

The application of genetic research to the conservation of fruit bats in the western Indian Ocean

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INTRODUCTION

The use of genetic methodologies to answer conservation-related questions makes sense intuitively. DNA is the basic unit of evolutionary change and is moulded by processes that leave characteristic 'signatures' that can accumulate over time, thereby recording the evolutionary history of a species or population. Thus, we can use DNA as a tool to infer the historical perturbations that have impacted a species or population and explain their current distribution and genetic diversity. We can then use this information to predict the response of a species or population to future pressures such as environmental change, habitat loss and population decline (LOESCHCKE *et al.* 1994).

Why is fruit bat conservation an issue?

Fruit bats of the genus *Pteropus* (Megachiroptera: Pteropodidae) are distributed from the Western Indian Ocean islands, eastward to the mid-Pacific islands in primarily tropical or sub-tropical climates (MICKLEBURGH *et al.* 1992). They act as important contributors to ecosystem maintenance, particularly on islands, through their role as seed dispersers and pollinators (FUJITA & TUTTLE 1991). Worryingly, island faunas are the most prone to extinction (ALCOVER *et al.* 1998) and 86% of *Pteropus* species inhabit islands (RAINEY & PIERSON 1992).

The most significant threats to fruit bats are habitat alteration, hunting and cyclonic storms that reduce populations and destroy food and roost trees (ROBERTSON 1992). There is a real need to investigate the viability of island populations of fruit bats in terms of extinction risk. Three of the seven extant species in the Western Indian Ocean are considered vulnerable (MICKLEBURGH *et al.* 2002) and are listed in the IUCN Red List (2003) [*P. rodricensis* (DOBSON 1878) from Rodrigues Island, *P. livingstonii* (GRAY 1866) from the Comoro Islands and *P. voeltzkowi* (MATSCHIE 1909) from Pemba Island].

There is a paucity of genetic research on bats. The order accounts for approximately one quarter of known mammalian diversity (NIKAIDO *et al.* 2000), but only 0.02% of DNA sequences accessioned under Mammalia in the DNA Sequence Databank (<http://www.ncbi.nlm.nih.gov/Genbank/>) are Chiropteran. In general fruit bats are a difficult group to study using standard observational techniques – they are highly mobile, nocturnal, have very fluid social systems and may form aggregations numbering thousands. In many cases parentage analysis is almost impossible using behavioural estimates due to

multiple mating. Genetic analysis can overcome many of these problems and its potential role in the conservation of fruit bats is becoming recognised.



Fig. 1. Distribution of *Pteropus* species in the western Indian Ocean: Seychelles – *P. s. seychellensis*; Aldabra – *P. (s.) aldabrensis*; Comoro I. – *P. (s.) comorensis* and *P. livingstonii*; Mayotte – *P. (s.) comorensis*; Pemba – *P. voeltzkowi*; Madagascar – *P. rufus*; Mauritius & Reunion – *P. niger*; Rodrigues – *P. rodricensis*

What is conservation genetics?

Conservation genetics can operate at two broad and often overlapping levels: between species (species-level) and within species (population-level).

Species-level studies.— One of the most fundamental units for conservation biologists is the ‘species’. However, what constitutes a ‘species’ is difficult to define as evidenced by the number of species concepts that are currently in existence (Wayne *et al.* 1994). Phylogenetics (defining species on the basis of genetic divergence) has proven to be of importance to conservation biology (AVISE 1996). There are numerous examples of cryptic species that are morphologically very similar but very different in genetic terms e.g. the existence of two species of European Pipistrelle bats (*Pipistrellus pipistrellus* spp.) with different echolocation frequencies was proven using genetic analysis (BARRATT *et al.* 1997). Thus, genetic analysis has a significant role to play in evaluating biodiversity.

Hybridisation has important implications for conservation management and allocation of limited resources. For example, the Ethiopian wolf (*Canis simiensis* RÜPPELL 1835) is the

most endangered of all canids. However, plans for an intensive breeding programme are hampered by difficulties in finding purebred animals due to introgression of dog (*Canis familiaris*) DNA (GOTTELLI *et al.* 1994). Some sympatric species of fruit bats are known to hybridise in overlapping parts of their respective ranges (MCWILLIAM 1985) and management plans should take this into account.

Phylogeography is the study of the relationship between species and their geographic distribution. By identifying the origins of species and subsequent dispersal or colonisation using genetic analysis, it is possible to put speciation processes in a geographic context. This facilitates the differentiation between dispersal and vicariance in a species history and is relevant to contemporary genetic variability (AVISE 1987). ANDERSEN (1912) admitted that his morphological taxonomy of fruit bats was hard to reconcile with their geographic distribution, and a genetic approach may prove useful in resolving this problem.

Population-level studies.— The comparison of genetic variability between populations is important for both *in situ* and *ex situ* conservation (BALLOU *et al.* 1995). In terms of wild populations, the impact of founder effects, genetic drift, gene flow and bottlenecks on variability are important questions. For captive populations, inbreeding and outbreeding are major concerns.

Range expansion or island colonisation by a species results in a founder effect since the initial colonisers generally represent a fraction of the genetic variability of the source population (also a factor in the establishment of captive populations as has occurred for three Western Indian Ocean fruit bats: *P. rodricensis*, *P. livingstonii* and *P. voeltzkowi*). If this founder population becomes isolated, random genetic drift will lead to further divergence. The extent of divergence will be determined by gene flow (SLATKIN 1994). If regular gene flow through mating occurs between meta-populations, they will tend to follow the same evolutionary trajectory. However, if it is absent, populations tend to evolve independently, potentially leading to speciation events

Any population that has been significantly reduced in number can be considered to have gone through a bottleneck. Rodrigues fruit bats (*P. rodricensis*) have suffered a number of population crashes with numbers as few as 70 individuals in 1974 making them, at the time, the world's most endangered bat (CHEKE & DAHL 1981). The implications of a bottleneck event will depend on a number of demographic traits but species with long generation lengths, low reproductive output and a skew in mating opportunities, as is typical of fruit bats, tend to suffer from eroded variability (LUIKART *et al.* 1998).

In instances where a bottlenecked population remains small (as might be the case in a captive population), the risk of inbreeding increases. Defined as mating between genetically similar individuals, inbreeding tends to reduce heterozygosity (a measure of genetic variability) and increases the likelihood of the expression of recessive lethal genes. Evidence is accruing for the negative impact of inbreeding on 'fitness' in terms of reproductive success and offspring survival (KELLER & WALLER 2002) and ultimately, extinction risk. However, breeding between genetically dissimilar populations can also have negative effects. This outbreeding tends to disrupt co-adapted gene complexes that may be important evolutionary adaptations to the local environment (ZSCHOKKE & BAUR 2002). The result is that both populations may suffer lower overall 'fitness'. Thus, replenishing a depleted population from another geographic area without considering their genetic relat-

edness can be detrimental.

How can conservation genetics be applied to fruit bats?

BURLAND & WORTHINGTON-WILMER (2001) recognised the value that molecular studies could play in answering some of the conservation issues faced by bats. The contribution of genetic research to conservation management is growing and no doubt will prove to be a vital element in the sustainability of island populations of fruit bats throughout their range. Most studies of *Pteropus* spp. fruit bats have concentrated on the Australasian region, the proposed region of origin for the genus (BERGMANS 1997) and the species from the Western Indian Ocean are under-represented in the literature. Our research is attempting to address some of this imbalance.

We have three main areas of interest that can be expressed in terms of conservation-related questions:

1. What are the genetic relationships between the seven extant and one recently extinct species of *Pteropus* fruit bat in the Western Indian Ocean?
In collaboration with colleagues in Yale University, USA, we are analysing the DNA sequences from four genetic loci in an effort to infer the colonisation patterns and degree of divergence between purported species. From this we can determine the evolutionary history of the species in this geographic region. This kind of information is useful to conservation managers by identifying taxonomic and management units. Preliminary results suggest a complex colonisation process with possibly three separate colonisation events beginning about one and a half million years ago. As more species are added to the phylogenetic tree and compared to the phylogenies produced from other genetic loci, the pattern of speciation in the Western Indian Ocean archipelagos will become clearer.
2. How has recent population declines impacted the genetic diversity of Rodrigues fruit bats (*Pteropus rodricensis*) and how effective is the captive breeding programme in retaining wild-type genetic variability?
In collaboration with the University of Tennessee, USA, we are analysing the genetic diversity of the wild population of fruit bats on Rodrigues Island and comparing it to a captive population at Dublin Zoo in Ireland. Using microsatellite (small repeated fragments of DNA) analysis, we have found relatively low but equivalent variability in both wild and captive populations. Our results to date suggest that the species has retained some genetic diversity despite being reduced to less than 70 individuals in the early 1970's – a significant population bottleneck. The biggest threat to the captive breeding programme may be genetic drift, indicated by some divergence between the captive and wild populations. Further microsatellite loci are being tested to clarify the pattern of variability.
3. What are the genetic relationships between island populations of *Pteropus seychellensis* and how can this data be used to formulate a conservation management plan?
There are three sub-species of *P. seychellensis* (MILNE-EDWARDS, 1878) currently recognised (HILL 1971) – *P. s. seychellensis* on the granitic islands of the Sey-

chelles; *P. s. aldabrensis* on Aldabra Island and *P. s. comorensis* on the Comoro Islands. The degree of genetic divergence between these subspecies is unknown and their geographic isolation may provide an argument for incipient speciation processes. In collaboration with the Nature Preservation Trust Seychelles, Seychelles Island Foundation and Yale University, we hope to investigate the genetic variability of island populations of this subspecies complex and to identify management units using microsatellites. Furthermore, we hope to determine the extent of gene flow within island groups with a view to elucidating population substructure. Sampling is underway on the relevant islands and DNA analysis will begin shortly on this element of our research.

CONCLUSION

Genetic research contributes to conservation by evaluating biodiversity, acting as a tool to understanding the evolutionary history of species and populations, and providing information useful for risk assessment in terms of sustainability at various hierarchical levels. Our current research project is a timely genetic analysis of the fruit bats of the Western Indian Ocean given their vulnerability as endemic faunas in highly threatened biodiversity hotspots.

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