J. Ornithol. 141, 152–159 (2000)

ISSN 0021-8375

Effects of forest fragmentation on genetic variation in endemic understory forest birds in central Madagascar

Aristide Andrianarimisa[1], Lutz Bachmann[2], Jörg U. Ganzhorn[3], Steven M. Goodman[2,4], and Jürgen Tomiuk[5]

[1] Peregrine Fund, BP 4113, Antananarivo (101), Madagascar;
[2] Field Museum of Natural History, Pritzker Laboratory for Molecular Systematics and Evolution, Roosevelt Road at Lake Shore Drive, Chicago, IL 60605, USA;
[3] Zoologisches Institut und Zoologisches Museum, Martin-Luther-King-Platz 3, D-20146 Hamburg, Germany (corresponding author: E-mail: ganzhorn@zoologie.uni-hamburg.de);
[4] WWF, BP 738, Antananarivo (101), Madagascar;
[5] Institut für Anthropologie und Humangenetik, Wilhelmstrasse 27, D-72074 Tübingen, Germany

Summary

This study focuses on some genetic consequences of habitat fragmentation in populations of four endemic bird species (*Monticola sharpei*, *Terpsiphone mutata*, *Foudia omissa*, and *Foudia madagascariensis*) living in the understory of forests in the Réserve Spéciale d'Ambohitantely on the Central High Plateau of Madagascar. The four species differ in their dependency on forest habitats, which may be related to their migration abilities between isolated forest fragments. Genetic variation was analysed on the basis of multi-locus fingerprints in order to study the influence of migration and habitat size on the genetic variability of local populations. There was no evidence that forest fragmentation affects any of the four species with respect to genetic variability.

Key words: DNA fingerprinting, genetic differentiation

Zusammenfassung

Effekte von Waldfragmentation auf die genetische Variabilität endemischer Vogelarten in Zentral-Madagaskar

Rund 53 % der Vogelarten Madagaskars sind endemisch. Viele dieser Arten sind an Waldhabitate gebunden und durch die Verinselung der madagassischen Wälder hochgradig bedroht. In dem vorliegenden Projekt wird die Auswirkung der Verinselung auf die genetische Variabilität und die genetische Differenzierung von vier endemischen Vogelarten am Beispiel des Reservats von Ambohitantely im zentralen Hochland Madagaskars untersucht. Ambohitantely beinhaltet mehr als 500 Waldfragmente von 0,64 ha bis 1250 ha. Untersucht wurden: 1. die Waldart *Foudia omissa*, 2. *Monticola* (früher *Pseudocossyphus*) *sharpei*, eine Art mit starker Bevorzugung für primäre Waldlebensräume, die aber gelegentlich auch in Sekundärvegetation auftritt, sowie 3. *Terpsiphone mutata* und 4. *Foudia madagascariensis*, beides Arten, die sowohl in Wäldern als auch in offenen Landschaften vorkommen. Die unterschiedliche Abhängigkeit dieser Arten von Waldhabitaten und der damit verbundenen Möglichkeit, offene Landschaften zwischen den Waldfragmenten zu überbrücken, führte zur Frage, ob die Verinselung die genetische Vielfalt der untersuchten

Populationen beeinflußt. Für die genetischen Analysen wurden Blutproben von Tieren aus drei Gebieten mit 1250 ha, 136 ha und 28 ha gesammelt. Die Proben wurden mit Multilocus-Fingerprints genetisch charakterisiert. Zum Untersuchungszeitpunkt konnte kein Einfluß der Fragmentierung auf die genetische Populationsstruktur der vier untersuchten Arten nachgewiesen werden.

Introduction

The tropical forests of the world are under tremendous human pressure and are being fragmented at an accelerating rate (Laurence & Bierregard 1997). Madagascar provides one of the most foreboding examples worldwide. Its eastern rain forest has been classified as one of the two terrestrial ecosystems with the highest plant species diversity (Barthlott et al. 1996). The vast majority of Madagascar's plant and animal species are endemic to the island (e. g. Jenkins 1987). It has been assumed that more than 70 % of Madagascar's eastern evergreen forest has been cleared since the arrival of humans on Madagascar some 2000 years ago (Green & Sussman 1990). The central portion of the island, often referred to as the Central High Plateau or Central Highlands, is a vast area, representing over 50 % of the land surface of Madagascar. East of this plateau is the eastern escarpment which descends into the eastern humid forest formation. Today the Central High Plateau is characterised by bare soil, extensive grass cover, and some highly fragmented and isolated remnant evergreen forests. In many cases these forests are in mesic ravines and valleys that provide some protection against the annual burning of pasture lands for cattle (Gade 1996).

Over the last two million years, the vegetation of Madagascar has experienced substantial and repeated changes during various glacial and interglacial cycles (Burney 1997). Recent climatic changes might have resulted in a mosaic of forest types and wooded savanna already since 7000 years (MacPhee & Burney 1991, Lowry et al. 1997). The isolation of certain forest formations on the Central High Plateau was not anthropogenic in nature, but rather part of a natural process. However, other sites, such as the remaining forests in and around the Réserve Spéciale (RS) d'Ambohitantely, have been isolated and fragmented by human activities (Langrand & Wilmé 1997). Though relatively recent, this fragmentation process has resulted in a typical nested pattern of bird communities where species drop out systematically with decreasing fragment size (Langrand & Wilmé 1997). This pattern is characteristic for deterministic extinction processes after fragmentation of once contiguous habitats (Patterson 1987). This poses the question whether or not habitat fragmentation is reflected in genetic differentiation of subpopulations. Understanding the consequences of forest fragmentation on the flora and fauna of Madagascar is of particular interest to allow for better management for conservation of the island's unique biota.

Here we analyse effects of forest fragmentation on the genetic variability of four bird species that show different habitat dependencies. The species have different abilities to migrate between forest fragments that are isolated by grassland.

Material and Methods

Study site

The study site is located in the Réserve Spéciale d'Ambohitantely on the Central High Plateau at 18° 10' S and 47° 15' E (Fig. 1). The forest area is part of the eastern Malagasy moist montane forest that grows between 800 and 2000 m above sea level. About 15 % of the reserve's surface consists of plantations of exotic trees and another 35 % are anthropogenic grassland (Langrand 1995). In the early 1960s it was estimated that the largest forest block

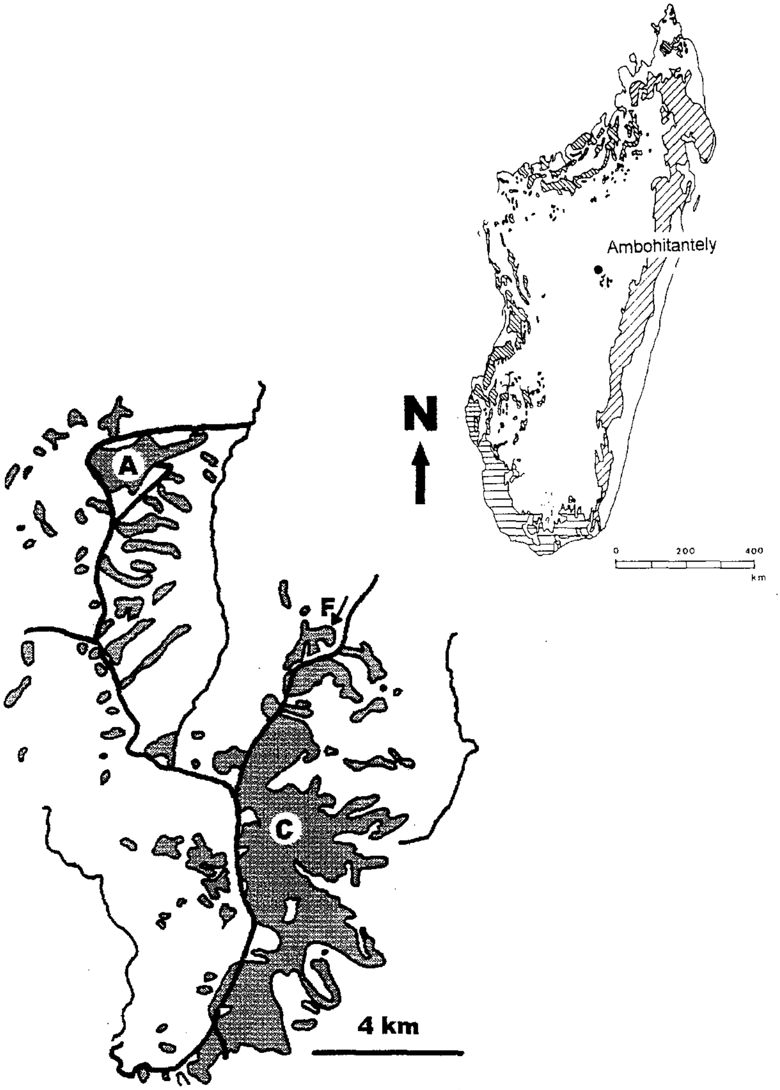

Fig. 1. Location of the reserve of Ambohitantely and extent of forest cover (gray) with the study sites *A*, *F*, and *C* (modified from Langrand & Wilmé 1997). Shaded areas in the insert mark different forest types: evergreen rain forest in the east, dry deciduous forest in the west and spiny forest in the south.

Abb. 1. Karte des Reservats von Ambohitantely mit den Untersuchungsgebieten *A*, *F*, und *C*. Waldfragmente sind grau markiert (verändert nach Langrand & Wilmé 1997). Schraffierte Bereiche der Übersichtskarte repräsentieren verschiedene Waldformationen: immergrüner Regenwald im Osten, regengrüner Trockenwald im Westen und Dornbuschformationen im Süden.

within the reserve was approximately 2000 ha in size and that 1000 ha of forest occured in the various fragments (Bastian 1964). By 1991, the natural forest of Ambohitantely had been fragmented into some 514forest blocks, ranging in size from the largest parcel of 1250 ha to fragments as small as 0.64 ha (Langrand 1995). For this study, birds were caught in eight of these fragments covering the complete range of fragment size from 0.64 up to 1250 ha (control site). All sites are separated by botanically depauperate grassland without secondary or regenerating forest habitat.

Bird species

Four species with different dependence on forest ecosystems were chosen for genetic analyses. Amongst them *Foudia omissa* is the only reputed forest-restricted species. In the RS d'Ambohitantely *Monticola sharpei* is forest dwelling, although in other areas of the Central High Plateau it is known to occur in exotic tree plantations and in open zones above the forest line. Two other species, *Terpsiphone mutata* and *Foudia madagascariensis*, do not depend on closed canopy natural forests but are found in various types of vegetation formation including forest habitats (Langrand 1990).

Population density and population size

Previously, relative population densities were estimated by standardized point counts in forest blocks of different size (Langrand & Wilmé 1997; Table 1). Point counts were spaced at 200 m intervals along transects, and each point count covered an area of 0.0625 ha. *Foudia madagascariensis*, however, was not recorded in fragment *A* but was instead caught with mist nets. Estimates of species' density were used to estimate the population size of each bird species in the various fragments. For all four species the total number of individuals living in the smallest forest fragment *F* (28 ha) is rather low. However, owing to its close proximity to the control site even forest depending bird species might be able to cross the small gap between the two fragments. Fragment A (136 ha) is five to six kilometers away from both fragment F and the control site. Therefore forest depending birds might have reduced capability to exchange birds via "fragment hopping".

Genetic analyses

Blood samples were taken from birds captured in mist nets and subsequently released. Samples were stored in Queen's buffer (Seutin et al. 1991). DNA was extracted according to standard procedures, using a genomic DNA isolation kit (*Biozym*). Duration of storage, however, affected the quality of DNA and thus reduced samples for genetic analysis. Genetic variation was determined by multilocus fingerprints. DNA was digested with the restriction endonuclease *Eco*RI, electrophoretically separated on 0.7 % agarose gels and subsequently transfered to Hybond N (Amersham) nylon membranes (Southern 1975). The immobilized DNA was hybridized with a Digoxigenin labeled $(GGAT)_4$ probe at 40 °C overnight. Detection of the hybridized fragments was according to the DIG detection system (Boehringer Mannheim) using CDP* as the substrate for the Anti-Dig conjugated alkaline phosphatase. The hybridizing fragments of 2–5 kb were scored for each individual.

The degree of genetic variability within populations was estimated by scoring the relative number of variable band positions *P*. Genetic similarity of two individuals within the same and from two different populations was quantified using the band-sharing index *F* that refers to the relative number of shared bands

$$F = \frac{2 \cdot n(xy)}{n(x) + n(y)}$$

where $n(xy)$ is the number of bands shared by both individuals, $n(x)$ and $n(y)$ are the total number of bands found in each of the individuals x and y respectively (Nei & Li 1979).

Small population size and low migration rate reduce genetic variability and can also increase inbreeding, both are sources of increased genetic similarity among individuals. To assess

Table 1. Genetic data of four Malagasy bird species of different dependency on forest habitats (forest dependent: *Foudia omissa*; moderately forest dependent: *Monticola sharpei*; forest independent: *Terpsiphone mutata* and *F. madagascariensis*) in three forest fragments (F, A and C). Sample sizes (N), percentage of polymorphic bands obtained with multilocus fingerprints (*P*), and mean band sharing index (*F*) of each forest fragment are given. Band sharing indices of all pairs were grouped into three classes of equal size: low, medium and high rank numbers (see text for details).

Tab. 1. Genetische Merkmale für vier madagassische Vogelarten mit unterschiedlichen Abhängigkeiten von Waldlebensräumen (reiner Waldbewohner: *Foudia omissa*; primär Waldbewohner: *Monticola sharpei*; Bewohner von Wäldern und offener Landschaften: *Terpsiphone mutata*, *F. madagascariensis*) in drei Waldfragmenten (F, A und C). Angegeben sind Stichprobengröße (N), Prozentsatz polymorpher Banden basieren auf Multilocus Fingerprints (*P*) und der Mittelwert der „band sharing indices“ aller Paarvergleiche (*F*) pro Waldfragment. Zur statistischen Analyse wurden die „band sharing indices“ aller Paarvergleiche in drei Klassen gleicher Größe eingeteilt.

Species		Fragment F (28 ha)	Fragment A (136 ha)	Fragment C (1250 ha)
F. omissa^{1}	Density	0.031	0.038	0.057
	Population size	14	83	1140
	N	1	7	3
	P (%)	–	88.1	52.2
	F	–	0.237	0.166
	Rank			
	Low		6	2
	Medium		8	0
	High		7	1
M. sharpei^{2}	Density	0.063	0.135	0.063
	Population size	28	294	1260
	N	9	11	8
	P (%)	89.9	88.4	82.6
	F	0.251	0.240	0.243
	Rank			
	Low	13	8	10
	Medium	6	13	12
	High	17	8	6
T. mutata^{3}	Density	0.188	0.173	0.074
	Population size	84	376	1480
	N	4	12	8
	P (%)	67.1	97.1	92.9
	F	0.251	0.246	0.231
	Rank			
	Low	2	20	11
	Medium	3	24	6
	High	1	23	10
F. madagascariensis^{4}	Density	0.031	0	0.006
	Population size	14	›0	120
	N	3	10	11
	P (%)	53.8	87.2	50.0
	F	0.160	0.217	0.169
	Rank			
	Low	1	14	2
	Medium	2	15	0
	High	0	16	1

Homogeneity of rank tables (permutation test): $^{1)}$ p = 0.54, $^{2)}$ p = 0.10, $^{3)}$ p = 0.75, $^{4)}$ p = 0.58

effects of fragmentation and isolation on genetic heterogeneity within populations, similarity indices of individual pairs within fragments from all three forest fragments were ranked and grouped into three categories (low/medium/high: band sharing index is within the lower/medium/higher third of all values). The differences of ranked similarity indices among forest fragments were analyzed with permutation tests which account for small class sizes (Roff & Bentzen 1989).

Results

The relative number of polymorphic bands obtained when hybridizing the DIG labeled $(GGAT)_4$ probe to *Eco*RI digested and immobilized genomic DNA of *F. omissa, M. sharpei, T. mutata* and *F. madagascariensis* ranges between 50 and 97 %. The degree of polymorphism was not related to the size of bird populations and the habitat preference of the respective species. Only the *F* population of *T. mutata* showed lower genetic variability than that of populations from the larger fragments. The data are summarized in Table 1. The mean band sharing indices of individuals within forest fragments were close to 0.2 for all species and slightly lower than those of individuals from different fragments (Table 2).

Genetic similarity within fragments was not affected by reduction of habitat sizes or isolation by distance. There was no significant increase of genetically similar individuals within small fragments and thereby also no indication that genetic relatedness of individuals increased through inbreeding (Table 1, $p > 0.10$ for all species).

Discussion

The fragmentation of tropical forests and the consequences for the remaining biodiversity are major conservation concerns. Many studies demonstrate that the number of forest dependent species declines with decreasing fragment size, even though different taxa may react differently, and the total number of species per unit area may not be altered all that much owing to edge effects and colonization by ubiquitous species (e.g., Brokaw 1998, Gascon & Lovejoy 1998). The bird fauna of the RS d'Ambohitantely is clearly affected by fragmentation. First, small forest fragments contain fewer bird species than larger ones ($p = 0.004$; $r_s = 0.84$; $n = 8$; data from Langrand & Wilmé 1997). Secondly, the composition of bird communities in the various fragments does not vary at random but follows a nested structure (Langrand & Wilmé 1997) with a fair number of "outliers" that are characteristic for nested bird communities (Patterson 1987; Cutler 1991). Further, several species are absent from the large control site that occur in the nearby large

Table 2. Mean band sharing indices (genetic similarity) of populations of *Foudia omissa, Monticola sharpei, Tersiphone mutata,* and *F. madagascariensis* from three different forest fragments. Band sharing indices are based on pairwise comparisons of individuals from different fragments.

Tab. 2. Genetische Ähnlichkeit (basierend auf band sharing indices) zwischen Populationen von *Foudia omissa, Monticola sharpei, Tersiphone mutata,* and *F. madagascariensis* aus drei verschiedenen Waldfragmenten. Band sharing Indices basieren auf paarweisen Vergleichen von Individuen aus verschiedenen Waldfragmenten.

Species	Comparison of forest fragments		
	C-F	A-F	CA
F. omissa	–	–	0.220
M. sharpei	0.241	0.246	0.267
T. mutata	0.288	0.267	0.243
F. madagascariensis	0.172	0.222	0.208

forested area of Anjozorobe which until a few years ago was attached to the eastern humid forest (Ravokatra et al. 1998). Comparisons between Ambohitantely and Anjozorobe reveal that there has already been a differential extirpation of certain forest-dwelling bird species.

Multilocus-fingerprint is a suitable and simple technique to characterize genetic similarity between individuals. However, the resulting data do not reflect allelic variation (e. g. Lubjuhn et al. 1994) and thus it is difficult to analyze them with standard population genetic procedures (Lynch 1990, 1991). Nevertheless, variation observed using fingerprints underlies the same population genetic principles as allelic variation, i.e. variability declines with decreasing population size and inbreeding increases similarity. With respect to our study, genetic variability and genetic similarity of individuals within fragments did not correlate with population densities or fragment size in all four species. This can indicate that populations are not completely isolated and individuals of all species are able to migrate among fragments. The small fragment *F* is located close to the large control site and even forest depending bird species might be able to cross this small grassland. Fragment *A* is about five times larger but more isolated than fragment *F* and therefore forest dependent birds might have reduced capability to migrate via "fragment hopping" between the fragments. However, the relatively large size of population *A* combined with low individual migration can minimize the risk of losing genetic variability rapidly, as can high migration combined with the small size of population in fragment *F*.

Alternatively, the observation that the deleterious effects of forest destruction and altered community composition in fragments of different size are not reflected in the genetic data could be due to the fact that the habitat fragmentation of Ambohitantely might have been too recent to be reflected in the genetic data.

In conclusion, ecological and genetic data indicate that bird communities of the reserve of Ambohitantely are neither in an ecologically nor in a genetically stable state. Neither population density nor genetic variability are correlated with the habitat size for any of the four species. There is evidence that several forest dependent birds have already disappeared from the RS d'Ambohitantely (Ravokatra et al. 1998), and this is probably a result of habitat fragmentation and population isolation. Although there is a clear negative relationship between forest size and bird species diversity (Langrand & Wilmé 1997), in the larger forest fragments, the decline in the number of species is not abrupt. Thus, at some level the remaining forest bird community of the RS d'Ambohitantely might largely be composed of the survivors, that is species that have been able to adapt to habitat degradation or that have no behavioral inhibitions against crossing non-forest habitat.

Acknowledgements

We thank the World Wide Fund for Nature Madagascar for logistical help and partial funding through its Ecology Training Programme. The Deutsche Ornithologen Gesellschaft supported the field work of A. Andrianarimisa at a crucial time. O. Langrand provided indispensable help at all stages of the project. E. Curio and two reviewers provided very helpful comments. Research permits were issued by the Direction des Eaux et Forêts and by the Association Nationale pour la Gestion des Aires Protégées.

References

Barthlott, W., Lauer, W. & Placke, A. (1996): Global distribution of species diversity in vascular plants: towards a world map of phytodiversity. Erdkunde 50: 317–327.

Bastian, G. (1964): La forêt d'Ambohitantely, Madagascar. Rev. Géographie, Madagascar 5: 1–42.

Brokaw, N. (1998): Fragments past, present and future. Trends Ecol. Evol. 13: 382–383.

Burney, D. A. (1997): Theories and facts regarding Holocene environmental change before and after human colonization. In Goodman, S. M. & Patterson, B. D. (Eds.): Natural change and human impact in Madagascar: 75–89. Washington, D. C.

Cutler, A. (1991): Nested faunas and extinctions in fragmented habitats. Cons. Biol. 5: 496–505.

Gade, D. W. (1996): Deforestation and ist effects in highland Madagascar. Mountain Research and Development 16: 101–116.

Gascon, C. & Lovejoy, T. E. (1998): Ecological impacts of forest fragmentation in central Amazonia. Zoology 101: 273–280.

Green, G. M. & Sussman, R. W. (1990): Deforestation history of the eastern rainforest of Madagascar from satellite images. Science 248: 212–215.

Jenkins, M. D. (1987): Madagascar: an environmental profile. Gland.

Langrand, O. (1990): Guide to the birds of Madagascar. New Haven.

Langrand, O. (1995): The effects of forest fragmentation on bird species in Madagascar: a case study from Ambohitantely Forest Reserve on the Central High Plateau. M.S. Thesis. University of Natal, Pietermaritzburg.

Langrand, O. & Wilmé, L. (1997): Effects of forest fragmentation on extinction patterns of the endemic avifauna on the central high plateau of Madagascar. In Goodman, S. M. & Patterson, B. D. (Eds.): Natural change and human impact in Madagascar: 280–305. Washington, D.C.

Laurance, W. F. & Bierregaard, R. O. (Eds. 1997): Tropical forest remnants. Chicago.

Lowry, P. P., Schatz, G. E. & Phillipson, P. B. (1997): The classification of natural and anthropogenic vegetation in Madagascar. In Goodman, S. M. & Patterson, B. D. (Eds.): Natural change and human impact in Madagascar: 93–123. Washington, D. C.

Lubjuhn, T., Schwaiger, F.-W. & Epplen, J. T. (1994): The analysis of simple repeat loci as applied in evolutionary and behavioral sciences. In Schierwater, B. Streit, B., Wagner, G. P. & DeSalle, R. (Eds): Molecular ecology and evolution: approaches and applications: 33–43. Basel.

Lynch, M. (1990): The similarity index and DNA fingerprinting. Mol. Biol. Evol. 5: 584–599.

Lynch, M. (1991): Analysis of population genetic structure by DNA fingerprinting. In Burke, T., Dolf, G., Jeffreys, A. J. & Wolff, R. (Eds): DNA fingerprinting: approaches and applications: 113–126. Basel.

MacPhee, R. D. E. & Burney, D. A. (1991): Dating of modified femora of extinct dwarf *Hippopotamus* from southern Madagascar: implications for constraining human colonization and vertebrate extinction events. J. Archaeol. Sci. 18: 695–706.

Nei, M. & Li, W. H. (1979): Mathematical models for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76: 5269–5273.

Patterson, B. D. (1987). The principle of nested subsets and its implication for biological conservation. Cons. Biol. 1: 323–334.

Ravokatra, M., Wilmé, L. & Goodman, S. M. (1998): Les oiseaux. In: Rakotondravony, D. & Goodman, S. M. (Eds.): Inventaire biologique, Forêt d'Andranomay, Anjozorobe, Recherches pour le Développement, Série Sciences biologiques. No-13: 60–79. Centre d'Information et de Documentation Scientifique et Technique, Antananarivo.

Roff, D. A. & Bentzen, P. (1989): The statistical analysis of mitochondrial DNA polymorphism: chi-square and the problem of small samples. Mol. Biol. Evol. 6: 539–545.

Seutin, G., White, B. N. & Boag, P. T. (1991): Preservation of avian blood and tissue samples for DNA analyses. Can. J. Zool. 69: 82–90.

Southern, E. M. (1975): Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98: 503–517.

Accepted: 12 July 1999