes de varianza, los porcentajes de la varianza total aportada por cada componente, y la probabilidad (P) de obtener un componente más extremo estimado únicamente al azar.

Source of variation	df	SSD	MSD	VCE	% TV	P***
Between populations	2	170.40	21.3	0.81	11.04	< 0.001
Individuals within population	34	1017.84	6.5	6.56	88.96	

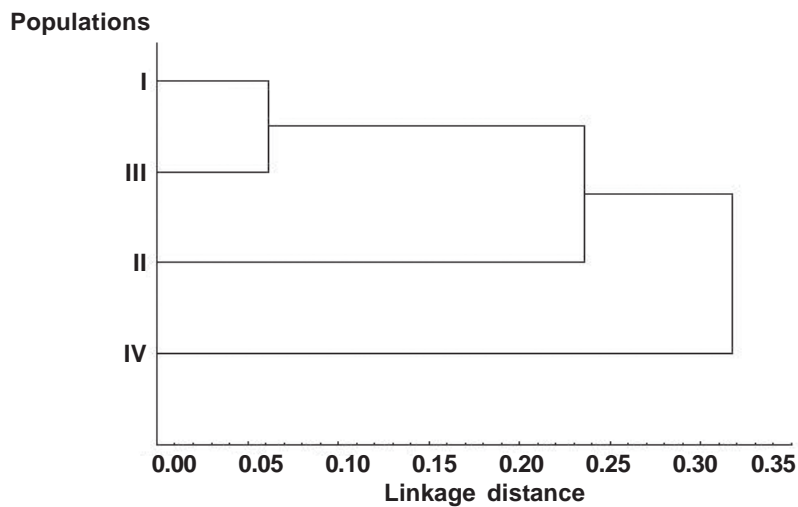


Fig. 3. Dendrogram showing the genetic relationship of the four populations of *M. lyra* based on the inter-population distance and cluster analysis by UPGMA

Fig. 3. Dendrograma que muestra las relaciones genéticas de cuatro poblaciones de *M. lyra*, basándose en la distancia entre poblaciones y el análisis de grupos mediante UPGMA.

(McCracken et al., 1994), the Australian flying-fox *Pteropus* spp (Webb & Tideman, 1996) —using conventional protein (allozymes) markers—, *Myotis myotis* (Petri et al., 1997) —using DNA markers, mtDNA and Simple Sequence Repeats—, and a gleaning bat *Plecotus auritus* (Burland et al., 1999).

The high values of N_m (1.41) and low values of G_{st} (0.15) indicate a large amount of gene flow between populations. Slatkin (1985) suggested that significant differentiation between populations would be expected only if the N_m value was < 1.0 . It is worth mentioning that despite the relatively high level of gene flow in these four populations, there were significant genetic differences among *M. lyra* in different regions. Similarly, there are a few other studies on genetic variation in megachiropterans in the Philippine islands (Peterson & Heaney, 1993) in which allozymes were used to estimate the level of gene flow for small fruit bats. Allele estimation of N_m was low at 0.05 for *Haplonycteris fischeri*, a species known to have low vagility compared with an N_m of 7.5 for *Cynopterus brachyotis*, which is an effective seed disperser and long distance forager (Peterson & Heaney, 1993). The level of genetic differentiation that we observed within and between populations of *M. lyra* compares favorably with other bat species such as the little red flying-fox *Pteropus scapulatus* (Sinclair et al., 1996) and the Indian leaf-nosed bat *Hipposideros speoris* (Emmanuvel Rajan & Marimuthu, 2000).

Gene flow between the colonies probably occurs mainly via extra-colony copulations without

permanent dispersal from the natal colony, but it may be limited by distance as well as availability of suitable mating sites or recently fragmented populations. Extra-colony copulation has been detected in most animal species, where interestingly no natal dispersal occurred in either sex (Amos et al., 1993). The recent mark-and-recapture studies at the roosting place show the fluctuation in population size of *M. lyra* during the breeding season (Sripathi et al., 2004). These observations support the extra-colony copulation and inbreeding avoidance behaviour, factors that may drive the maternity roost and determine the immigration/emigration dynamics of a particular colony. However, population 1 and 3 are genetically more related than the other populations estimated in the present study. Future studies using advanced molecular markers in a greater number of samples (especially samples from populations 1 and 3) and distant populations will add additional insights to our understanding of the genetic diversity and contribute to conservation assessment of *M. lyra* in the Indian subcontinent.

Acknowledgements

We thank Prof. G. Shanmugam for providing laboratory facilities and Dr. K. Kannan for helping in the experiments. Thanks also go to Francis C. Yeh and Rong-Cai Yang for providing the Popgene Genetic Analysis software program and to the Bioinformatics Centre, MKU for providing facilities to analyse the data.

References

- Advani, R., 1981. Seasonal fluctuations in the feeding ecology of the Indian false vampire *Megaderma lyra*. *International Journal of Mammalian Biology*, 46: 90–93.
- Allnutt, T. R., Newton, A. C., Lara, A., Premali, A., Armesto, I. J., Vergara, R. & Gardner, M., 1999. Genetic variations in *Fitzroya cupressoides* (alerce) a threatened South American conifer. *Molecular Ecology*, 8: 975–987.
- Amos, W., Schlötterer, C. & Tautz, D., 1993. Social structure of pilot whales revealed by analytical DNA profiling. *Science*, 260: 670–672.
- Bowen, B. W. & Avise, J. C., 1995. Conservation genetics of marine turtles. In: *Conservation genetics: case histories from nature*: 190–237 (J. C. Avise & J. L. Hamrick, Eds.). Columbia Univ. Press, New York.
- Brosset, A., 1962. Bats of central and western India. *Journal of Bombay Natural History and Society*, 59: 608–613.
- Borowsky, R. J., 2000. Estimating nucleotide diversity from random amplified polymorphic DNA and amplified fragment length polymorphism data. *Molecular Phylogenetic and Evolution*, 18: 143–148.
- Burland, T. M., Barratt, E. M., Beaumont, M. A. & Racey, P. A., 1999. Population genetic structure and gene flow in a gleaning bat *Plecotus auritus*. *Proceeding of Royal Society of London (B)*, 266: 975–980.
- Burland, T. M., Barratt, E. M., Nichols, R. A. & Racey, P. A., 2001. Mating patterns, relatedness and the basis of natal philopatry in the brown long-eared bat, *Plecotus auritus*. *Molecular Ecology*, 10: 1309–1321.
- Burland, T. M. & Wilmer, J. W., 2001. Seeing in the dark: molecular approaches to the study of bat populations. *Biological Review*, 76: 389–409.
- Dallimer, M., Blackburn, C., Jones, P. J. & Pemberton, J. M., 2002. Genetic evidence for male biased dispersal in the red-billed quelea *Quelea quelea*. *Molecular Ecology*, 11: 526–533.
- Emmanuvel Rajan, K. & Marimuthu, G., 2000. Genetic diversity among populations of the microchiropteran bat *Hipposideros speoris* based on RAPD analysis. *International Journal of Mammalian Biology*, 65: 301–306.
- Excoffier, L., Smouse, P. E. & Quattro, J. M., 1992. Analysis of molecular variation inferred from metric distances between DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131: 479–491.
- Haig, S. M., Bowman, R. & Mullins, T. D., 1996. Population structure of red-cockaded woodpeckers in South Florida – RAPDs revisited. *Molecular Ecology*, 5: 725–734.
- Haig, S. M., Rhymer, J. M. & Heckel, D. G., 1994. Population differentiation in randomly amplified polymorphic DNA of red-cockaded woodpeckers *Picoides borealis*. *Molecular Ecology*, 3: 581–595.
- Heckel, D. G. & Helversen, O. V., 2003. Genetic mating system and the significance of harem associations in the bat *Saccopteryx bilineata*. *Molecular Ecology*, 12: 219–227.
- Kim, I., Philips, C. J., Monjeau, J. A., Birney, E. C., Noack, K., Pumo, D. E., Sikes, R. S. & Dole, J. A., 1998. Habitat islands, genetic diversity, and gene flow in patagonian rodent. *Molecular Ecology*, 7: 667–678.
- Kimberling, D. N., Ferreira, A. R., Shuster, S. M. & Keim, P., 1996. RAPD marker estimation of genetic structure among isolated northern leopard frog populations in the south-west USA. *Molecular Ecology*, 5: 521–529.
- Maharadatunkamsi, H. S., Kitchener, D. J. & Schmitt, L. H., 2000. Genetic and morphometric diversity in Wallacea: geographical patterning in the horseshoe bat, *Rhinolophus affinis*. *Journal of Biogeography*, 27: 193–201.
- McCracken, G. F., McCracken, M. K. & Vawter, T., 1994. Genetic structure in migratory populations of the bat *Tadarida brasiliensis mexicana*. *Journal of Mammalogy*, 75: 500–514.
- Miller, M. P., 1998. AMOVA-PREP, a program for the preparation of AMOVA input files from dominant makers raw data release 1.01, Department of Biological Sciences, Northern Arizona Univ., Flagstaff.
- Nei, M., 1973. Analysis of genetic diversity in subdivided populations. *Proceedings of National Academy of Sciences USA*, 70: 3321–3323.
- Peterson, A. T. & Heaney, L. R., 1993. Genetic differentiation in Philippine bats of genera *Cynopterus* and *Haplonycteris*. *Biological Journal of Linnean Society*, 49: 203–218.
- Petri, B., Pääbo, S., Haeseler, A. V. & Tautz, D., 1997. Paternity assessment and population subdivision in a natural population of the larger mouse-eared bat *Myotis myotis*. *Molecular Ecology*, 6: 235–242.
- Pusey, A. & Wolf, M., 1996. Inbreeding avoidance in animals. *Trends in Ecology and Evolution*, 11: 201–206.
- Rossiter, S. J., Jones, G., Ransome, R. D. & Barratt, E. M., 2000. Genetic variation and population structure in the endangered greater horseshoe bat *Rhinolophus ferrumequinum*. *Molecular Ecology*, 9: 1131–1135.
- Rubin, C. S., Warner, R. E., Bouzat, J. L. & Paige, K. N., 2001. Population genetic structure of Blanding's turtles (*Emydoidea blandingii*) in an urban landscape. *Biological Conservation*, 99: 323–330.
- Sambrook, J., Fritsch, E. F. & Maniatis, T., 1989. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, New York.
- Sanguinetti, G. J., Neto, E. D. & Simpson, A. J. G., 1994. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques*, 17: 915–917.

- Sinclair, E. A., Webb, N. J., Marchant, A. D. & Tidemann, C. R., 1996. Genetic variation in the little red flying-fox *Pteropus scapulatus* (Chiroptera: Pteropodidae): implication for management. *Biological Conservation*, 76: 45–50.
- Slatkin, M., 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics*, 16: 393–430.
- Slatkin, M. & Barton, N. H., 1989. A comparison of three indirect methods for estimating average levels of gene flow. *Evolution*, 43: 1349–1368.
- Sripathi, K., Raghuram, H., Rajasekar, R., Karuppudurai, T. & Abraham, S. G., 2004. Population size and survival in the Indian False vampire bat *Megaderma lyra*. *Acta Chiropterologica*, 6: 145–154.
- Sneath, P. H. A & Sokal, R. R., 1973. *Numerical Taxonomy*. Freeman, San Francisco.
- Vandwoestijne, S. & Baguette, M., 2004. Genetic population structure of the vulnerable bog fritillary butterfly. *Hereditas*, 141: 119–126.
- Virjenhoek, R. C., 1995. Conservation genetics of North American desert fishes. In: *Conservation genetics: case histories from nature: 367–397* (J. C. Avise & J. L. Hamrick, Eds.). Columbia Univ. Press, New York.
- Watt, E. M. & Fenton, M. B., 1995. DNA fingerprinting provides evidence of discriminate suckling and non-random mating in little brown bats *Myotis lucifugus*. *Molecular Ecology*, 4: 261–264.
- Webb, N. J. & Tidemann, C. R., 1996. Mobility of Australian flying-foxes, *Pteropus spp.* (Megachiroptera): evidence from genetic variation. *Proceedings of Royal Society of London (B)*, 263: 497–502.
- Welsh, J. & McClelland, M., 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research*, 18: 7213–7218.
- Williams, J. G. K., Kubeilk, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V., 1990. DNA poly-morphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, 18: 6531–653.
-