Genetics and Molecular Biology, 23, 4, 793-797 (2000)

RAPD analysis of *Nectomys squamipes* (Rodentia, Sigmodontinae) populations

Francisca C. Almeida[1,2], Miguel A.M. Moreira[1], Cibele R. Bonvicino[1] and Rui Cerqueira[2]

Abstract

Random amplified of polymorphic DNA (RAPD) analysis was used to assess genetic distance and the genetic structure of populations of *Nectomys squamipes*, a semiaquatic rodent species distributed along watercourses. DNA samples of five populations were analyzed using three primers, producing 45 scorable bands, 31 of which were polymorphic. There was a significant differentiation among populations [$F_{ST} = 0.17$; $\Phi_{ST} = 0.14$ ($P < 0.004$)] but gene flow ($Nm = 1.25$) was sufficient to overcome genetic drift effects. No fixed specific markers were found for any population. The Mantel's test and UPGMA cluster analysis showed a lack of relationship between genetic and geographic distances. The apparent homogeneity indicated by RAPD markers coincided with morphometric data, despite the wide geographic range of *N. squamipes*. Alternative hypotheses for explaining our results include recurrent processes of local extinction and recolonization or a recent and sudden increase in the geographic distribution of this species.

INTRODUCTION

Water rats of the genus *Nectomys* (Rodentia, Sigmodontinae) comprise 11 species that can be distinguished from one another by their morphometric and karyotypic attributes (Bonvicino, 1994). This genus is widely distributed in the South American continent, with all species inhabiting watercourse banks (Ernest and Mares, 1986). *Nectomys squamipes* is a medium-sized rodent, with a body weight ranging from 160 to 400 g, distributed along the São Francisco and Paraná River basins and small independent basins in eastern Brazil (Musser and Carleton, 1993). Karyotypic analysis indicated that *N. squamipes* has a basic diploid number (2n) of 56 chromosomes with an autosomic fundamental number (FN) of 56 (Maia *et al.*, 1984; Yonenaga-Yassuda *et al.*, 1987; Bonvicino *et al.*, 1996).

Nectomys squamipes is a natural host of *Schistosoma mansoni*, with high prevalence in endemic areas, and is an important experimental model for schistosomiasis (Rey, 1993; D'Andrea *et al.*, 1996). Reproduction of *N. squamipes* occurs throughout the year, peaking during the rainy season, with a gestation period of approximately 30 days, and a most frequent litter size of five (ranging from two to seven; D'Andrea *et al.*, 1996). This species is nocturnal, with an omnivorous diet that includes leaves, fruits, fungi, invertebrates and small vertebrates (D'Andrea *et al.*, 1996). Field studies reported that the home range of *N. squamipes* is small, with a maximum recapture distance of 90.4 + 55.3 m for males and 42.8 + 28.9 m for females. A preliminary report on the geographic variation of *N. squamipes* showed limited morphometric differentiation among populations (Bonvicino, 1994).

Studies on genetic variability within and between populations can provide insights on speciation and a better understanding of the evolutionary trends. This is important for assessing prospective evolutionary potentials as well as for risk assessments and conservation strategies. During the course of a survey on the geographic variation of *N. squamipes*, liver samples were obtained from specimens of five different localities. We used random amplified of polymorphic DNA (RAPD; Williams *et al.*, 1990; Welsh and McClelland, 1990) for analyzing the genetic structure of these populations and the genetic variability within and among them. We chose RAPD because it is expeditious and inexpensive and because it does not require a previous knowledge of the genome of species under study (Lynch and Milligan, 1994). Earlier comparisons showed that RAPD and isoenzyme results are very similar, with the RAPD technique revealing even higher amounts of variation (Baruffi *et al.*, 1995; Sinclair *et al.*, 1996).

MATERIAL AND METHODS

Samples

Five natural populations of *Nectomys squamipes* were sampled: 18 individuals from Glicério (22°14'S, 42°03'W, in three trapping transects), 17 from Sumidouro (22°03'S, 42°40'W, in six trapping transects), six from Fazenda União (22°25'S, 42°02'W, in two trapping transects), Rio de Janeiro State, eight from Pedreira (22°43'S, 46°55'W, in four trapping transects), São Paulo State, and six from Fazenda Canoas (16°50'S, 43°35'W, in one trapping transect), Minas Gerais State, Brazil (Figure 1). At least one individual from each locality was karyotyped. Livers were stored in 100% ethanol, at -70°C or in liquid nitrogen.

[1]*Divisão de Genética, Coordenação de Pesquisa, Instituto Nacional de Câncer, Rio de Janeiro, RJ, Brasil.*
[2]*Departamento de Ecologia, Laboratório de Vertebrados, Universidade Federal do Rio de Janeiro, Caixa Postal 68020, 21491-590 Rio de Janeiro, RJ, Brasil. Send correspondence to F.C.A. Fax: +55-21-290-3008. E-mail: falmeida@biologia.ufrj.br*

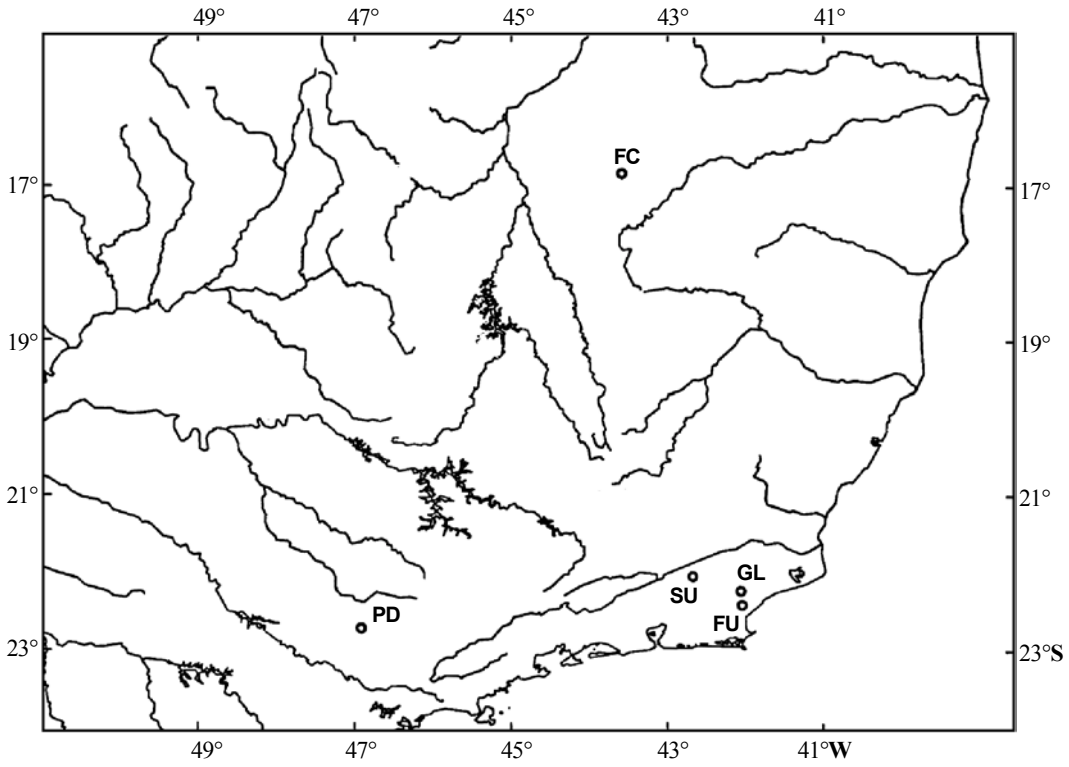

Figure 1 - Map showing sample sites for the five populations of *Nectomys squamipes*. GL = Glicério, SU = Sumidouro, FU = Fazenda União, PD = Pedreira, and FC = Fazenda Canoas.

DNA extraction

DNA from ethanol-preserved liver samples was extracted as described by Smith *et al.* (1987) while extraction from samples stored in liquid nitrogen and at -70°C followed the procedure of Sambrook *et al.* (1989). DNA was stored in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) at 4°C. DNA samples were run in 0.8% agarose gels stained with ethidium bromide to assure that degradation had not occurred. DNA concentrations were estimated by comparisons with a standard sample in an agarose gel.

RAPD-PCR

PCR reactions were carried out with 200 ng of template DNA in 67 mM Tris, pH 8.8, 6.7 mM $MgSO_4$, 16.6 mM $(NH_4)_2SO_4$, 10 mM β-mercaptoethanol, 1 mM of each dNTP, 250 ng of primer and 1 unit of Taq DNA polymerase in a final volume of 50 µl. Amplifications were carried out in a DNA Thermal Cycler (Perkin-Elmer 480) under the following conditions: one initial cycle of 3' at 95°C, 5' at 35°C, and 5' at 72°C followed by 43 cycles of 1' at 95°C, 1' at 35°C and 2' at 72°C, with a final extension period of 15' at 72°C. Tubes containing all reaction components except for template DNA were included as controls. Amplification products were observed in silver-stained 5% polyacrylamide gels following vertical electrophoresis.

Eight primers of different size were tested and those resulting in visible, reproducible and easily scorable bands were selected. Mendelian patterns of inheritance were tested in a family comprising both progenitors and two male offspring from the Sumidouro population. Patterns of inheritance were deduced by comparing fragment profiles and by careful verification that any band present in the offspring was at least shared with one progenitor (Stott *et al.*, 1997).

Data analysis

Bands were scored on dried gels and analyzed twice by two different observers. One randomly chosen specimen was used as control for band migration and staining quality in all gels. Ambiguous and extremely light bands were disconsidered. For every individual, bands were scored as present (1) or absent (0) in a matrix for estimating genetic dissimilarity between individuals of each locality. The dissimilarity index (D) was estimated by $D = NAB/(NA + NB)$, where NA and NB are the numbers of fragments of individuals A and B, respectively, and NAB is the number of fragments that are not shared between these individuals (Gilbert *et al.*, 1990). Intrapopulational dissimilarity was estimated as the average D value of all pairwise comparisons within each population. Estimates of the mean standard error were calculated as in Gilbert *et al.* (1990).

Genotypic analysis based on allele frequencies (Lynch and Milligan, 1994) was carried out for assessing genetic variability among populations and the degree of subdivi-

sion of genetic diversity among populations (F_{ST}; Wright, 1951) was calculated. Gene flow was estimated by Wright's (1951) formula, $Nm = 0.25(1/F_{ST} - 1)$, where N is population size, and m is the immigration rate among populations, assuming equilibrium between migration and drift in the "island model". Subdivision of genetic variation was also assessed by analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) using D values from pairwise comparisons. Geographically close populations (from Glicério, Sumidouro and Fazenda União) were grouped and compared with those from other localities in order to assess intergroup variance. AMOVA analyses were performed using the WINAMOVA 1.55 program (Excoffier *et al.*, 1992).

The genetic distance between any two populations was estimated by averaging D values between pairwise comparisons from individuals of these populations (Gilbert *et al.*, 1990). Genetic distances were used in UPGMA cluster analysis with the MEGA program (Kumar *et al.*, 1993). The relationship between genetic and geographic linear distance was analyzed by a Mantel's test with the NTSYS-PC program (Rohlf, 1993).

RESULTS

PCR amplifications resulted in visible products with four of the eight primers tested. When analyzing amplified products from two separate, albeit identical, reactions on contiguous lanes, these four primers produced reproducible results (Figure 2). Moreover, these primers amplified fragments whose pattern of inheritance was clearly Mendelian, although one of these primers (ROJS2) was discarded because amplified fragments produced faint bands.

One hundred and ten different bands were scored in an analysis of 55 individuals with the three selected primers, but only 45 different bands were used for analyses. Assuming that these 45 bands represented different loci, 31 of them were found to be polymorphic. For each primer, the number of scored, useful and polymorphic bands was determined (Table I).

D estimates of pairwise comparisons within localities were used for constructing UPGMA clusters to analyze inter-individual relationships within populations, among trapping transects. None of the localities where trapping occurred in more than one transect showed transect-specific clusters, suggesting that each sample locality could be considered a single population sharing the same genetic pool. Estimates of the intrapopulational dissimilarity index were very similar among populations (Table II).

There were no population-specific alleles. The estimated F_{ST} value was 0.17, indicating that approximately 17% of genetic variability was due to differences between populations. The Nm value, equivalent to the number of immigrants exchanged per generation, was 1.25. AMOVA indicated a significant level of differentiation, with an estimated Φ_{ST} value among populations of 0.14 ($P < 0.0004$). This indicated that 86% of total variation occurred within populations while only 14% was due to population subdivision. When we grouped the three geographically close populations (Sumidouro, Glicério and Fazenda União) and compared them to the two distant ones (Fazenda Canoas and Pedreira), the variance subdivision estimate among

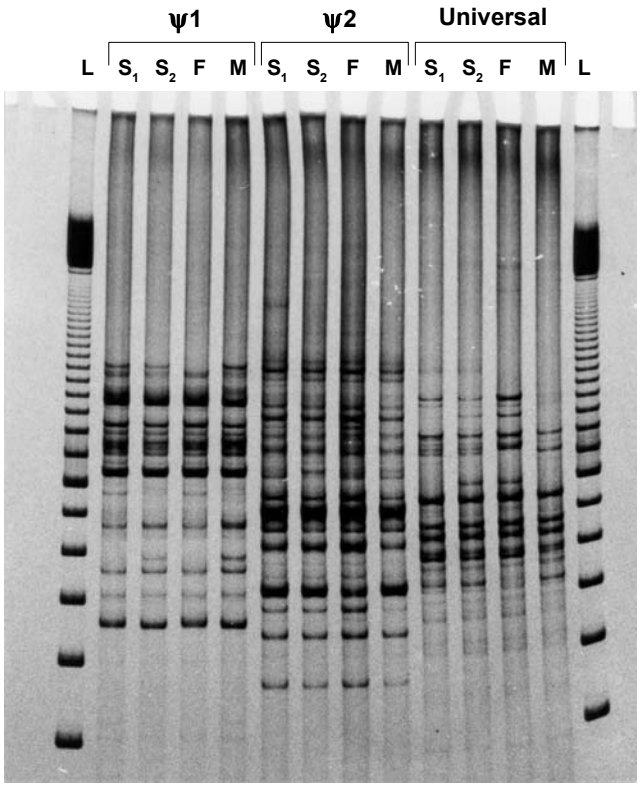

Figure 2 - Silver-stained polyacrylamide gel with RAPD products for the control family, obtained using the primers: ψ1, ψ2 and universal. S_1 and S_2 = Offspring, F = father, M = mother, L = molecular size standard 100-bp DNA ladder.

Table I - Primers used and the number of scored, useful and polymorphic bands obtained in the RAPD analysis.

Name	Nucleotide sequences 5'→3'	Scored bands	Useful bands	Polymorphic bands
Universal	GTAAAACGACGGCCAGT	38	15	12
ψ1	ATGAAGAATACGGATGGC	33	17	9
ψ2	GCCTCTTCCTCCACACGA	39	13	10

Table II - Intrapopulational genetic dissimilarity indexes (D), standard error of the mean (SEM), and expected heterozygosity (with standard error, SE) for each *Nectomys squamipes* population.

Site	D	SEM	Heterozygosity + SE
Glicério	0.1254	0.0237	0.295 + 0.038
Sumidouro	0.1212	0.0235	0.326 + 0.036
Pedreira	0.1276	0.0259	0.319 + 0.049
Faz. Canoas	0.1185	0.0248	0.264 + 0.048
Faz. União	0.1271	0.0267	0.224 + 0.044

groups (Φ_{CT}) was not significant (P = 0.57). This indicated that the three geographically closest populations showed the same genetic differentiation as between the more distant ones.

Distance estimates were made between populations (or interpopulation dissimilarity index values; Table III). The relationship between populations is represented in the UPGMA topology (Figure 3). This confirmed Mantel's test estimates in showing a lack of significant correlation between genetic and linear geographic distance (r = -0.228; t = -0.588; P = 0.278), which indicates that there was no isolation by distance in the *N. squamipes*.

DISCUSSION

The RAPD procedures proved to be a useful tool for assessing genetic variability because band profiles with the three selected primers were reproducible and their patterns of inheritance proved to be Mendelian for a dominant marker. Despite the high proportion of excluded bands a substantial number of polymorphic markers was detected.

Few studies of terrestrial mammals using the RAPD technique have been reported and different similarity and dissimilarity indexes have been used for RAPD analysis. The lack of standard procedures for RAPD analysis makes comparisons with other studies very difficult. Baruffi *et al.* (1995) using the same *D* index herein used, found *D* values ranging from 0.158 to 0.387 in six wild population samples of fruit flies (*Ceratitis capitata*), each consisting of five individuals. Conversely, our within-population *D* values were very similar to one another, ranging between 0.118 and 0.128, despite differences in sample size.

Sinclair *et al.* (1996) found a smaller F_{ST} value (0.028) between flying-fox (*Pteropus scapulatus*) populations than the ones we found (F_{ST} = 0.17, Φ_{ST} = 0.14), while studies with bird species found similar values (F_{ST} = 0.17, Haig *et al.*, 1996; Φ_{ST} = 0.20, Nusser *et al.*, 1996). This is interesting because birds have a higher dispersion ability, with potentially higher gene flow between populations.

In the *N. squamipes* populations the F_{ST} value corresponds to an *Nm* of 1.25, which is above the minimum number of migrants per generation needed to avoid differentiation by genetic drift (*Nm* > 1, Slatkin, 1987). Thus, although differentiation estimates were significant, the level of differentiation was not very high. This was coincident with the lack of population-specific alleles and with previous data showing absence of major morphometric differentiation in *N. squamipes* across a very extensive geographic distribution (Bonvicino, 1994).

The lack of substantial differentiation contrasts with the dispersion pattern in *N. squamipes*, which occurs along watercourses with a discontinuous distribution along hydrographic basins (Ernest and Mares, 1986). Based on the dispersion data, it was expected that genetic drift would result in a higher differentiation between populations than that found by us. Alternative hypotheses which might explain our results include recurrent processes of local extinction and recolonization (Maruyama and Kimura, 1980; Slatkin, 1987) or recent, sudden increases in the geographic distribution of this species (Slatkin, 1993).

Table III - Dissimilarity index between pairs of populations (*D*, in the lower-left matrix) and standard error of the mean (SEM, in the upper-right matrix).

	GL	SU	PD	FC	FU
GL	–	0.028	0.029	0.029	0.031
SU	0.129	–	0.028	0.024	0.026
PD	0.151	0.146	–	0.026	0.030
FC	0.151	0.124	0.126	–	0.027
FU	0.160	0.134	0.146	0.130	–

For abbreviations, see legend to Figure 1.

Figure 3 - UPGMA cluster analysis of *Nectomys squamipes* populations derived from dissimilarity values.

ACKNOWLEDGMENTS

We thank D. Astúa de Moraes, E. Hingst-Zaher, L. Geise, R. Gentile and M. Weksler for their collaboration during fieldwork. We are indebted to P.S. D'Andrea for providing samples from Sumidouro and R. Santori and M.V. Vieira for additional ecological information. We are grateful to H. Seuánez, G. Marroig and R. Gentile for useful discussions, to H. Seuánez for reviewing the text, to N.P. Barros and C.V. Silva for their help in the laboratory, and to A.M. Marcondes for secretarial work. Research supported by CPEG/UFRJ, CNPq grant to Drs. L. Rey and R. Cerqueira, MacArthur Foundation (to Fundação Biodiversitas), FAPERJ, FUJB and PRONEX/CNPq.

RESUMO

Uma análise baseada em amplificação aleatória de ADN polimórfico (*random amplified polymorphic DNA*, RAPD) foi feita a fim de se estimar distâncias genéticas e a estruturação da variabilidade genética em populações de *Nectomys squamipes*, uma espécie de roedor de hábito semi-aquático, que está sempre associada a cursos d'água. Amostras de ADN de cinco populações foram analisadas utilizando-se três *primers*, que produziram um total de 45 bandas úteis, sendo 69% delas polimórficas. Os resultados mostraram uma diferenciação significativa entre as populações [F_{ST} = 0,17; Φ_{ST} = 0,14 (P < 0,004)], mas com uma taxa

de fluxo gênico suficiente ($Nm = 1,25$) para minimizar os efeitos da deriva genética. Não foram encontrados marcadores exclusivos para nenhuma das populações. O teste de Mantel e a análise de agrupamento por UPGMA não evidenciaram relação entre as distâncias genéticas e as geográficas, indicando que não há um padrão de isolamento por distância. A aparente homogeneidade indicada pelos marcadores de RAPD vai ao encontro de dados morfométricos prévios, que não indicaram uma diferenciação substancial na extensa área de distribuição de *N. squamipes*. Algumas hipóteses alternativas podem ser levantadas para explicar estes resultados, como processos recorrentes de extinção e recolonização locais, ou um aumento súbito e recente da distribuição geográfica desta espécie.

REFERENCES

Baruffi, L., Damiani, G., Guglielmino, C.R., Bandis, C., Malacrida, A.R. and **Gasperi, G.** (1995). Polymorphism within and between populations of *Ceratitis capitata*: comparison between RAPD and multilocus enzyme electrophoresis data. *Heredity 74*: 425-437.

Bonvicino, C.R. (1994). Especiação do rato d'água *Nectomys*. Abordagem cariológica, morfológica e geográfica. PhD thesis, Universidade Federal do Rio de Janeiro, Rio de Janeiro.

Bonvicino, C.R., D'Andrea, P.S., Cerqueira, R. and **Seuánez, H.N.** (1996). The chromosomes of *Nectomys* (Rodentia, Cricetidae) with 2n = 52, 2n = 56, and interspecific hybrids (2n = 54). *Cytogenet. Cell Genet. 73*: 190-193.

D'Andrea, P.S., Horta, C., Cerqueira, R. and **Rey, L.** (1996). Breeding of the water rat *Nectomys squamipes* in the laboratory. *Lab. Anim. 30*: 369-376.

Ernest, K.A. and **Mares, M.A.** (1986). Ecology of *Nectomys squamipes*, the neotropical water rat, in central Brazil: home range, habitat selection, reproduction and behaviour. *J. Zool. 210*: 599-612.

Excoffier, L., Smouse, P.E. and **Quattro, J.M.** (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics 131*: 479-491.

Gilbert, A.D., Lelunan, N., O'Brien, S.J. and **Wayne, R.K.** (1990). Genetic fingerprinting reflects population differentiation in the California Channel Island fox. *Nature 344*: 764-767.

Haig, S.M., Bowman, R. and **Mullins, T.D.** (1996). Population structure of red-cockaded woodpeckers in south Florida: RAPDs revisited. *Mol. Ecol. 5*: 725-734.

Kumar, S., Tamura, K. and **Nei, M.** (1993). *MEGA: Molecular Evolutionary Genetics Analysis*, *Version 1.02*. The Pennsylvania State University, University Park, PA.

Lynch, M. and **Milligan, B.G.** (1994). Analysis of population genetic structure with RAPD markers. *Mol. Ecol. 3*: 91-99.

Maia, V., Yonenaga-Yassuda, Y., Freitas, T.R.O., Kasahara, S., Suñé-Mattevi, M., Oliveira, L.F., Galindo, M.A. and **Sbalqueiro, I.J.** (1984). Supernumerary chromosomes in *Nectomys squamipes* (Cricetidae-Rodentia). *Genética 63*: 121-128.

Maruyama, T. and **Kimura, M.** (1980). Genetic variability and effective population size when local extinction and recolonization of subpopulation are frequent. *Proc. Natl. Acad. Sci. 77*: 6710-6714.

Musser, G.G. and **Carleton, M.D.** (1993). Family Muridae. In: *Mammal Species of the World* (Wilson, D.E. and Reeder, D.M., eds.). 2nd edn. Smithsonian Institution, Washington, pp. 501-753.

Nusser, J.A., Goto, R.M., Ledig, D.B., Fleischer, R.C. and **Miller, M.M.** (1996). RAPD analysis reveals low genetic variability in the endangered light-footed clapper rail. *Mol. Ecol. 5*: 463-472.

Rey, L. (1993). Non-human vertebrate hosts of *Schistosoma mansoni* and schistosomiasis transmission in Brazil. *Res. Rev. Parasitol. 53*: 13-25.

Rohlf, F.J. (1993). *NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System*. Exeter Software, New York.

Sambrook, J., Fritsch, E.F. and **Maniatis, T.** (1989). *Molecular Cloning: A Laboratory Manual*. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sinclair, E.A., Webb, N.J., Marchant, A.D. and **Tidemann, C.R.** (1996). Genetic variation in the little red flying-fox *Pteropus scaputatus* (Chiroptera: Pteropodidae) implications for management. *Biol. Conserv. 76*: 45-50.

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science 236*: 787-792.

Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution 47*: 264-279.

Smith, L.J., Braylan, R.C., Nutkis, J.E., Edmundson, K.B., Downing, J.R. and **Wakeland, E.K.** (1987). Extraction of cellular DNA from human cells and tissues fixed with ethanol. *Anal. Biochem. 160*: 135-138.

Stott, W., Ihssen, P.E. and **White, B.N.** (1997). Inheritance of RAPD molecular markers in lake trout *Salvelinus namaycush*. *Mol. Ecol. 6*: 609-613.

Welsh, J. and **McClelland, M.** (1990). Fingerprint genomes using PCR with arbitrary primers. *Nucleic Acids Res. 18*: 7213-7218.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and **Tingey, S.V.** (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res. 18*: 6531-6535.

Wright, S. (1951). The genetical structure of populations. *Ann. Eugen. 15*: 323-335.

Yonenaga-Yassuda, Y., Maia, V. and **L'Abbate, M.** (1987). Two tandem fusions and supernumerary chromosomes in *Nectomys squamipes* (Cricetidae, Rodentia). *Caryologia 41*: 25-39.

(Received January 3, 2000)