Conservation of the Seychelles paradise flycatcher

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Abstract

The main goal of recovery programmes for threatened species is to reverse declines in population trajectory, distribution and abundance that have been caused directly or indirectly by human activities. One conservation strategy increasingly used to reduce the threat of extinction is (re) introduction. However reintroduction is a daunting task, given the complex suite of genetic and demographic factors influencing the ecological and evolutionary processes of natural populations, some of which are still relatively poorly understood. I use the Seychelles paradise flycatcher (SPF) as a study system in order to undertake a series of scientific studies to address questions relevant to the species' conservation and to the wider field of reintroduction biology.

I construct a molecular phylogeny of the *Terpsiphone* flycatchers of the Indian Ocean and use it to evaluate conservation priorities based on evolutionary distinctiveness and place conservation of the SPF into a wider context. I assess the genetic consequences of reintroductions by comparing the loss of genetic diversity across a historical bottleneck that reduced the SPF to c.28 individuals to the loss of genetic diversity due to a recent conservation introduction. I then assess a suite of individual, ecological, and evolutionary variables on SPF productivity within remnant and reintroduced SPF populations.

I find severely depleted genetic diversity following a historical bottleneck does not render a species immune to further genetic erosion upon reintroduction. I find the main drivers of flycatcher productivity are food abundance and predators, and importantly food abundance predicts offspring sex with a bias towards males in low quality habitat. Lastly I combine my findings on the relative influences of genetic, demographic, ecological and evolutionary factors to inform future conservation and reintroduction strategy for the both the SPF and threatened species in general.

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Chapter 1 Introduction

1.1 Conservation of threatened species

The world is facing a global biodiversity crisis as a direct result of human activities (Isaac et al. 2007; Jones & Merton 2012). Extinctions of known species over the past 100 years indicate that current extinction rates are approximately 100 times greater than background rates of extinction estimated from fossil records (Mace et al. 2009), and modelling scenarios indicate that future rates could exceed recent rates by more than two orders of magnitude (Pereira et al. 2010). Currently approximately 19% of the world's vertebrates are considered threatened with extinction (Baillie et al. 2010) and predictions forecast that we could lose 20% of all vertebrate species within the next century (Baillie et al. 2010; Sinervo et al. 2010). While there is no doubt that extinction is a natural process, the current unprecedentedly high rate is outpacing speciation rates leading to a substantial loss of biodiversity (Butchart 2004; Millennium Ecosystem Assessment 2005; Baillie et al. 2010). Conserving current levels of biodiversity in the face of increasing human demands on natural resources is clearly a daunting task. The main goal of recovery programmes for threatened species is to reverse declines in population trajectory, distribution and abundance that have been caused directly or indirectly by human activities.

1.2 Reintroduction as a conservation tool

For threatened species with restricted distributions, conservation biologists often face problems that require direct intervention (Stockwell et al. 1996). One conservation strategy increasingly used to reduce the threat of extinction is the translocation of animals to new localities to establish new populations (Griffith et al. 1989; Stockwell et al. 1996; Seddon 1999; Seddon et al. 2007). *Translocation* is human-mediated movement of living organisms from one area with free release in another (IUCN, 1987; IUCN/SSC 2013). *Reintroduction* is the intentional movement and release of an organism inside its indigenous range from which it has disappeared (IUCN 1998; IUCN/SSC 2013). *Conservation introduction* is an attempt to establish a species, for the purpose of conservation, outside its recorded distribution but within an appropriate habitat and eco-geographical area (IUCN 1998; IUCN/SSC 2013). Published estimates of the total number of conservation translocations undertaken worldwide are lacking however Griffith et al. (1989) estimated around 700 per year in the 1980's (though this included both conservation and native game reintroductions), Seddon et al. (2005) recorded 699 recent, current or planned conservation translocations in 2005 using both IUCN Reintroduction Specialist Group (RSG) records and queries to all RSG members, Germano & Bishop (2009) estimate thousands of translocations have occurred worldwide and Seddon et al.

(2012) state that reintroduction numbers are increasing almost exponentially each year. We can therefore assume that conservation translocations number in the thousands and that they are becoming increasingly common. Conservation translocations are also taxonomically biased towards mammals and birds (Seddon et al. 2005; Germano & Bishop 2009).

Throughout this thesis the word 'reintroduction' is used to refer to both reintroductions and conservation introductions. Reintroduction is a daunting task, given the complex suite of genetic and demographic factors influencing the ecological and evolutionary processes of natural populations (Robichaux et al. 1997). The research conducted as part of this thesis aims to add to this literature through applied conservation scientific research that focuses on a threatened island species. Below, a number of key areas in the fields of conservation and reintroduction biology that need more research are briefly introduced. These sections are followed by a description of an island species system that forms the platform upon which a series of scientific studies were carried out to address questions relevant to the species' conservation and reintroduction biology.

1.3 Ecological and genetic considerations for reintroduction

Despite the extensive use of reintroductions, the biological and genetic implications of this practice remain poorly understood (Stockwell et al. 1996; Robichaux et al. 1997; Groombridge et al. 2012). Prior to any reintroduction numerous factors need to be considered. Most importantly, the causes of decline or local extinction must be addressed (either removed or neutralised) before any reintroduction can be undertaken (IUCN 1998). The most common reasons for species declines are habitat loss and introduced predators (Frankham 1998; Baillie et al. 2010). The rapid increase in the number of reintroductions taking place worldwide and the high failure rates documented for reintroduction initiatives prompted the formation of the IUCN reintroduction specialist group in1988 and the production of a set of guidelines for reintroductions (IUCN 1998) These guidelines have recently been updated in response to accelerating ecological change and the accompanying increase in pressure on global biodiversity due to habitat degradation, loss and fragmentation, biological invasions and clmate change (IUCN/SSG 2013). Numerous reasons have been cited as contributing to low success rates of reintroductions. The most commonly cited reason is poor quality habitat at the reintroduction site (Wolf et al. 1996, 1998; Germano & Bishop 2009; Moorhouse et al. 2009; White et al. 2012). Other reasons cited as influencing reintroduction outcome are the number of individuals released (higher numbers released increase the chance of success) (Griffith et al. 1989; Wolf et al 1996, 1998; Fischer & Lindenmayer 2000), the source of the released individuals (wild individuals generally establish more successfully than captive reared individuals) (Fischer & Lindenmayer 2000; Jule et al. 2008; Aaltonen et al. 2009), the presence of predators (Moorhouse et al. 2009; White et al. 2012), provision of supplementary food (White et al. 2012) and failure to rectify the cause of the historical decline/loss of the species in the area where the reintroduction was undertaken (Fischer &

Lindenmayer 2000). Consequently, assessment of habitat quality and its effects on reintroduced populations has become an important area of focus for improving reintroduction success.

An equally important but until recently largely overlooked aspect of reintroduction is the genetic consequences of such initiatives. The magnitude of genetic consequences for reintroduced populations remains largely unknown (Armstrong & Seddon 2008; Groombridge et al. 2012). However we can assume that reintroduced populations founded from low numbers of individuals will likely be compromised by the same genetic problems that are associated with bottlenecked populations, such as loss of genetic variation, inbreeding and inbreeding depression, genetic attributes which are widely accepted to increase extinction risk in natural populations (Saccheri et al. 1998; Bijlsma et al. 2000; Keller & Waller 2002; Frankham 2005). However, genetic factors do not usually work in isolation, but rather they most often interact with non-genetic factors that cause populations to decline in the first place (e.g. introduced predators or habitat loss or disease) (Reed 2010; Jamieson & Lacy 2012). Consequently, genetic and ecological factors need to be examined alongside each other in order to provide an informed perspective of their effects on reintroduced populations.

1.4 Maintaining genetic diversity for long-term evolutionary potential

To be successful in the long-term, reintroduced populations need to retain sufficient genetic diversity to enable evolutionary adaptation to future environmental change (Robichaux et al. 1997; Reed & Frankham 2003; Keller et al. 2012). Reintroduced populations, particularly those carried out for conservation purposes, are vulnerable to both low initial levels of genetic diversity (as a consequence of the species' demographic history) and to subsequent loss of that diversity (due to management-induced bottlenecks). Reintroduced populations have, therefore, usually passed through at least two bottlenecks: an initial one that reduced the species' population to endangered levels prompting conservation intervention, and a second bottleneck experienced upon reintroduction. Without doubt, maintaining a species' evolutionary potential presents a long-term challenge for conservation managers, alongside ensuring its survival in the short-term. There is disagreement in the literature concerning minimum viable population sizes required for maintenance of sufficient quantitative genetic variation to allow future adaptive change. Effective population sizes (N_e) in the low thousands have been proposed by several authors as necessary to maintain adequate adaptive potential for long term persistence (Willi et al. 2006; Keller et al. 2012)while others have proposed values considerably lower suggesting census populations in the low thousands may be sufficient. For exmple Franklin (1980) calculated an effective population size of at least 500 would be required for the long-term maintenance of sufficient quantitative genetic variation to allow future adaptive change. Later Frankham (1995) reviewed N_e to census population size (N) ratios and found that they averaged 0.10. Therefore a N_e of 500 would equate to a census population of 5000. In a meta-analysis of

minimum viable population size (MVP) required for long-term survival covering 212 species Traill et al. (2007) found a median MVP of 4169 individuals (95% CI=3577-5129).

For most Critically Endangered species conservation programmes, achieving effective population sizes in the low thousands, which equates to census population sizes an order of magnitude larger, will not be possible. However achieving census population sizes in the low thousands may be realistic for many Critically Endangered species, though equally it may not be possible for many others. It is worth noting however that some species restricted to small islands for many thousands of years may never have had effective population sizes or census population sizes in this range due to naturally small range sizes. Most conservation sceintists however do not advocate taking no action because a species has too few individuals and/or is unlikely to be able to achieve the MVP advocated in the foreseeable future. In order to achieve the goal of maximising evolutionary potential in reintroduced populations, managers can begin by incorporating genetic management into recovery programmes and by managing reintroduced populations to maximise retention of genetic diversity.

1.5 Using phylogenetic approaches to identify evolutionarily distinct units

As the global biodiversity crisis intensifies with rising human activity, there is an increasing need to prioritise the allocation of finite conservation resources. The number of species worldwide threatened with extinction far exceeds the conservation resources available and predictions suggest this trend is worsening (Pimm et al. 1995; Hazevoet 1996; Myers et al. 2000; Butchart et al. 2004; Isaac et al. 2007), forcing conservation planners to increasingly focus on prioritizing which populations to protect or restore. Priority-setting approaches have frequently focused on measures of endemism and restricted range (e.g. Stattersfield et al. 1988; Myers et al. 2000; Olson et al. 2001), however numerous studies advocate that evolutionary distinctiveness should also be an important consideration (e.g. Faith 1992; Witting & Loeschcke, 1995; Crozier 1997; Isaac et al. 2007). In practice however, evolutionary distinctiveness has often been overlooked, largely due to a lack of availability of taxonomically-complete species and subspecies-level phylogenies for the taxa of concern (Isaac et al. 2007). However recent advances in molecular techniques have meant that molecular phylogenetic studies are more affordable and accessible, paving the way for incorporation of measurement of evolutionary distinctiveness into conservation priority-setting.

1.6 The Seychelles

The Seychelles are a nation comprising approximately 140 islands spreading almost 1000 kilometres from 4-10^{o} South and 46-54^{o} East. The North eastern most group comprises c.40 ancient granite islands that once formed part of the supercontinent Gondwanaland some 130 million years ago. The granitic Seychelles split away from India around 65 million years ago and have been isolated

from other landmasses since. The coralline islands (c.100 in total) are much younger, comprising either sand cays which are approximately 2,000 years old or raised reef platform islands that emerged most recently approximately 125,000 years ago (Braithwaite 1984).

Four endemic land birds have been recorded to go extinct in the granitic islands since human colonisation in 1768; the Seychelles parakeet (*Psittacula wardi*), the Seychelles chestnut flanked white-eye (*Zosterops semiflava*), the Seychelles turtle dove (*Streptopelia picturata rostratus*) and the 'Poule ble' which went extinct before it was officially identified though from descriptions it must have been a species of *Porphyrio* (Lionnet 1984). Of the remaining 12 endemic bird species, six have been subjected to intensive recovery programmes involving reintroductions and conservation introductions; the Seychelles white-eye (*Zosterops modestus*), the Seychelles warbler (*Acrocephalus sechellensis*), the Seychelles fody (*Foudia sechellarum*), the Seychelles kestrel (*Falco araea*), the Seychelles magpie-robin (*Copsychus sechellarum*) and the Seychelles paradise flycatcher (*Terpsiphone corvina*).

Reasons cited for the population declines and extinctions of Seychelles avifauna since human colonisation in 1768 are habitat loss and destruction, predation by introduced rats and persecution, and in the case of the Seychelles turtle dove dilution through interbreeding with the introduced Madagascar turtle dove (*Streptopelia picturata picturata*) (Lionnet 1984; Watson 1984). Recovery programmes have for these species have involved predator (rat and cat) eradications, habitat rehabilitation (the removal of invasive species and replanting with native vegetation) and reintroductions and conservation introductions of the target species concentrated on several of the smaller granitic islands.

1.7 The Seychelles Paradise Flycatcher

The Seychelles paradise flycatcher (*Terpsiphone corvina*), (Newton 1968), known locally as Vev, and referred to as SPF or flycatcher throughout this thesis, belongs to the family Monarchidae, a diverse family of passerine birds containing 18 genera and 97 species with a largely Old World Paleotropical distribution (Coates et al. 2006). The paradise flycatchers in the *Terpsiphone* genus are the most widely distribted occurring over most of sub-Saharan Africa, southern and eastern Asia, the Philippines and the western Indian Ocean islands (Coates et al. 2006). There are 12 or 13 recognised species in the genus depending on authors (Sibley & Monroe 1990; Coates et al. 2006; BirdLife International 2011; IUCN 2013). Most are widespread and are listed as Least Concern, apart from *T. bedfordi*, *T. atrocaudata*, and *T. cyanescens* which are listed as Near Threatened and *T.corvina* which is listed as Critically Endangered (IUCN 2013) and the SPF is the only species in the genus that is subject to intensive conservation management. The SPF is a lowland forest-dwelling, insectivorous c.18 gram passerine (Watson 1981, 1991; Currie et al. 2003b) endemic to the granitic islands of the Republic of Seychelles. The species is highly sexually dichromatic. Males have entirely glossy black

plumage with very elongated central tail feathers up to 33 cm long and electric blue fleshy eye rings and bills. Females are tri-coloured with black heads, chestnut wings, tails and upper parts and dirty white under parts and lack the elongated tails of the males.

SPF lay single-egg clutches in small open cup-shaped nests woven from coconut fibre, moss, spiders' web and other vegetation, but have multiple nesting attempts per year. Successful breeding attempts take c.4 months to complete. Breeding occurs throughout the year but is at its highest in the rainy North-west monsoon season between November-April and at its lowest during the dry South-east trade wind season from May-October. SPF are relatively long-lived; adults have an annual mortality rate of 21% (Currie et al. 2005) and several ringed individuals have been re-sighted 10 years after ringing (RM Bristol pers. obs.; Watson 1991). The SPF is behaviourally monogamous and highly territorial. Pairs maintain and defend exclusive territories year round. Pairs maintain long-term pair bonds, usually for life; only very rarely have adult territory-holding individuals been observed to move territory (RM Bristol pers. obs.).

1.8 Previous research on the SPF

Watson (1981, 1988, 1991) surveyed the SPF population and conducted initial research into their habitat requirements on the La Digue western plateau during 1977-78. He found an association between SPF and broadleaved native woodland in proximity to wetlands with highest SPF densities in woodland close to wetland. This finding resulted in the widespread belief that SPF were dependant on native broadleaved forest associated with wetland. These findings were instrumental in the creation of the 'Veuve Special Reserve'. Eight hectares of plateau woodland were leased in 1979, formally protected in 1991 and subsequently expanded to 21 hectares in 2003 to encompass a significant area of wetland.

Currie et al. (2003a, 2003b, 2003c, 2005) conducted extensive field research for 2 ½ years from 1999- 2001 into the habitat requirements and ecology of the SPF on the coastal western plateau of La Digue in order facilitate prioritisation of islands for creating additional sustainable populations through translocations. The study quantified, habitat requirements, foraging and breeding success, juvenile and adult mortality, determined flycatcher distribution and estimated population size. I was the main field researcher on this project.

Currie et al. (2003b) found that native broadleaved forest was an important flycatcher habitat requirement with flycatcher territories containing significantly more native tree species than predicted by their availability on the plateau. Native tree species were used significantly more for both foraging and nesting than predicted by their availability within territories and there was an inverse correlation between the density of native tree species and territory size. However this study also found that the importance of wetland areas had been exaggerated. There was no significant effect of proximity to

water on either foraging or breeding success, and only a marginal effect of distance to water on total invertebrate numbers (Currie et al. 2003b). Additionally the majority of identified flycatcher prey species were not dependant on water at any stage of their life cycle (Currie et al. 2003b). Currie et al. (2003b) also found the density of flycatcher territories was higher near water, however impotantly noted that this result did not take into account that the majority of the remaining woodland on the La Digue plateau is near water and that the distribution of flycatcher territories on the La Digue plateau could be explained by the distribution of native broadleaved habitat. Additionally a La Digue Island wide survey (Currie et al 2003a) found significant numbers of flycatchers residing off plateau and the only variable explaining flycatcher presence was canopy height with individuals more likely to be present in high canopy broadleaved woodland. Historically SPF were recorded on islands without extensive wetland areas and only very small areas of coastal plateau (e.g. Aride, Félicité, Marianne) lending support to the finding that the importance of water and wetland areas to SPF may have been exaggerated (Currie et al 2003c). Ship rats (*Rattus rattus*) and native Seychelles bulbuls (*Hypsipetes crassirostris*) were positively identified as egg predators and cats (*Felis catus*) as predators of adult flycatchers during this study (Currie et al. 2005).

SPF were first recorded away from coastal platea areas during a population survey in 1997 with a few indiviuals recorded on the the La Digue mountain (referred to as 'hill' throughout this thesis) (Rocamora 1997). Currie et al. (2005) found higher numbers of SPF on the La Digue hill in 2001 than Roacmora (1997) but at significantly lower densities than the plateau, and not in all hill areas.

1.9 Species status and rationale behind choice of island for reintroduction.

The SPF is listed as listed as Critically Endangered (B1ab(iii), ver3.1; IUCN 2013) based on small species range and decreasing extent of habitat. Historically recorded on five islands in the Seychelles archipelago (Diamond 1984) (see Figure 1.1), the species experienced a dramatic reduction in range and numbers in the late 19th-20th century, attributed to habitat loss through large-scale forest clearance for plantation agriculture, and predation by introduced rats and cats (Gaymer et al. 1969; Watson 1984; Currie et al. 2001, 2003c, 2005). The species disappeared from Aride (68 ha), Félicité (268 ha) and Marianne (9.5ha) by the early 1900s (Nicoll 1906; Vesey-Fitzgerald, 1940; Diamond 1984) and from Praslin (2750 ha) by the 1980s (Gerlach 1997), leaving it restricted to La Digue (1000 ha) where the population declined to its lowest estimate in 1965 of 28 individuals, restricted to c.300 ha of coastal plateau on La Digue (Gaymer et al. 1969). The species then began a relatively unassisted and steady recovery to the current population estimate of 218-290 individuals (Currie et al. 2003a), distributed across the coastal plateau on La Digue with recent expansion of the species distribution up onto the hill of La Digue (Currie et al. 2003a, RM Bristol pers. obs.).

The major current threat to the flycatcher on La Digue is habitat loss through deforestation for housing and tourist developments. Currently the vast majority (>90%) of flycatcher pairs occur outside the small 21 hectare Veuve (SPF) Special Reserve, and mainly on privately owned land on the La Digue plateau, making their territories more vulnerable to loss or degradation through development. Consequently, there is little opportunity to increase the amount of suitable habitat on the La Digue coastal plateau, the stronghold of the species. Therefore establishment of additional populations on other suitable islands has long been considered a major priority in order to improve the prospects of long-term survival and reduce the risk of extinction (Watson 1984, 1991; Hambler 1992; Rocamora 1997; Marshall 1997; Currie et al. 2001; Hill 2002; Currie et al. 2003c).

1.10 Choice of island for 'reintroduction'

Prior to any translocation, particular research and decision-making must be undertaken as part of reintroduction planning in order to ensure the most appropriate choice of site and methods that will yield the highest chance of a successful reintroduction. The reintroduction planning for SPF included qualitative and quantitative assessment of potential islands using criteria regarded as important for flycatchers and a review of potential translocation methods. A selection of 15 potential islands was critically and objectively assessed for suitability to support a flycatcher population.

Islands were assessed on criteria believed to be important for flycatchers including area of lowland native broad-leafed woodland, presence of known SPF predators (rats *Rattus* species, cats *Felis catus* and Seychelles bulbuls *Hypsipetes crassirostris*), flycatcher food invertebrate abundance and presence of wetland areas. The future potential of each island for supporting flycatchers i.e. 'rehabilitation potential' was also assessed on area of lowland plateau and island management's support for conservation initiatives and willingness to implement habitat rehabilitation programmes. Denis Island was selected as the site for a conservation introduction of this species, because although outside the known historical distribution of the species, it was the only island with a sufficient area of suitable habitat and an absence of known predators (Currie et al. 2003c; Bristol & Groombridge 2007). A copy of the island assessment report can be found in Appendix 1.

Disease and health screening of donor and recipient island avifauna populations was then undertaken to ensure no potentially harmful diseases would be introduced to Denis Island along with the flycatchers and also that the flycatchers would not be exposed to potentially harmful diseases on Denis Island that they had not already been exposed to on La Digue. Habitat rehabilitation efforts to convert 20 hectares of abandoned coconut plantation into native broad-leafed woodland was also undertaken to provide additional habitat for flycatchers to expand into once the woodland is established.

In order to proceed with the conservation introduction, support was required from all national and local stakeholders, particularly the La Digue inhabitants (Diguois), the Seychelles government and Denis Island management. The Diguois felt a strong ownership of the flycatcher and were reluctant to see it introduced to any other island. Therefore an education and awareness campaign was mounted on La Digue, led by RM Bristol and Terence Vel (Nature Seychelles) under Darwin Initiative Funded project 15/009 "*Investing in island biodiversity; restoring the Seychelles Paradise Flycatcher"* to raise knowledge of the flycatchers critical status, the threats it faces on La Digue and the necessity of the species' introduction to other islands to provide safety net populations and to secure the long-term future of the species. Permission was gained from the Diguois in 2008 together with permission from the Seychelles Government, and the conservation introduction was undertaken to Denis island in November 2008, funded by the Darwin Initiative-funded project 15/009 on which I was the Project Officer from late 2006-late 2009. The initial proposal to introduce flycatchers to Denis Island was favourably reviewed by the IUCN Reintroduction Specialist Group (by the bird section Chair, Dr P Seddon) and was subsequently approved by the Seychelles Government (see Appendix 2 for full proposal).

1.11 Brief description of the conservation introduction

Twenty three individuals (comprising 13 males and 10 females [7 adult males; 7 adult females; 6 immature males and 3 immature females]) were introduced to Denis Island on the mornings of 25th and 26th November 2008. Birds were captured early morning in mist-nets, weighed, measured, blood sampled and ringed with unique colour ring combinations. Birds were transferred by helicopter from La Digue to Denis Island (30 minute flight) in individual cardboard boxes with a perch, some foliage and a small water container and covered in closed cell foam mats to reduce helicopter noise levels. Upon arrival on Denis the flycatchers were checked over, given a drink of rehydration fluid and immediately released into broad-leafed forest that had been liberally sprayed with water. All birds were released before midday. Following their release the new population intensively monitored for 22 months: all individuals were closely tracked to determine movements, survival, and to detect and monitor all breeding attempts. After the 22 months intensive monitoring (November 2008-September 2010) where the SPF were continuously monitored, annual population censuses were undertaken to monitor population growth.

1.12 Brief summary of conservation introduction outcome

Initial survival of individuals post-translocation was high with only two individuals not seen after release. The remaining 21 individuals settled and were all still regularly re-sighted five months post release (Bristol 2009). The released birds started breeding one month post-release. By 11 months post release the population stood at 24 individuals with five chicks successfully fledged on Denis

Island and four of the original released stock missing and assumed dead (Bristol & Nourrice 2009). By the end of the intensive monitoring period, 22 months post release, the population was estimated at c.24 individuals with eight of the original released stock assumed dead and 11 chicks successfully fledged on Denis Island (French & Bristol 2010). A population census in December 2011 estimated the population on Denis Island to be between 30-33 individuals (Henriette & Laboudallon 2011) and by July 2013 the population had grown to an estimated size of 39-41 individuals (Bristol 2013).

1.13 Overall aim of the thesis

Conservation practice has received criticism for failing to incorporate science into conservation and neglecting to systematically and objectively evaluate conservation evidence (Sutherland et al. 2004, 2010; Seddon et al. 2007; Armstrong & Seddon 2008). In order to inform conservation management and to learn from actions taken, what is needed are genetic and ecological studies designed to answer specific questions which have been identified *a priori* (Armstrong & Seddon 2008). In order to inform conservation management of the Seychelles paradise flycatcher I undertook research centred on the following themes:

(i) Assessment of the evolutionary distinctiveness of the *Terpsiphone* paradise flycatchers of the Indian Ocean in order to characterise the evolutionary radiation of this group of flycatchers and to quantify the evolutionary distinctiveness, and subsequently the conservation priority, of the Seychelles paradise flycatcher.

(ii) Assessment of the genetic consequences of the historical bottleneck experienced by the species and of the genetic consequences of the contemporary conservation management actions on the genetic profile of the species.

(iii) All previous SPF research has been undertaken exclusively on the large western plateau of La Digue. I undertake an assessment of evolutionary, ecological and demographic variables on flycatcher productivity between remnant and reintroduced flycatcher populations on the LaDigue coastal plateau, the La Digue hill and the population introduced to Denis Island

I then use my findings to inform future conservation management of the SPF and the wider avian reintroduction biology field.

1.14 Thesis Outline

The thesis is structured as follows:

In **Chapter 2** I construct a molecular phylogeny of *Terpsiphone* flycatchers of the Indian Ocean using a total of 4.4kb of mitochondrial (cyt-*b*, *ND3*, *ND2*, control region) and nuclear (*G3PDH*, *MC1R*) sequence data obtained from all species, subspecies and island populations of the region. I use the molecular phylogeny to investigate the evolutionary relationships between the *Terpsiphone* species

of the Indian Ocean and to determine routes, chronology and timing of *Terpsiphone* flycatcher colonisation of the western Indian Ocean islands. I then compare the molecular phylogeny to the current species nomenclature and use evolutionarily significant units to prioritise conservation investment within the *Terpsiphone* flycatchers of the region.

Reintroduction is an important tool for recovering endangered species; however the magnitude of genetic consequences for reintroduced populations remains largely unknown, in particular the relative impacts of historical population bottlenecks compared to those induced by conservation management. In **Chapter 3** I characterise 14 microsatellite loci developed for the SPF and use them to quantify temporal and spatial measures of genetic variation across a 134-year timeframe encompassing a historical bottleneck that reduced the species to ~28 individuals in the 1960s, through the initial stages of recovery and across a second contemporary conservation-introduction-induced bottleneck. I then evaluate the relative impacts of the two bottlenecks, and finally apply the findings to inform broader reintroduction strategy.

The generally low success rates of reintroductions of threatened species has led to calls from the reintroduction biology community to strategically research, monitor and evaluate reintroductions in order to understand the drivers of success and failure and to provide feedback to improve future success rates. In **Chapter 4** I quantify differences in levels of productivity in the SPF between three sites; the remnant population on the La Digue plateau, the recently self-colonised population on the La Digue hill, and the population introduced to Denis Island. I then quantify the drivers of SPF productivity within and across these three sites, and lastly use the findings to make recommendations for future reintroductions.

In **Chapter 5** I examine ecological and evolutionary determinants of parental investment, fledging weights and survival to independence in the SPF. I quantify parental feeding rate and food volume delivery, offspring fledging weight, survival to independence and extra-pair paternity (EPP) for source and reintroduced populations of the SPF. I analyse these variables alongside a suite of territory-specific and site-specific measures of habitat quality to (i) identify biological and ecological predictors of nestling feeding rate, fledging weight, fledgling sex, and survival to independence (ii) quantify levels and effects of EPP, and (iii) examine how an understanding of these processes can help guide future reintroduction strategy for this endemic island species and for other threatened passerines.

In **Chapter 6** I provide a synopsis of the key findings and discuss their implications for providing guidance for future reintroduction strategy of the SPF and other threatened species.

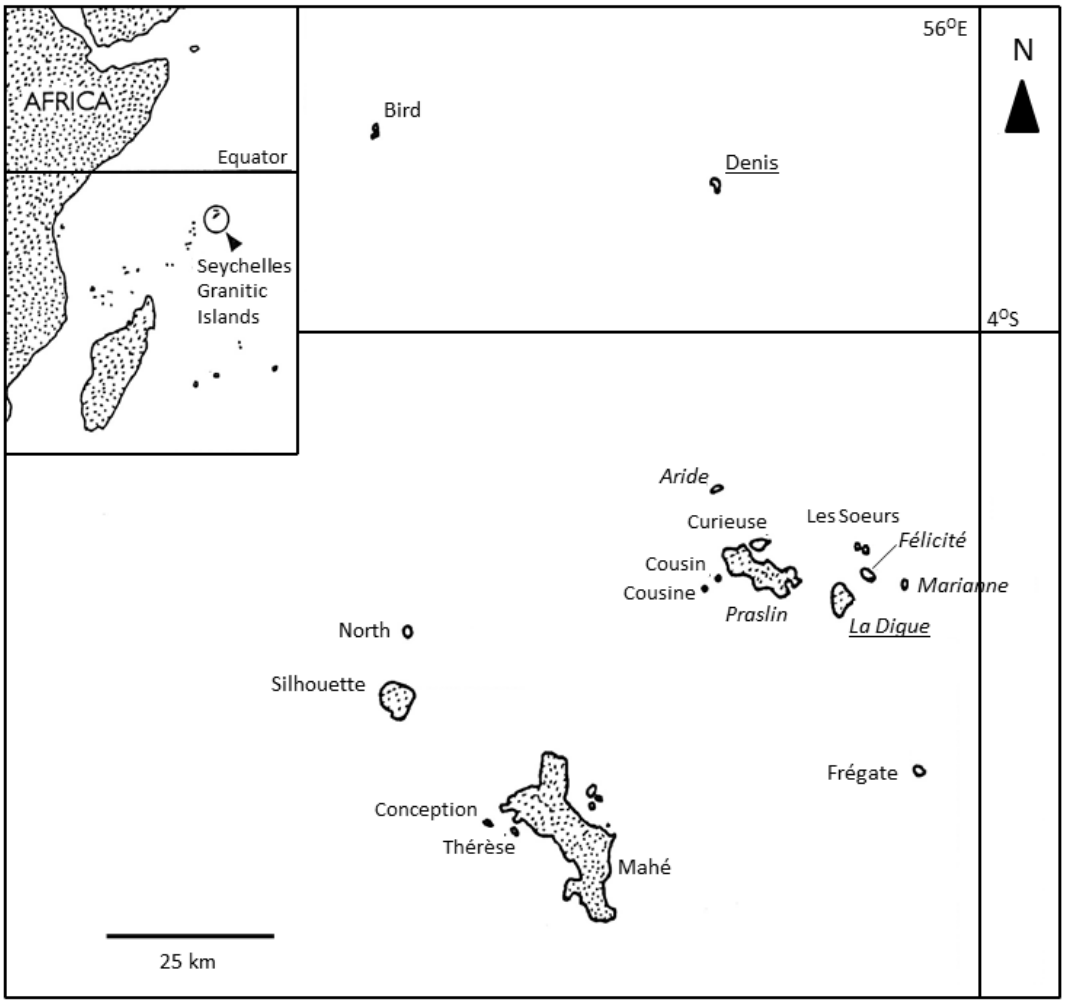

Figure 1.1 Map of the granitic Seychelles Islands showing documented Seychelles parasdise flycatcher historical distribution (*italics*) and current distribution (underlined).

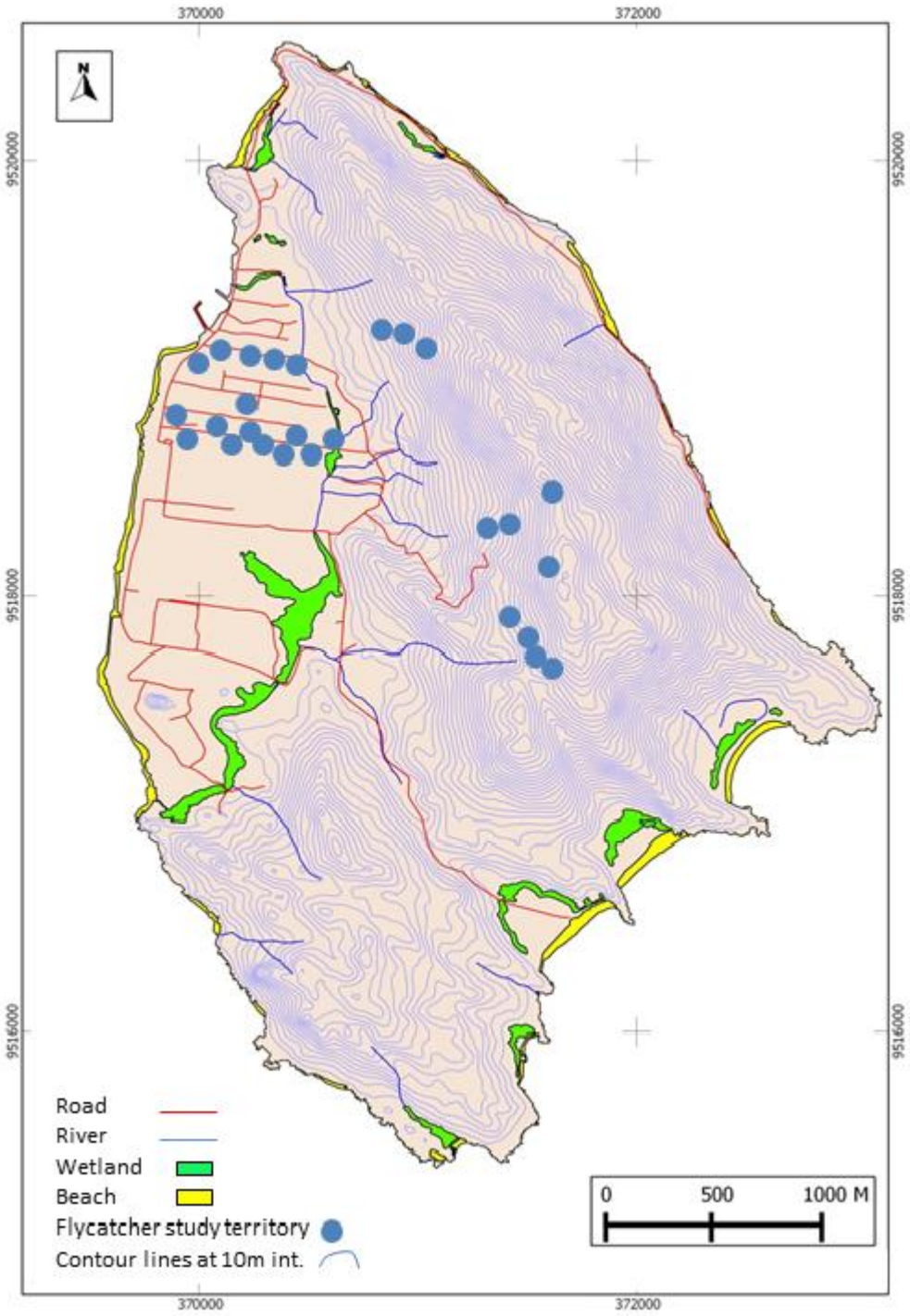

Figure 1.2 Map of La Digue Island showing locations of flycatcher study territories.

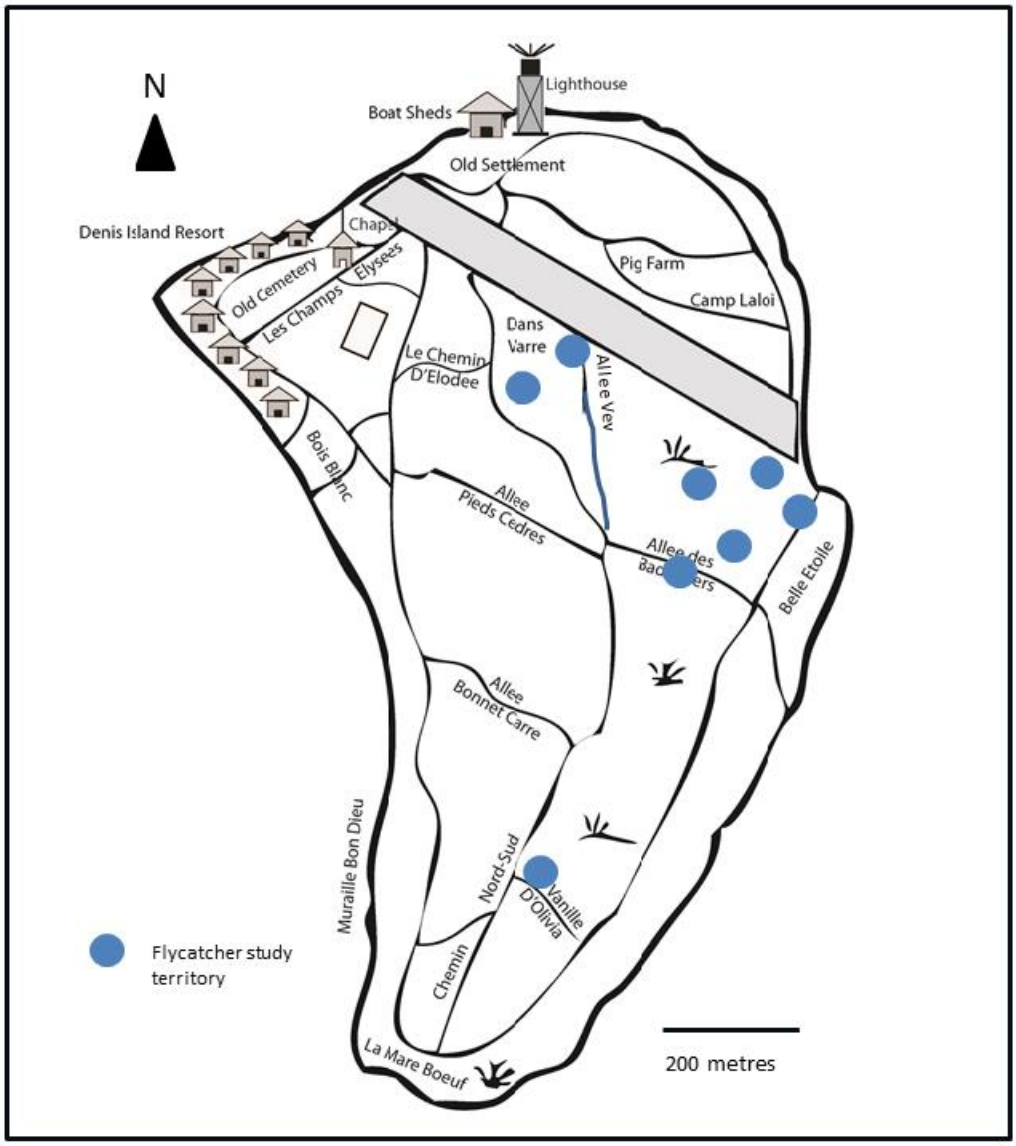

Figure 1.3 Map of Denis Island showing locations of flycatcher study territories.

Chapter 2. Molecular phylogeny of the Indian Ocean *Terpsiphone* paradise flycatchers: undetected evolutionary diversity revealed amongst island populations

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Key Words: *Terpsiphone*, molecular phylogeny, biogeography, island populations, evolutionary distinctiveness, conservation.

ABSTRACT

We construct a molecular phylogeny of *Terpsiphone* flycatchers of the Indian Ocean and use this to investigate their evolutionary relationships. A total of 4.4kb of mitochondrial (cyt-*b*, *ND3*, *ND2*, control region) and nuclear (*G3PDH*, *MC1R*) sequence data were obtained from all species, subspecies and island populations of the region.

Colonisation of the western Indian Ocean has been within the last two million years and greatly postdates the formation of the older islands of the region. A minimum of two independent continent-island colonisation events must have taken place in order to explain the current distribution and phylogenetic placement of *Terpsiphone* in this region. While five well-diverged Indian Ocean clades are detected, the relationship between them is unclear. Short intermodal branches are indicative of rapid range expansion across the region, masking exact routes and chronology of colonisation.

The Indian Ocean *Terpsiphone* taxa fall into five well supported clades, two of which (the Seychelles paradise flycatcher and the Mascarene paradise flycatcher) correspond with currently recognised species, whilst a further three (within the Madagascar paradise flycatcher) are not entirely predicted by taxonomy, and are neither consistent with distance-based nor island age-based models of colonisation. We identify the four non-Mascarene clades as evolutionarily significant units (ESUs), while the Mascarene paradise flycatcher contains two ESUs corresponding to the Mauritius and Réunion subspecies. All six ESUs are sufficiently diverged to be worthy of management as if they were separate species.

This phylogenetic reconstruction highlights the importance of subspecific molecular phylogenetic reconstructions in complex island archipelago settings in clarifying phylogenetic history and ESUs that may otherwise be overlooked and inadvertently lost. Our phylogenetic reconstruction has identified hidden pockets of evolutionary distinctiveness, which provide a valuable platform upon which to re-evaluate investment of conservation resources within the *Terpsiphone* flycatchers of the Indian Ocean.

2.1 INTRODUCTION

As the global biodiversity crisis intensifies with rising human activity, there is an increasing need to prioritise the allocation of finite conservation resources. The number of species worldwide threatened with extinction far exceeds the conservation resources available and predictions suggest this trend is worsening (Pimm et al. 1995; Hazevoet 1996; Myers et al. 2000; Butchart et al. 2004; Isaac et al. 2007), forcing conservation planners to increasingly prioritise which populations to protect or restore. Priority setting approaches have frequently focused on measures of endemism and restricted range (e.g. Stattersfield et al. 1988; Myers et al. 2000; Olson et al. 2001), however numerous studies advocate that evolutionary distinctiveness should also be an important consideration (e.g. Faith 1992; Witting & Loeschcke 1995; Crozier 1997; Isaac et al. 2007). In practice however, evolutionary distinctiveness is often overlooked, largely due to a lack of complete species and subspecies-level phylogenies (Isaac et al. 2007).

The concept of Evolutionarily Significant Units (ESUs) was developed to provide an objective approach for prioritising taxa for conservation management and to ensure important phylogenetic diversity is not overlooked (Ryder 1986) as taxonomy does not necessarily reflect underlying phylogenetic diversity (Avise 1989; Zink 2004). A recent advance in the objective identification of ESUs is Pons et al.'s (2006) general mixed Yule coalescent (GMYC) model. The method makes use of coalescence theory to identify a point of transition between species-level and population-level evolutionary processes. The success in the application of this method across a wide range of taxa (e.g. Vuataz et al. 2011; Poulakakis et al. 2012) suggests that it is likely to become a key tool in the objective allocation of finite conservation resources across regions and communities. Nowhere is this approach likely to be more important than in island systems, as a result of their high frequency of cryptic evolutionary distinctiveness.

The islands of the western Indian Ocean are known for their high levels of biodiversity, endemism and investment of conservation efforts, and as a result have become a natural focus for evolutionary research (e.g. Groombridge et al. 2002; Raxworthy et al. 2002; Fuchs et al. 2008; Rocha et al. 2009; Warren et al. 2003, 2005, 2006, 2010; Kundu et al. 2012). This region's endemic biodiversity has suffered high levels of extinction including well-documented cases such as the dodo (*Raphus cucullatus*), and solitaire (*Pezophaps solitaria*), as well as several species of parrot, owl, rail and giant tortoise. Réunion Island alone has lost 61% of its native landbird fauna since human arrival, of which at least 12 species were endemic to the island (Probst & Brial 2002; Cheke & Hume 2008). Fortunately, these islands still contain remnant populations of a plethora of other endemic species however many have suffered drastic declines and are now reduced to tiny relict populations relying on intensive and sustained conservation efforts to prevent further extinctions. Consequently, phylogenetic studies focused on radiations of island populations can play a particularly important role; identifying

evolutionary significant units can help streamline biodiversity conservation within these island settings.

Against this backdrop of historical extinctions, the endemic taxa of the Indian Ocean islands are frequently comprised of different island forms that collectively show the full range of extinction threat status between them, from very common to extremely endangered, making this oceanic region an important focus for examining evolutionary processes within a conservation context. Previous molecular phylogenies of subspecies and island forms within the region have exposed pockets of phylogenetic diversity that do not align to current taxonomy, e.g. *Hypsipetes* bulbuls (Warren et al. 2005), *Phelsuma* geckos (Austin et al. 2003) and *Coracopsis* parrots (Kundu et al. 2012), highlighting a widespread need to clarify phylogenetic history and refocus conservation priorities.

The *Terpsiphone* paradise flycatchers of the western Indian Ocean are a visually stunning and intriguing group that illustrate the need for fine-scale molecular phylogenetic information to determine evolutionary distinctiveness amongst their different island forms in order to prioritise conservation efforts. *Terpsiphone* paradise flycatchers are a globally widespread and highly speciose genus of Monarchidae passerines, occurring over most of sub-Saharan Africa, southern and eastern Asia, the Philippines and the western Indian Ocean islands. There are 12 or 13 recognised species depending on authors (Sibley & Monroe 1990; Coates et al. 2006; BirdLife International 2011; IUCN 2011). Levels of threat and conservation status vary enormously. For example, the Seychelles paradise flycatcher (*Terpsiphone corvina*) is listed as Critically Endangered, three other species in the genus (*T. bedfordi, T. atrocaudata, T. cyanescens*) are listed as Near Threatened and the remainder are widespread and listed as Least Concern (IUCN 2011). Three *Terpsiphone* species are endemic to the western Indian Ocean, (*T. corvina* in the Seychelles, *T. mutata* in Madagascar and the Comoros and *T. bourbonnensis* in Mauritius and Réunion). These three species are split into eight subspecies found amongst the different islands (see Figure 2.1 for locations of different species and subspecies). The Critically Endangered Seychelles paradise flycatcher is restricted to just $10km^2$ with a total population of c. 300 individuals (Currie et al. 2003a, 2003b). Several studies have examined habitat requirements, threats and conservation action strategies for this species, but its evolutionary distinctiveness remains unconfirmed. Elsewhere, the Mascarene paradise flycatcher (*T. bourbonnensis*) is common on Réunion (*T. b. bourbonnensis*), but the subspecies on Mauritius (*T. b. desolata*) is extremely rare with a population of 100-223 pairs (Safford 1997) while accurate population estimates for the *T. mutata* subspecies and island forms are lacking.

The high levels of biodiversity and endemism that have evolved in this part of the world have stemmed from two key characteristics, namely the position of the western Indian Ocean in relation to the neighbouring continental land masses, and the region's unusually complex geological history (Duncan & Hargraves 1990; Raxworthy et al. 2002.) Situated between Africa to the west and Asia to

the north-east, the western Indian Ocean islands' biota displays affinities with both Africa and Asia, with the origins of different taxonomic groups appearing to come from either one or the other of these continents. For example the western Indian Ocean islands were colonised by kestrels and sunbirds from Africa (Groombridge et al. 2002; Warren et al., 2003) and Scops owls and bulbuls from Asia (Fuchs et al. 2008; Warren et al. 2005). Additionally the western Indian Ocean islands show an unusually diverse range of geological ages and origins. They can be grouped into three broad categories based on geology and age (Warren et al. 2003; see Table 2.1 for details of island ages, geology and sources of information). A geological anomaly within the Indian Ocean is the presence of very shallow regions, such as the Seychelles Bank, a shallow submarine platform of some 43,000km^2 that seldom exceeds 65m depth (Camoin et al. 2004) and other shallow, currently submarine areas of substantial size. Sea level low-stands are known to have exceeded 80 m below present levels at least six times during the past 500,000 years (Rohling et al. 1998; Camoin et al. 2004; Bintanja et al. 2005) with at least 12 further episodes where sea level exceeded 65m below present levels within the last 3.5 million years (Rohling et al. 1998; Siddall et al. 2003; Bintanja et al. 2005; Miller et al. 2005). During these sea-level low stands, some of which persisted for thousands of years, the Seychelles' land mass has been up to 180 times its present size and additional large islands would have been present in the western Indian Ocean providing stepping-stones between landmasses facilitating dispersal of individuals across the region during the Pliocene and Pleistocene (Cheke & Hume 2008; Warren et al. 2010). Consequently, the evolutionary history of *Terpsiphone* flycatchers is likely to be complex and not easily discerned from current geography alone.

Here, we present a comprehensive molecular phylogenetic reconstruction for the western Indian Ocean *Terpsiphone* flycatchers based on a 4,429 base pair (bp) DNA sequence dataset comprising six genes (two nuclear and four mitochondrial loci). We use this molecular phylogeny to: (i) identify the evolutionary origins and routes of radiation of the western Indian Ocean paradise flycatchers, and (ii) clarify the evolutionary distinctiveness of the different island forms, in particular the Critically Endangered Seychelles paradise flycatcher, and re-evaluate priorities for their conservation.

2.2 MATERIALS AND METHODS

2.2.1 Taxon sampling

We obtained samples for genetic analysis from 34 individuals covering all three species (*Terpsiphone mutata*, *Terpsiphone corvina* and *Terpsiphone bourbonnensis*) and nine populations (see Figure 2.1) from the western Indian Ocean islands. Our sample contained representatives of between

two and six individuals from each population (see Table 2.2 for details of all samples used in this analysis). In addition we included representatives of both African and Asian *Terpsiphone* flycatchers to determine closest continental affinities of the western Indian Ocean island *Terpsiphone* flycatchers. We also included the São Tomé paradise flycatcher *Terpsiphone atrochalybeia* as its plumage colouration is very similar to *T. corvina*. The Black-naped Monarch *Hypothymus azurea* was chosen as an outgroup to root the phylogenetic reconstructions because *Hypothymus* is the sister genus to *Terpsiphone* (Coates et al. 2006; Fabre et al. 2012).

2.2.2 DNA extraction, PCR, sequencing and alignment

All DNA extractions from blood samples were carried out using the Ammonium Acetate method following Nicholls et al. (2000). Fragments from the following six loci were amplified and sequenced: cytochrome-b (cyt-*b*) (888 bp), NADH dehydrogenase subunit 3 (*ND3*) (467 bp), NADH dehydrogenase subunit 2 (*ND2*) (1030 bp), control region (888 bp), glyceraldehyde-3-phosphodehydrogenase intron 11 (*G3PDH*) (398 bp), and the melanocortin-1 receptor gene (*MC1R*) (758 bp).

Loci were amplified by Polymerase chain reaction (PCR) and sequenced using the primers listed in Table 2.3. Each PCR reaction comprised the following reagents; 1-4ul template DNA, 5ul NH4 reaction buffer (10x), 1.5ul $MgCl_2$ (50mM), 8ul dNTP's, 1ul of each of the forward and reverse primers, 0.4ul of 5U/µl Biotaq DNA polymerase (Bioline) and UV sterilised DNA grade distilled water to mix to a total volume of 50ul. PCR thermal cycling conditions were as follows for all genes: an initial denaturing step of 94^0C for 4 minutes followed by 30 cycles of [94^0C for 30 seconds, 49-63.4^0C (specific to primer pair) for 45 seconds, 72^oC for 60 seconds,] ending with 10 minutes extension at 72^oC. The annealing temperatures used for each primer pair are listed in Table 2.3.

PCR products were purified using the GENECLEAN Turbo kit (MP Biomedicals, LLC). Purified PCR product was sequenced by Macrogen-South Korea, Macrogen-Europe and Eurofins MWG Operon-Germany. Sequence reads were manually checked and then aligned and edited using the programme FinchTV 1.4 (Geospiza). Consensus sequences were aligned using the programme ClustalX 2.1.12 (Larkin et al. 2007) and GeneDoc 2.7.000 (Nicholas & Nicholas 1997).

For some of the outgroup taxa for which we did not have fresh blood samples, we extracted DNA from museum specimens. For the museum samples we amplified and sequenced DNA for four loci (cyt-*b, ND3, ND2, G3PDH*). For extractions, amplifications, and sequencing procedures from museum skin samples we followed the methods described in Irestedt et al. (2006). However, 20µl of DTT (dithiothreitol) was added in the lysis phase during the extractions and we amplified shorter fragments (around 250 bp including primer sequence lengths) in order to increase the ratio of

successful amplifications. The additional primers used for museum skin samples can be found in Fabre et al. (2012).

2.2.3 Phylogenetic Analysis

Maximum likelihood analyses on the single and concatenated dataset

Phylogenetic tree inferences were computed on each single gene matrix and on the concatenated datasets using the Maximum Likelihood (ML) criterion. The MODELTEST 3.07 software (Posada and Crandall 1998) was employed in order to determine the best fitting model for the DNA sequence evolution using the Akaike Information Criterion (AIC). This method can implement partitioned analyses by appropriating to each partition either a GTR (general time reversible) model with rate heterogeneity accommodated with a gamma (Γ) distribution (GTR+Γ), or a GTR+CAT model (general time reversible model with rate heterogeneity accommodated with a number of discrete rate categories). For the partitioned datasets [6 gene partitions and 3 codon partitions (for cyt-*b*, ND2 and ND3) for coding genes], we used the GTR+MIX option of RAxML, which assumes the faster GTR+CAT model for topological tree searches, but assumes the GTR+Γ model when computing the likelihood value of each topology. We used RAxML default parameters and specified 1000 tree search replicates. Node stability on partitioned supermatrices was computed with 1000 non-parametric bootstrap replicates (Felsenstein 1985). Bootstrap percentages (BP) were calculated using RAxML under a GTR+MIX model. ML searches for the best trees were performed using the PAUP* program (Swofford 2002), version 4b10. We conducted our analyses in two steps. Firstly, we used a heuristic search to estimate ML model parameters on a neighbour-joining (NJ) starting tree. Secondly, the previously estimated parameters were entered in a new search with tree bisection reconnection (TBR) branch swapping. The robustness of nodes was estimated by ML bootstrap percentages after 100 replicates using previously estimated parameters, NJ starting tree and TBR branch swapping.

Bayesian analyses on the partitioned supermatrices

Phylogenetic tree inferences were performed on each single gene matrix and on the concatenated datasets using Bayesian methods. We applied a partitioned strategy to the supermatrices in which each gene was assigned to its own partition ("gene partitioned"). Bayesian analyses used MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) which allows different models for each gene partition. Models for the partitioned Bayesian analyses were identified using the MrModeltest 2.2 software (Nylander et al. 2004), and models preferred by the AIC were implemented. All parameters except topology were unlinked across partitions and two independent runs (with one cold and three heated chains) were computed simultaneously, with trees sampled every 100 generations. The MrBayes

analyses were run for 5 x 10^7 generations. Majority rule consensus was constructed, with burn-ins of 5 x 10^5 generations. Support for different clades was calculated by posterior probabilities.

In order to test hypotheses regarding monophyly of the Indian Ocean lineages and several other potential scenarios inferred by our phylogenetic trees, we employed the approximately unbiased (AU) test (Shimodaira 2002) and the Shimodaira-Hasegawa (SH) test (Shimodaira & Hasegawa 1999) as implemented in CONSEL (Shimodaira & Hasegawa 2001). The six gene supermatrix dataset was used for these tests and Programme PAUP* version 4.0b10 (Swofford 2002) was used to calculate the site likelihoods for each of the test topologies with the gene partitioning scheme assumed and the appropriate model for each partition specified using the output from Modeltest. The CONSEL analyses employed 10 batches of 1x10^6 bootstrap replicates.

Molecular dating and DNA based species delimitation

We used BEAST V1.6 (Drummond et al. 2002; Drummond & Rambaut 2007) to estimate the divergence dates within Indian Ocean *Terpsiphone*, applying the best fitting model, as estimated by MODELTEST 2.0, to each of the partitions. We assumed a Yule Speciation Process for the tree prior and an uncorrelated lognormal distribution for the molecular clock model (Ho et al. 2007). We used default prior distributions for all other parameters and ran MCMC chains for 200 million generations. The program Tracer (Rambaut & Drummond 2007) was used to assess convergence diagnostics. Because no fossil data are available for this group, we used a molecular clock approach in order to estimate the divergence dates among Indian Ocean *Terpsiphone* species. Using the Tajima's relative test (Tajima 1993) implemented in pegas package (Paradis 2010) of the R software we tested if the molecular clock hypothesis could be applied to our dataset. Because a molecular clock hypothesis could not be rejected, we applied both a strict and a relaxed molecular clock to our matrix using partitions by genes and codon positions. The Hawaiian honeycreeper rate of evolution of 1.6% sequence divergence per million years (My) was used to obtain the absolute date. This estimate is based on the geology of Hawaii and may be inaccurate as Hawaiian island emergence provides a maximum age for taxa inhabiting the particular island (Fleisher et al. 1998). Consequently, the date estimates generated with island age calibrations in this study can be regarded as maxima.

Geological calibration points have been applied to several avian groups of oceanic islands (e.g. Tarr & Fleischer 1993; Warren et al. 2003; Fuchs et al. 2008; Moyle et al. 2009; Fabre et al. 2012). Assumptions and criteria regarding geological calibration points (see Fleischer et al. 1998; Emerson et al. 2000; Warren et al. 2003; Heads 2011) are generally fulfilled for the Indian Ocean islands of Mauritius and Réunion. We therefore used the split between *Terpsiphone bourbonnensis desolata* from Mauritius (age c.7.8 My; McDougall & Chamalaun 1969) and *T. b. bourbonnensis* from Réunion (age c.2.1 My; Chevallier & Vatin-Perignon 1982) in the Indian Ocean as a geological

calibration point. We assume that the divergence between the lineages on Mauritius and Réunion cannot be older than the younger of the two islands (Réunion, 2.1 My). Thus, to obtain a calibration point based on the split between these two species, we applied a lognormally distributed prior at 1.5 million years ago (Mya) ±0.25 standard deviations (95% confidence interval=1.089-1.911 Mya). Finally, we used a 2%/My rule to corroborate the dates resulting from the island calibrations.

In order to delineate Indian Ocean taxa and to discuss the importance of our phylogenetic results for conservation purposes, we employed the Pons et al. (2006) method. This likelihood approach detects the switch in the rate of lineage branching to intraspecific short budding branching and identifies clusters of specimens corresponding to potential taxonomic units. Two models can be applied to account for different branching processes within the phylogeny. Within the null model, the sample grows from a single population following a coalescent process. The other model follows a general mixed Yule coalescent (GMYC) model which takes into account branching at the population level (coalescent process) and at the macro-evolutionary level (with extinction and speciation rate inferred from the Yule process). The GMYC model optimized a threshold (T) from which we could consider the species number estimation and then delineate taxonomic units. The fit of both models were compared using Likelihood-ratio test (LRT). We used the package SPLITS (Pons et al. 2006) within R version 2.10.1 (R Development Core Team 2009). In addition, uncorrected pairwise distances between each island population were calculated using our four gene mitochondrial DNA dataset in the programme PAUP* (Swofford 2002).

2.3 RESULTS

2.3.1 Phylogenetic results

The results from the phylogenetic analyses are displayed in Figure 2.2. Both Maximum Likelihood (ML) and Bayesian (BI) trees converged to produce very congruent topologies, so only the ML tree is shown in Figure 2.2 however both the Ml and BI values for each node are given on the ML tree, the ML value above and the BI value below. The full BI tree can be found in the Supplementary Information SI 2.3. The single gene and concatenated datasets produced congruent results (See Supplementary Information SI 2.1), although the nuclear gene trees provided little resolution, reflecting the recent diversification of the group. Thus the species trees are largely driven by the mitochondrial data. Six main biogeographic monophyletic lineages are supported by our analyses: (i) a *Terpsiphone bourbonnensis* clade (Mascarenes), (ii) a *Terpsiphone corvina* clade (Seychelles), (iii) a *Terpsiphone mutata mutata*+*T. m. singetra*+*T. m. pretiosa* clade (Madagascar and Mayotte), (iv) a *Terpsiphone mutata vulpina*+*T. m. voeltzkowiana* clade (Anjouan and Moheli), (v) a *Terpsiphone*

mutata comorensis clade (Grande Comore) and (vi) a *Terpsiphone viridis*+*T. batesi* clade (central Africa). Of these, five are Indian Ocean lineages which are nested alongside African *Terpsiphone* clades but within an Asian clade (see also Fabre et al. 2012). However, due to an absence of branch support for divergences between these lineages in both analyses, their relationships remain uncertain. Within the Mascarene clade the subspecies *T. bourbonnensis bourbonnensis* and *T. b. desolata* constitute two distinct clades, however the relationship of *T. bourbonnensis* to the other western Indian Ocean taxa remains unresolved. Within the *T. mutata* lineages, our analyses strongly support (cf. PP=1) the monophyly of each of the Comoros subspecies (*T. m. comorensis, T. m. voeltzkowiana, T. m. vulpina* and *T. m. pretiosa*), however there appears to be no separation of the two *T. mutata* subspecies from Madagascar (*T. m. mutata and T. m. singetra*). The occurrence of several poorly supported nodes separated by short internodal branches in our analyses may indicate a case of simultaneous dispersal and/or rapid diversification or possibly gene flow between populations. AU tests allow us to reject a hypothesis of monophyly of Indian Ocean taxa ($p=4.00 \times 10^{-15}$). However, we are unable to reject the monophyly of a clade including *T. atrochalybeia* and the Indian Ocean taxa ($p \geq 0.341$), the monophyly of *T. atrochalybeia* and *T. corvina* ($p \geq 0.398$), and the monophyly of *T. mutata* ($p \geq 0.440$) (see supporting information SI 2.3). The five biogeographically different monophyletic Indian Ocean lineages revealed by our analyses are well-supported and provide a valuable delineation of *Terpsiphone* evolutionary history across the Indian Ocean.

2.3.2 Molecular dating and species delimitation

A time scale for the evolution of the Indian Ocean *Terpsiphone* derived from the Bayesian dating analysis is shown in Figure 2.3. Divergence times of Indian Ocean *Terpsiphone* clades with high nodal support (see Figure 2.2) (Mya) with 95% highest posterior densities obtained using a relaxed clock rate and island constraints are listed in Table 2.4. Using the calibration point provided by the islands of Mauritius and Réunion, we provide maximum estimates for several key phylogenetic events in the diversification of the *Terpsiphone* in order to delineate ESUs for conservation purposes. The arrival of *Terpsiphone* in the Indian Ocean dates back to the Pliocene, around 2 Mya (see Figure 2.3A for detail).

The analyses of the branching rate pattern revealed the existence of six lineages within the Indian Ocean islands (Figure 2.3). The lineage-through-time plot derived from the BEAST ultrametric tree displayed an increase in branching rate towards the present, which corresponds to intraspecific splitting events. To delineate between older interspecific and more recent intraspecific lineage splitting, the Pons et al. (2006) methodology was applied to our dated phylogeny (Figure 2.3). Both GMYC models showed a significantly better fit compared to the null model of uniform branching rates; with respectively the multiple threshold model (*log*L=105.51, compared to the null model *log*L=96.91; $2\Delta L=17.20$, χ^2 test, df=3, $p<0.0006$) and the single threshold model (*log*L=106.29,

compared to the null model *log*L=96.91; 2ΔL=18.75, χ^2 test, df=3, p<0.002). The GMYC multiple threshold model was a slightly better fit than the single threshold model but was only marginally significant (χ^2 test, df=6, p=0.95). The GMYC single threshold model delineated the switch in the branching pattern around 0.35 Mya leading to an estimate of six putative Indian Ocean *Terpsiphone* lineages (estimated number of species ranged from 6 to 7; see Figure 2.3, lineages are highlighted in red). One *T. corvina* lineage (IOL1), three *T. mutata* lineages (IOL2, IOL3, IOL4) and two *T. bourbonnensis* lineages (IOL5, IOL6) are identified (as indicated in Figure 2.3) corresponding to six putative species following Pons et al.'s (2006) approach. The GMYC multiple threshold model indicated the three thresholds ranged from 0.35/0.12/0.06 Mya and the estimated number of species ranged from five to seven (i.e. estimates falling within 2 log-likelihood units of the ML solution).

Mitochondrial DNA mean uncorrected pairwise distances between the three species-level taxa within the Indian Ocean region are as follows: 3.71% (range 2.85-4.49%) between *T. corvina* and *T. bourbonnensis*; 3.60% (range 2.94-4.49%) between *T. corvina* and *T. mutata*; and 3.77% (range 2.06-4.95%) between *T. bourbonnensis* and *T. mutata*. Mean uncorrected pairwise distances observed between the different Indian Ocean lineages (IOL) (delineated as described above) are as follows: 1.38% (range 1.12-1.81%) between the two Mascarene flycatcher subspecies, IOL5 (*T. b. bourbonnensis* on Réunion) and IOL6 (*T. b. desolata* on Mauritius), (c.f. mean within population pairwise distances of 0.29% and 0.49% respectively); 3.46% (range 2.64-4.24%) between IOL2 (*T. m. vulpina* on Anjouan and *T. m. voeltzkowiana* on Moheli) and IOL3 (*T. m. singetra+T. m. mutata* on Madagascar and *T. m. pretiosa* on Mayotte); 3.27% (range 2.50- 3.90%) between IOL2 and IOL4 (*T. m. comorensis* on Grande Comore); and 2.92% (range 2.24-3.41%) between IOL3 and IOL4. The uncorrected pairwise distance matrix for our four gene mtDNA dataset is provided as Supplementary Information SI 2.4.

2.4 DISCUSSION

2.4.1 Previously undetected taxonomic diversity

Within the Indian Ocean islands, *Terpsiphone corvina* on the Seychelles and *T. bourbonnensis* on the Mascarenes are clearly divergent, reflecting their taxonomic status as different species. Remarkably, however, our analyses have revealed a high degree of divergence within the Madagascar paradise flycatcher *T. mutata* species found on Madagascar and the Comoros. Our molecular phylogeny shows three distinct clades within *T. mutata* that are almost as diverged from each other as they are from the Seychelles paradise flycatcher *T. corvina* and from the Mascarene paradise flycatcher *T. bourbonnensis*, two species which are both morphologically more divergent and

geographically more distant. Interspecific uncorrected pairwise distances between full species of closely related taxa reported in other studies are within the range of our findings for our Indian Ocean lineages; Johnson & Cicero (2004) report mtDNA mean uncorrected pairwise distances of 1.86% (range 0-8.2%) amongst 39 pairs of sister species of North American birds, whilst Lovette & Bermingham (1999) report interspecific distances of 0.9 to 1.7% between sister species of *Dendroica* warblers. Results from our study indicate the three different *T. mutata* lineages (mtDNA mean uncorrected pairwise distances of between 2.92% and 3.46%) are more genetically differentiated from each other than are some other avian taxa with full species status (see Lovette & Bermingham 1999; Johnson & Cicero 2004). Our analysis has revealed a similar pattern of clearly diverged island subspecies within the Mascarene paradise flycatcher, with mtDNA mean uncorrected pairwise distance of 1.38% between *T. bourbonnensis desolata* on Mauritius *and T. b. bourbonnensis* on Réunion. Given these levels of genetic differentiation we observe within *T. bourbonnensis* and *T. mutata*, our findings suggest that the two island lineages on Mauritius and Reunion and the three island lineages within *T. mutata* (Madagascar+Mayotte; Anjouan+Moheli; and Grande Comore) should be considered as separate ESUs and that they should be managed separately for conservation.

Within *T. corvina* on the Seychelles, sequence from one sample was obtained from a historical museum specimen collected in 1888 and stands out as divergent from the six other samples collected from modern specimens on the Seychelles. Careful examination of the DNA sequence traces showed the nucleotide differences to be authentic. The museum specimen was collected on Praslin Island, where the species is now extinct, whereas the six modern samples were all collected from La Digue Island, the only remaining population of this Critically Endangered species. This result is an example of loss of genetic diversity as a result of the extinction of *T. corvina* on Praslin (see chapter 3).

2.4.2 Prioritizing conservation effort based on evolutionary distinctiveness

Our results show the Mascarene clade (encompassing Mauritius and Réunion taxa) to be the most deeply diverging Indian Ocean clade, and likely the earliest colonisation of the Indian Ocean islands. Within this clade the Mauritius and Réunion populations are sufficiently diverged to warrant management as separate ESUs. This information is likely to be important because the population of *T. b .desolata* on Mauritius, consisting of 100-223 pairs, is considered to be under threat from habitat degradation, fragmentation and impacts of invasive species (Safford, 1997). Currently, due to the subspecific status afforded to the flycatcher population on Mauritius, and the fact that the Réunion population is still fairly widespread and common, the population on Mauritius has struggled to attract conservation resources, despite local efforts to obtain funds for basic survey and ecological studies of this island form. Our findings may help to improve the conservation attention that this island population receives.

The Seychelles paradise flycatcher *T. corvina* is highly evolutionarily distinct and forms its own monophyletic clade dating back to the early Pleistocene. Given its critical conservation status (IUCN 2011), the current conservation efforts to improve this species' long term survival prospects are supported by our findings and should be continued.

One of the most unexpected findings from this study is the considerable evolutionary diversity amongst the Madagascar paradise flycatcher *T. mutata*. The three *T. mutata* lineages (IOL2, IOL3 and IOL4; see Figure 2.3) are all highly divergent from each other. While *T. mutata* is currently divided into subspecies, our molecular reconstruction has revealed that there is a strong evolutionary case for, at minimum, treatment of these three lineages as separate ESUs, warranting conservation management as if they were separate species. This information is likely to be important for conservation efforts on the Comoro islands as little conservation work is currently undertaken on their *T. mutata* subspecies due to the species' wide range and healthy overall numbers. Knowledge that there are three highly diverged lineages amongst these nearby islands may encourage baseline survey work to determine in more detail population sizes and distributions of these unique lineages and allow this novel phylogenetic diversity to be conserved.

2.4.3 Biogeography and chronology of dispersal and colonisation

Our phylogeny agrees with the results of Fabre et al. (2012), supporting an Asian origin of the *Terpsiphone* species' on the Indian Ocean islands and the African continent. It does not, however, resolve whether the Indian Ocean was colonised directly from Asia or via Africa, or whether the Indian Ocean was colonised independently from both Africa and Asia. A characteristic of this phylogeny is the short internal branch lengths and low branch support for nodes separating the major western Indian Ocean lineages, meaning that the phylogeny is less able to determine the precise chronology of dispersal and island colonisation within the Indian Ocean. Other phylogenetic studies have reported patterns of hard polytomies for several taxonomic groups and attributed this occurrence to rapid radiation (Lara et al. 1996; Leite & Patton 2002; Rabosky & Lovette 2008; Jønsson et al. 2012), or the extinction of some taxa (e.g. Marshall & Baker 1999).

That the Indian Ocean taxa are not recovered as monophyletic in our study indicates it is likely that more than one colonisation event between the continent and western Indian Ocean occurred to explain the distribution and phylogenetic placement of taxa. The most likely scenario would appear to be two or more independent colonisations of the western Indian Ocean. However, we cannot rule out the possibility of a single colonisation of the western Indian Ocean, followed by a colonisation (or back colonisation) from the Indian Ocean to other landmasses. Likewise, our inability to reject the monophyly of a clade containing *T. atrochalybeia* and the Indian Ocean taxa is most likely explained by the independent colonisation of the Indian Ocean and São Tomé by a common ancestor on Africa

that has either become extinct on Africa since these colonisations, or has not been sampled. An alternative scenario is the colonisation of the Indian Ocean from Africa or Asia, followed by (back-) colonisation of Africa from the Indian Ocean, and colonisation of São Tomé thereafter. While our data do not allow us to rule out the latter scenario, it requires more steps and therefore seems less likely. Since the Mascarene clade (encompassing Mauritius and Réunion taxa) is the most deeply-diverging Indian Ocean clade, it was likely an early colonisation of the region, either from Asia or Africa.

The estimated divergence times based on island calibrations and the pairwise genetic distances generated from our study are broadly consistent with a rate of 2% per My, an observation that adds confidence to our date estimations. Our estimation of maximum divergence times implies that the *Terpsiphone* genus colonised the Indian Ocean relatively recently (approximately 2 Mya) and that the genus has subsequently rapidly expanded its range and diversified across the region. The Seychelles paradise flycatcher (*T. corvina*) appears to have been isolated for c. 1.75 My and the three *T. mutata* lineages have all had continuous evolutionary independence for c. 1.5 My. Sea level low stands of 70-80m below present levels (bpl) occurred at approximately 1.9 and 1.5 Mya (Miller et al. 2005) and these events may have facilitated range expansion by flycatchers. Elsewhere in the region, shallow-water plateaus exist that would have been exposed during particular low stands (e.g. Saya da Malha, 40,000 km^2 , 8-150 m bpl and Nazareth, 7,000-20,000 km^2 and 30-150m bpl lying between India, the Seychelles and the Mascarenes, with additional shallow areas between Madagascar and the Comoros archipelago). These would have resulted in (i) much larger landmasses in the western Indian Ocean including the granitic Seychelles and other islands along the Mascarene bank between the Seychelles and the current Mascarene islands, (ii) a chain of islands extending from India to the Seychelles, and (iii) additional islands between Madagascar and Mayotte, creating stepping stones from Asia through the Indian Ocean islands to Africa. These additional islands would have greatly reduced distances across large expanses of ocean from one landmass to another. The timing of these sea level low stands aligns well with our estimates of species divergence times, and is therefore consistent with an island hopping scenario for the rapid range expansion and divergence shown in the Indian Ocean *Terpsiphone*. Sea level rises between these times would have reduced the number and size of landmasses and may have prevented dispersal between islands. During this time, effects of genetic drift and evolutionary adaptation to island life may have reduced the resulting species tendencies for dispersal, a phenomenon displayed by many island taxa (Bennett & Owens 2002). More recent sea level low stands during the last glaciation c. 18-23 thousand years ago (Rohling et al. 1998; Siddall et al. 2003) may have facilitated dispersal of *T. mutata* between Madagascar and Mayotte (c.250km apart) where at least two additional stepping stone islands would have been present at this time. It is not surprising that the Moheli and Anjouan island populations are so similar as the islands are only 42 km apart; what is surprising is how diverged the Grande Comore island population is given that Moheli is only 35 km away. The monophyly of lineages on Madagascar and Mayotte allows us to rule

out a simple conveyor belt ‘volcanic islands colonised as they emerge’ scenario. Additionally, at least within *T. mutata*, evolutionary affinity does not correlate with geographical distances.

2.4.4 Plumage as an indicator of phylogeny

The Seychelles paradise flycatcher *T. corvina* has very similar plumage to the São Tomé flycatcher *T. atrochalybeia*. Males of both species are entirely black and possess elongated central tail feathers while the females of both species also possess very similar black, rufous and white plumage. Maximum Likelihood analyses revealed a possible relationship between *T. corvina* and *T. atrochalybeia* but without strong support. Since we could not reject monophyly of *T. corvina* and *T. atrochalybeia*, it is possible that the phenotypic similarities observed in these two species results from a shared common ancestor where males were black with long tails. However given the lack of branch support (bootstrap=54, PP=0.55) for their monophyly, convergent or parallel evolution of their phenotype is also a possibility; melanin deposition obscuring ancestral plumage patterns is a common occurrence particularly in island populations, and the tri-colour plumage of the females of both species (black head, rufous wings and tail and light under parts), is thought to be the ancestral *Terpsiphone* plumage type (Fabre et al. 2012).

The Mascarene paradise flycatcher is the only species in the western Indian Ocean lacking elongated central tail feathers, aligning with our phylogenetic reconstruction indicating that this species is the most diverged of the Indian Ocean taxa, and likely the result of a separate earlier colonisation of the region.

2.4.5 Summary and conclusion

Our phylogenetic reconstruction shows relatively recent colonisation of the western Indian Ocean by *Terpsiphone* flycatchers, that greatly postdates the formation of the older islands of the region. A minimum of two colonisations between the continent and Indian Ocean must have occurred to explain current *Terpsiphone* distribution. Subsequent radiation has not followed a stepwise succession of populations on older islands colonising newer islands as they emerge, but rather appears to have involved rapid range expansions. The resulting lineages, however, are well diverged following relatively long periods of isolation. Within *T. mutata*, only one of the three most diverged lineages corresponds to a current taxonomic unit (*T. m. comorensis*), while the other two lineages group two or more subspecies. Surprisingly, the phylogenetic placement of *T. mutata* subspecies are neither consistent with a distance-based nor island age-based model of colonisation. The Seychelles paradise flycatcher *T. corvina*, the only Critically Endangered species in the genus, is highly diverged and worthy of the conservation attention it receives. *Terpsiphone bourbonnensis* is the most diverged of the Indian Ocean *Terpsiphone* taxa and likely results from an earlier colonisation of the region.

This phylogenetic reconstruction highlights the importance of subspecific molecular phylogenies in complex island archipelago settings in clarifying phylogenetic history and evolutionary significant units that may otherwise be overlooked and inadvertently lost. The ability of the Pons et al. (2006) GMYC method to objectively delimit species units based on DNA sequence data makes it a powerful tool to assist conservation planners with the difficult task of objective allocation of finite conservation resources. Our phylogenetic reconstruction has provided a valuable platform upon which to identify hidden pockets of evolutionary distinctiveness, and re-evaluate investment of conservation resources within the *Terpsiphone* flycatchers of the Indian Ocean.

ACKNOWLEDGEMENTS

Unpublished primers to amplify *Terpsiphone* mtDNA control region were kindly provided by R Kimball, Department of Zoology, University of Florida. A Uy provided Monarchidae MC1R sequence from which we designed MC1R primers for *Terpsiphone*. We would like to thank the CNDRS, CNDRS d'Anjouan, Conservation de la Biodiversite Moheli, DAF-SEF, MICET, Nature Seychelles, Seychelles Department of Environment, Mauritian Wildlife Foundation and the Government of Mauritius National Parks and Conservation Service for support, S Anli, T Ghestemme, C Moussa Iboura, B Milá, F Ratrimomanarivo, I Saïd and S Tollington for help in the field and C Raisin and S Kundu for guidance in the lab. We are grateful to the following people and institutions for granting access to toe-pad, blood and tissue samples, Eric Pasquet (MNHN), Jan Bolding Kristensen and Jon Fjeldså (ZMUC), Michael Brooke (UMZC), Pascal Eckhoff and Sylke Frahnert (ZMB). P-HF and KAJ acknowledge the Danish National Research Foundation for support to the Center for Macroecology, Evolution and Climate.

2.5 FIGURES AND TABLES

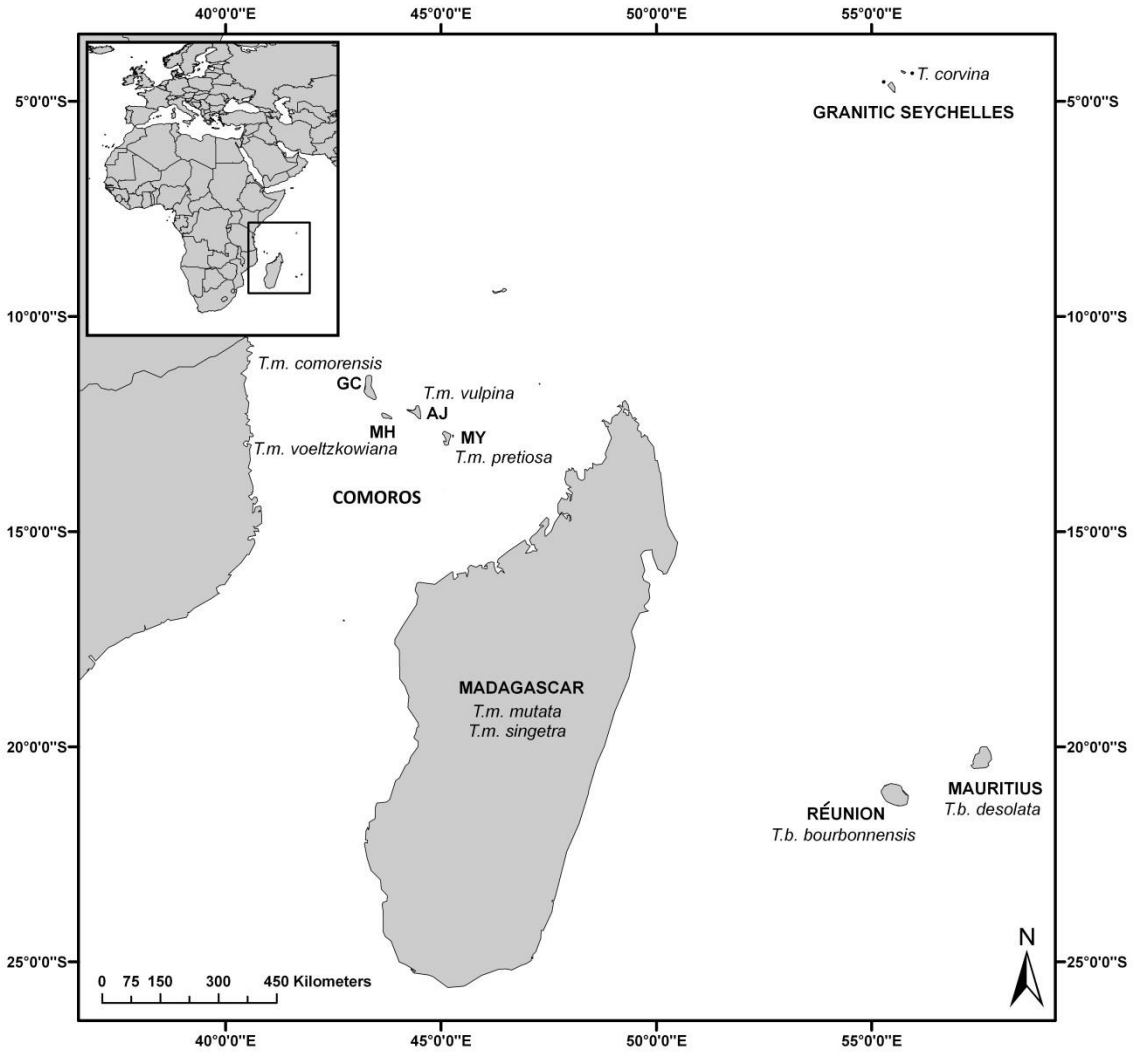


Figure 2.1. Distribution of *Terpsiphone* taxa of the western Indian Ocean. GC, Grande Comore; MH, Moheli; AJ, Anjouan; MY, Mayotte.

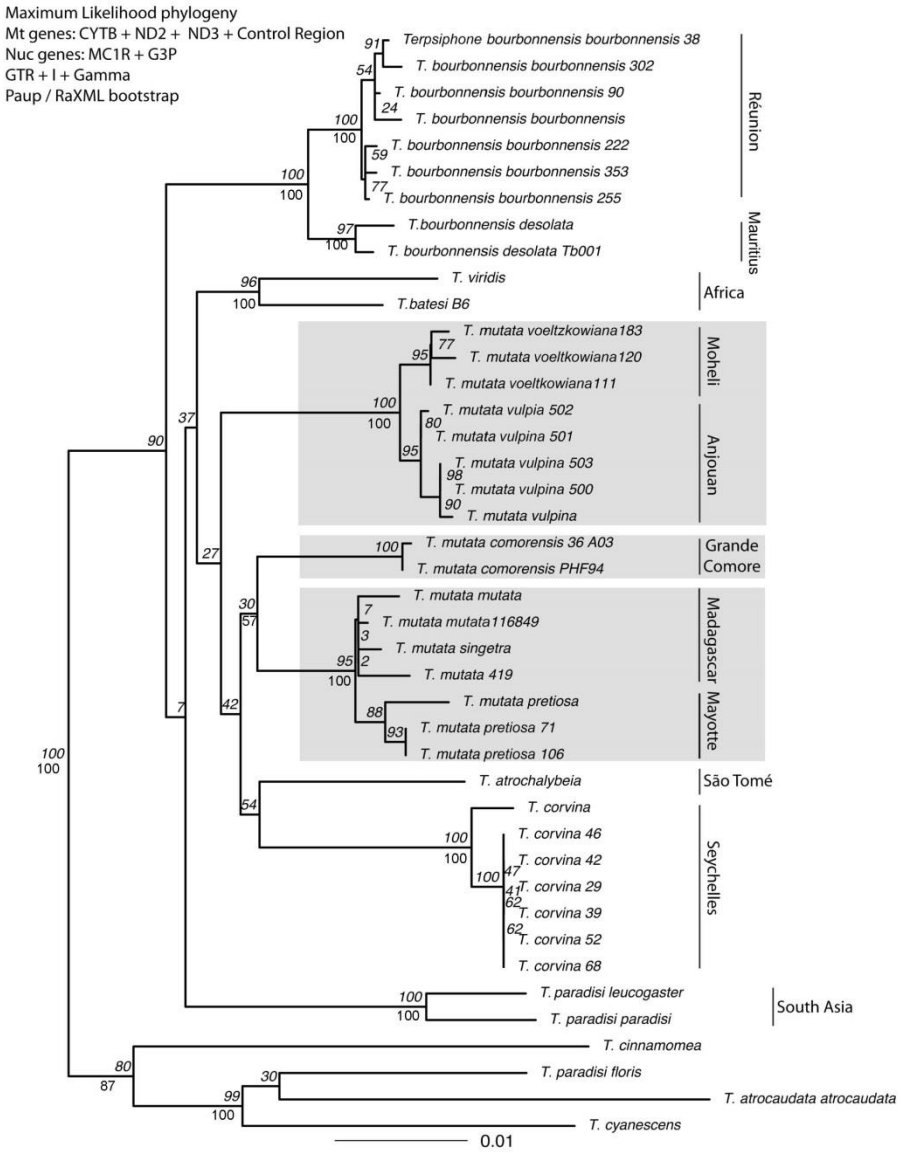


Figure 2.2. Maximum Likelihood topology of Indian Ocean paradise flycatcher produced from the mito-nuclear supermatrix analyses.

Biogeographic origins are shown on the side of the phylogeny. BP=Bootstrap proportion issued from the PAUP analysis. Voucher numbers are indicated for each specimen used for this study in Table 2.2. *T. mutata* clades indicated by grey shading. The posterior probabilities from the partitioned Bayesian analysis are shown below the Maximum Likelihood Bootstrap values for each node.

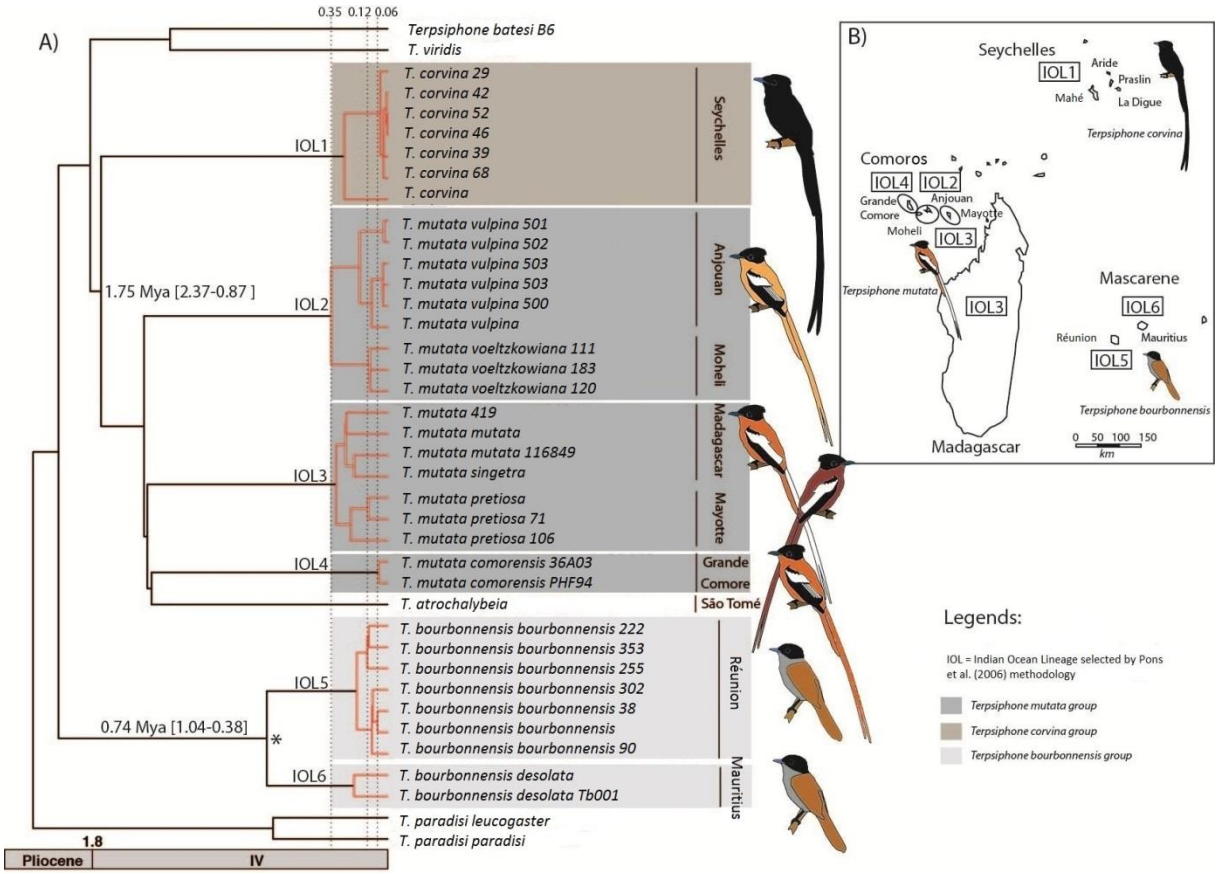


Figure 2.3. Indian Ocean *Terpsiphone* dated tree with Indian Ocean geographical map.

A) Indian Ocean *Terpsiphone* ultrametric tree obtained with BEAST and cluster of specimens as putative species by the methods of Pons et al. (2006). Genetic cluster recognized as a putative species are coloured in red. The vertical bars group all sequences within each significant clusters, labelled IOL1 to IOL6. B) Map of Indian Ocean with significant clusters mapped.

Table 2.1. List of western Indian Ocean island ages, geology and sources of information.

Island	Geology	Age	Source
Granitic Seychelles Islands	granite	Separated from Africa c.130 Mya/ India c.64 Mya	Coffin & Rabinowitz 1987; Kingdom 1990; Rabinowitz et al. 1983
Madagascar	granite	Separated from Africa c.130 Mya/ India c.88Mya	Coffin & Rabinowitz 1987; Kingdom 1990; Rabinowitz et al. 1983
Mayotte	volcanic	7.7-15 My	Emerick & Duncan 1982; Nougier et al. 1986
Moheli	volcanic	c.5 My	Emerick & Duncan, 1982; Nougier et al. 1986
Anjouan	volcanic	c. 3.9-11.5 My	Emerick & Duncan, 1982; Nougier et al. 1986
Grande Comore	volcanic	c.0.13-0.5 My	Emerick & Duncan, 1982; Nougier et al. 1986; R. Duncan pers. comm. in Warren et al. 2003
Mauritius	volcanic	c.7.8 My	Duncan & Hargraves 1990; McDougall & Chamalaun 1969
Reunion	volcanic	c.2.1 My	Chevallier & Vatin-Perigno 1982; Duncan & Hargraves 1990
South-east Seychelles Islands	coral/sand	$\leq$ 0.015-0.125 My	Radtkey 1986; Thompson & Walton 1972

Table 2.2. List of all samples used in this analysis. [a]All fresh blood samples were collected from mistnetted individuals that were then released unharmed. Museum samples were provided by the respective museums as listed under sample ID number.

Species	Sample ID number	Location	Sample type[a]
Terpsiphone bourbonnensis bourbonnensis	38	Réunion	fresh blood
Terpsiphone bourbonnensis bourbonnensis	90	Réunion	fresh blood
Terpsiphone bourbonnensis bourbonnensis	222	Réunion	fresh blood
Terpsiphone bourbonnensis bourbonnensis	255	Réunion	fresh blood
Terpsiphone bourbonnensis bourbonnensis	302	Réunion	fresh blood
Terpsiphone bourbonnensis bourbonnensis	353	Réunion	fresh blood
Terpsiphone mutata pretiosa	71	Mayotte	fresh blood
Terpsiphone mutata pretiosa	98	Mayotte	fresh blood
Terpsiphone mutata pretiosa	106	Mayotte	fresh blood
Terpsiphone mutata vulpina	120	Moheli	fresh blood
Terpsiphone mutata vulpina	183	Moheli	fresh blood
Terpsiphone mutata vulpina	111	Moheli	fresh blood
Terpsiphone mutata voeltzkowiana	500	Anjouan	fresh blood
Terpsiphone mutata voeltzkowiana	501	Anjouan	fresh blood
Terpsiphone mutata voeltzkowiana	502	Anjouan	fresh blood
Terpsiphone mutata voeltzkowiana	503	Anjouan	fresh blood
Terpsiphone mutata	419	Madagascar	fresh blood
Terpsiphone corvina	29	La Digue	fresh blood
Terpsiphone corvina	39	La Digue	fresh blood
Terpsiphone corvina	42	La Digue	fresh blood
Terpsiphone corvina	46	La Digue	fresh blood
Terpsiphone corvina	52	La Digue	fresh blood
Terpsiphone corvina	68	La Digue	fresh blood
Terpsiphone bourbonnensis desolata	Tb001	Mauritius	fresh blood
Terpsiphone mutata comorensis	PH94	Grande Comore	fresh blood
Terpsiphone cinnamomea	116848	Philippines	fresh blood
Terpsiphone viridis	134397	Tanzania	fresh blood
Terpsiphone mutata vulpina	ZMB 2000/17393	Anjouan	museum tissue
Terpsiphone mutata pretiosa	ZMB 2000/17408	Mayotte	museum tissue
Terpsiphone mutata mutata	ZMUC 116849	Berenty, Madagascar	museum tissue
Terpsiphone mutata comorensis	MNHN 36 A03	Grande Comore	museum tissue
Terpsiphone mutata mutata	ZMUC 28229	Vondrozo, Madagascar	museum tissue
Terpsiphone mutata singetra	ZMUC 28258	Ranpotaka, Madagascar	museum tissue
Terpsiphone bourbonnensis bourbonnensis	NRM 553920	Réunion	museum tissue
Terpsiphone bourbonnensis desolata	NRM 556022	Mauritius	museum tissue
Terpsiphone atrochalybeia	ZMUC 59966	São Tomé	museum tissue
Terpsiphone corvina	UMZC 27/Mus/51/f/6	Praslin	museum tissue
Terpsiphone atrocaudata atrocaudata	NRM 68533	North China	museum tissue
Tterpsiphone cyanensis	ZMUC 105237	Palawan	museum tissue

Terpsiphone paradisi floris	RMNH 85100	Flores	museum tissue
Terpsiphone batesi	RMCA 107459	DR Congo	museum tissue
Terpsiphone paradisi leucogaster	ZMUC 28233	Afghanistan	museum tissue
Terpsiphone paradisi paradisi	ZMUC 28237	India	fresh tissue
Hypothymis azurea	MNHN 5 40 4 1997	Laos	fresh blood

Table 2.3. List of primers used to amplify and sequence the genes used in this study.

Loci	**Primer Name/Sequence (5'-3')**	**Source**	**Ta (^{O}C)**
cyt-*b*	F: TerpCytb_F (CCCCCAACCTACGTAAAAA+TC) R: TerpCytb_R (TTTGTGATAGGGGTCGGAAG)	designed for this research from existing *Terpsiphone paradisi* sequence (GenBank Accession Number EF081356)	60.0
ND3	L10755	Chesser, 1999	49.0
	H11151		
ND2	L5216	Sorenson et al. 1999	55.0
	H6313		
control region (PCR)	F: TerpCRF (GGACTTTCTCCAAGATCTATGGC) R: TerpCRR (GCAACCATGACACTATTAGCTAC)	Rebecca Kimball pers. comm.	59.0
control region (internal sequencing primers)	F: TerpCRIntSeq20_F (CCCCATGTTTTTACATGGTTT) F: TerpCRIntSeq400_F (TCGTGTTTCTCACGCTACCC)	designed for this research	
G3PDH	G3P13b	Fjeldså et al. 2003	60.0
	G3P14b		
MC1R	F: MC1R_F (TGGACATTCCCAACGAGCTG) R: MC1R_R (AGATGAGGGGGTCAATCACTG)	designed for this research from chestnut-bellied and melanic monarch sequences provided by Albert Uy pers. comm.	63.4

Ta, annealing temperature

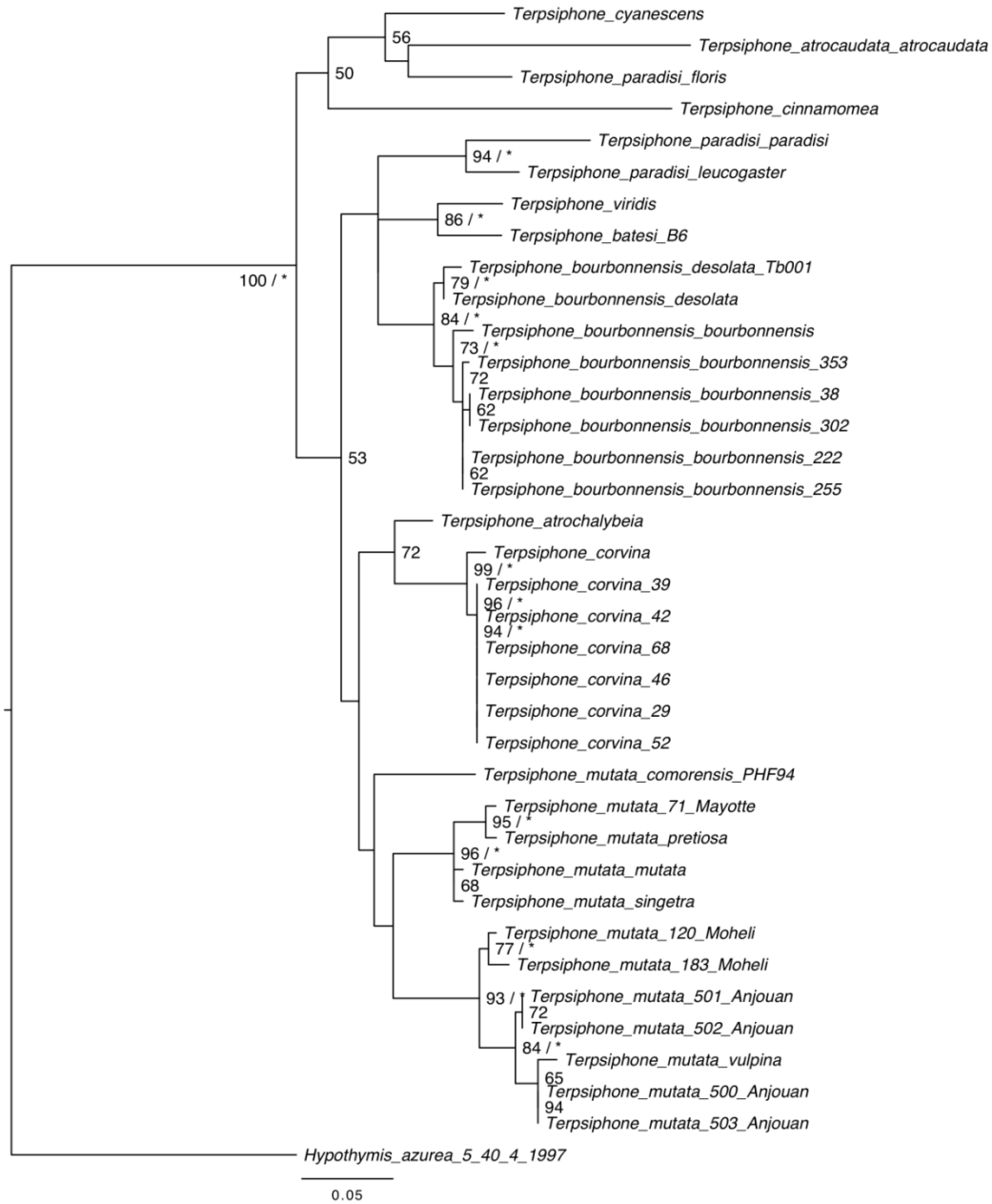
Table 2.4. Divergence times of Indian Ocean *Terpsiphone* clades with high nodal support in million years ago (Mya) with 95% highest posterior densities obtained using a relaxed clock rate and island constraints. IOL, Indian Ocean lineages as described in Figure 2.3 and section 2.3.2 of the text.

	Divergence times (Mya)	
Clade	Mean	[Min-Max]
Terpsiphone clade	4.26	[3.22-5.44]
South East Asian clade	3.66	[2.53-4.85]
Node *T. bourbonnensis*/*T. paradisi*/*T. mutata*	2.92	[2.09-3.79]
T. corvina IOL1	0.35	[0.13-0.64]
T. mutata	1.75	[2.37-0.87]
IOL2	0.58	[0.29-0.91]
IOL3	0.53	[0.23-0.89]
IOL4	0.14	[0.10-0.42]
T. bourbonnensis	0.74	[0.38-1.04]
IOL5	0.35	[0.02-0.43]
IOL6	0.24	[0.05-0.47]

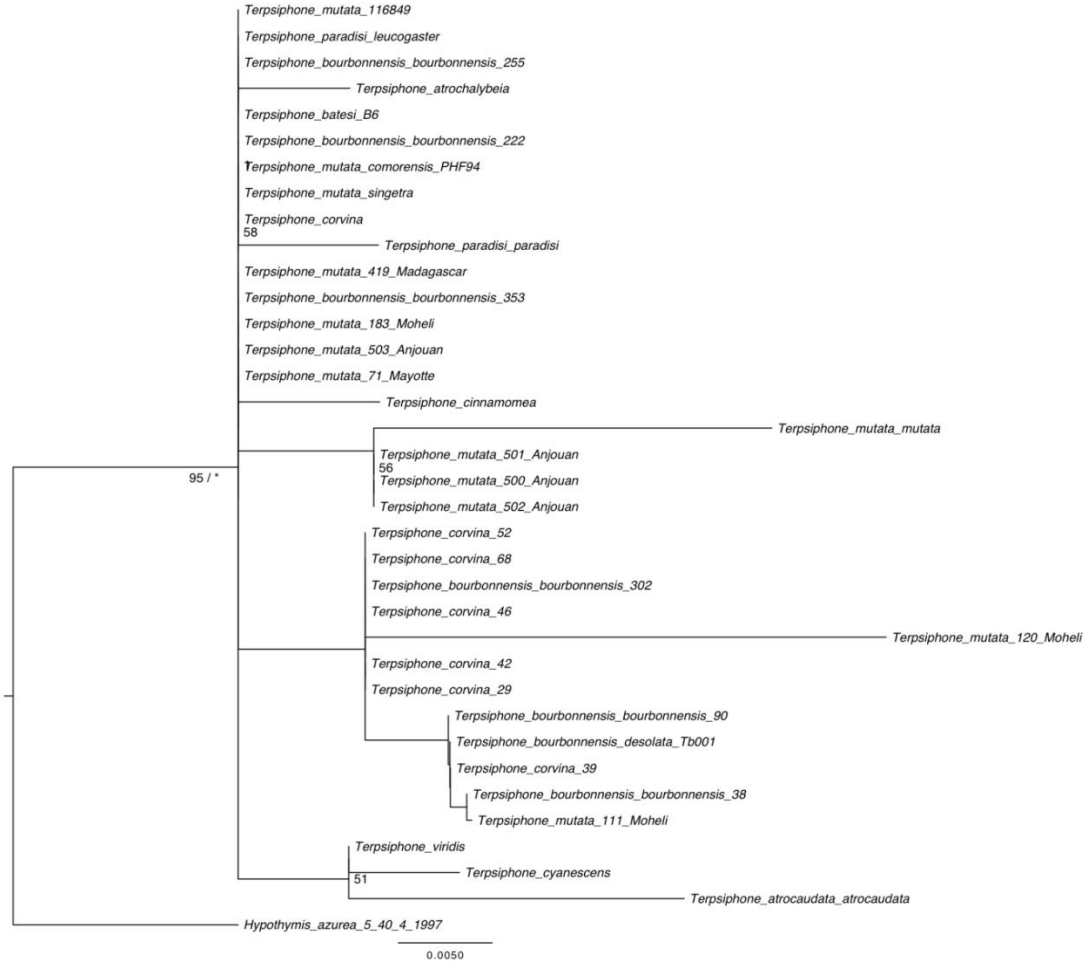
2.6 SUPPORTING INFORMATION

SI 2.1 The maximum likelihood trees of the Indian Ocean *Terpsiphone* obtained from the RAxML analysis of cyt *b*, *GAPDH*, *MC1R*, *ND2*, *ND3* and control region.

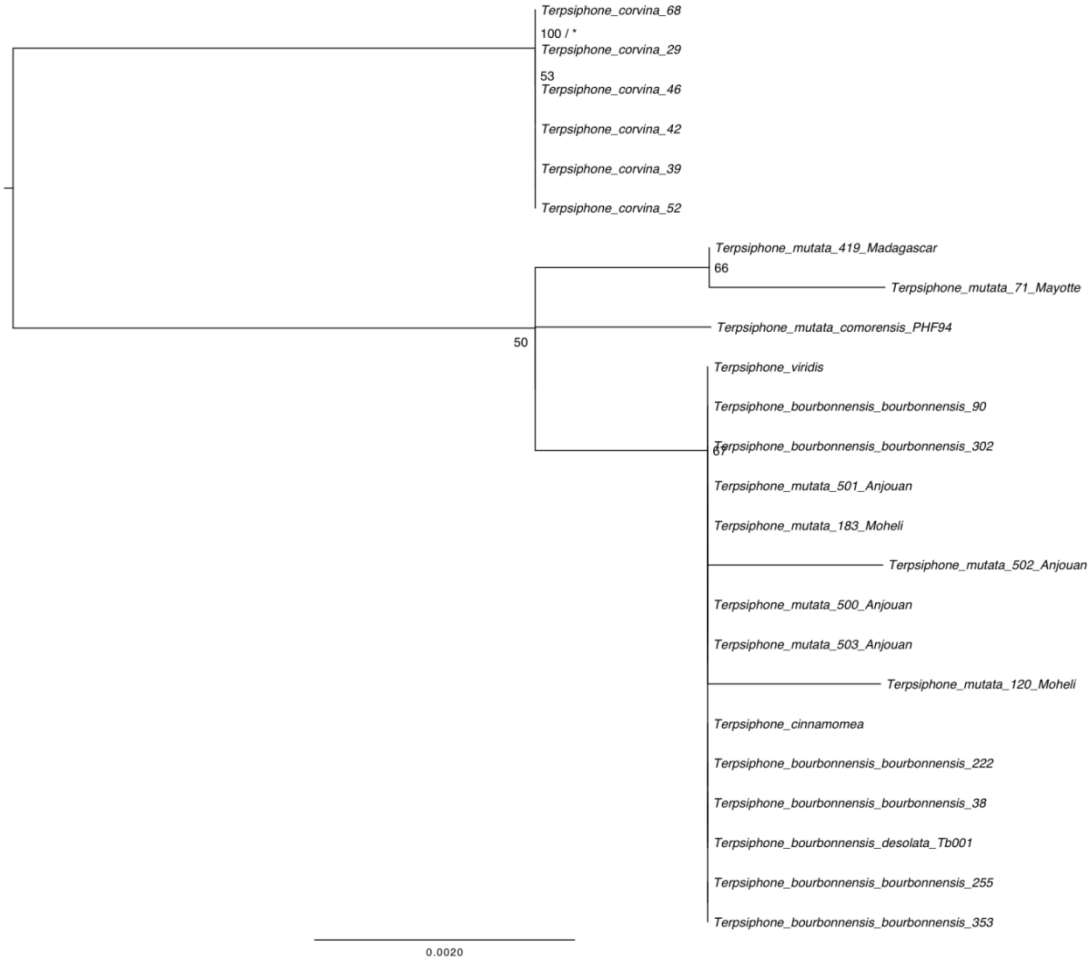
(a) The maximum likelihood tree of the Indian Ocean *Terpsiphone* obtained from the RAxML analysis of cyt *b*. Maximum likelihood bootstrap values from 100 replicates are indicated to the right of the nodes. Posterior probabilities of one are indicated by an asterisk (*) to the right. Scale bar indicates the number of nucleotide substitutions per site.



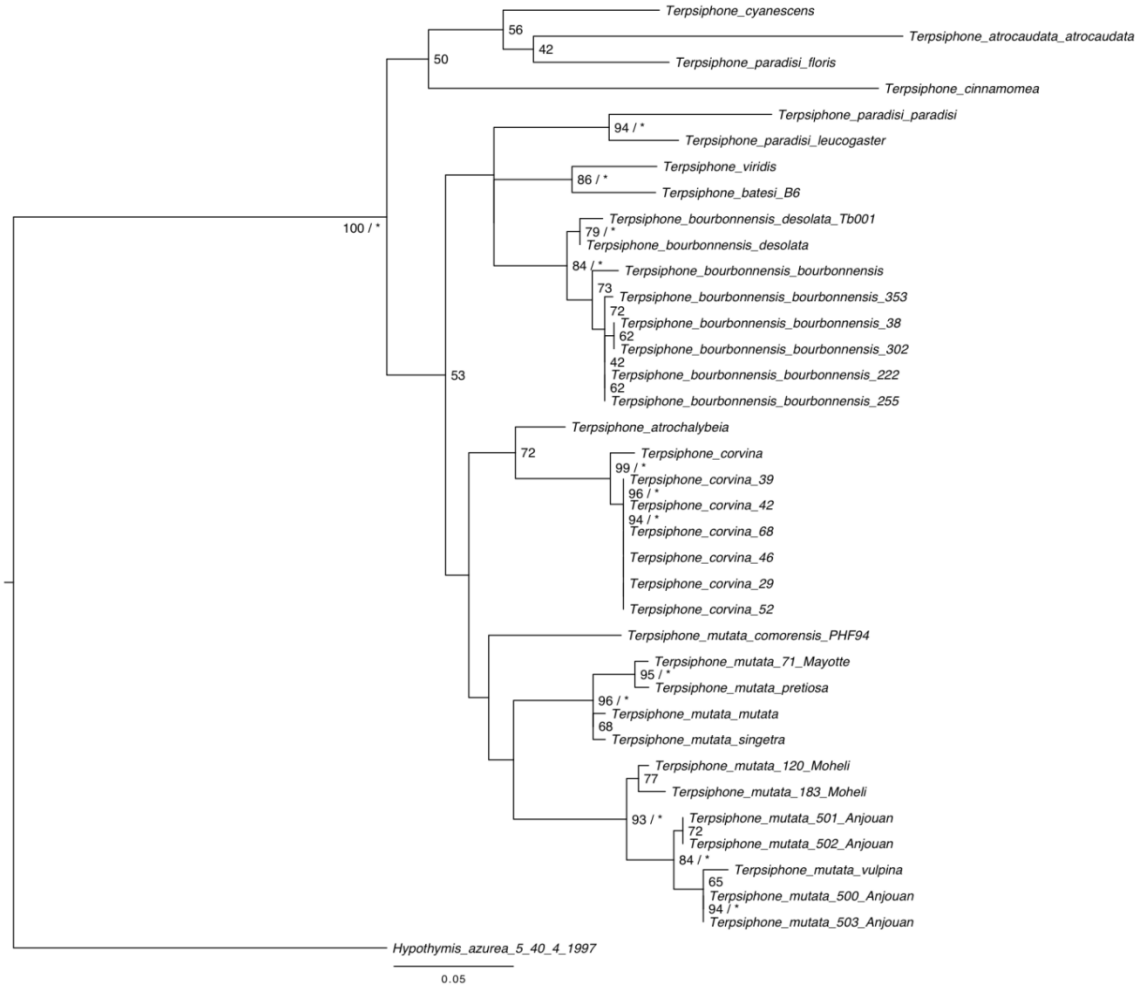
(b) The maximum likelihood tree of the Indian Ocean *Terpsiphone* obtained from the RAxML analysis of *GAPDH*. Maximum likelihood bootstrap values from 100 replicates are indicated to the right of the nodes. Posterior probabilities of one are indicated by an asterisk (*) to the right. Scale bar indicates the number of nucleotide substitutions per site.



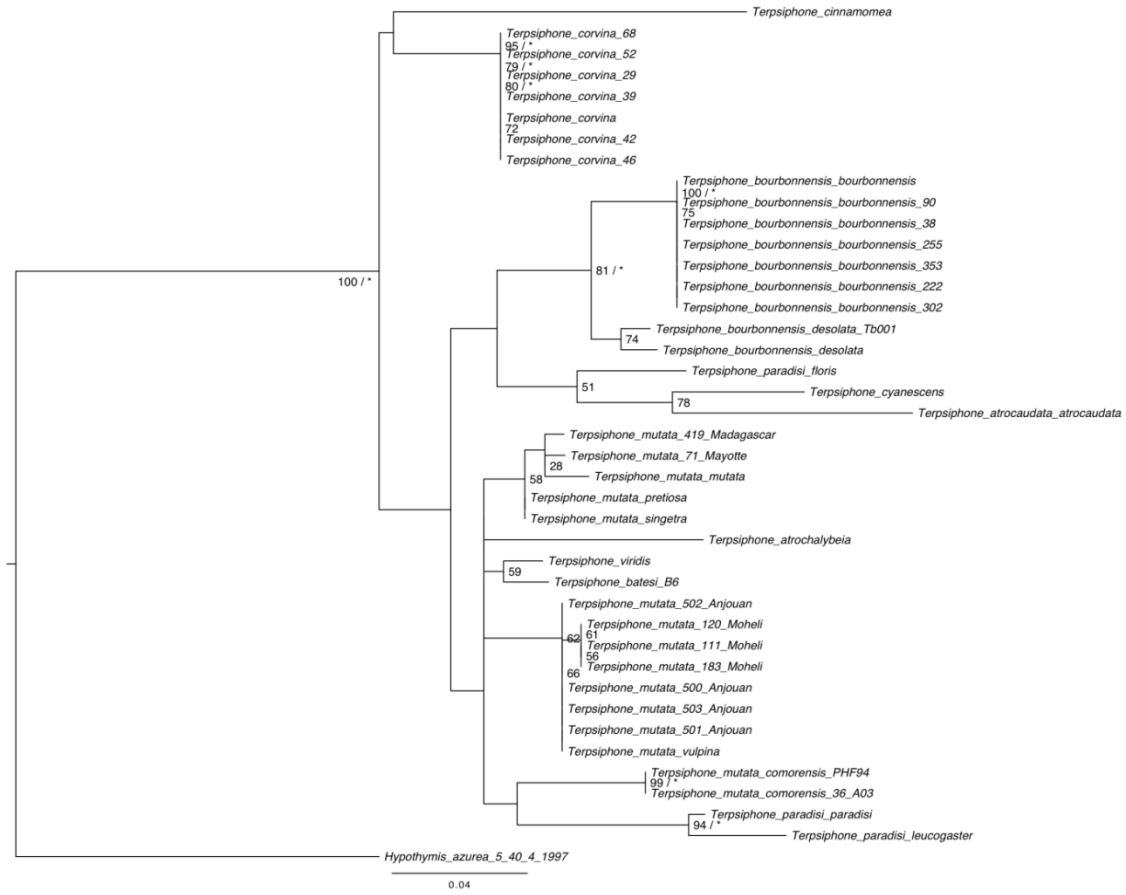
(c) The maximum likelihood tree of the Indian Ocean *Terpsiphone* obtained from the RAxML analysis of *MC1R*. Maximum likelihood bootstrap values from 100 replicates are indicated to the right of the nodes. Posterior probabilities of one are indicated by an asterisk (*) to the right. Scale bar indicates the number of nucleotide substitutions per site.



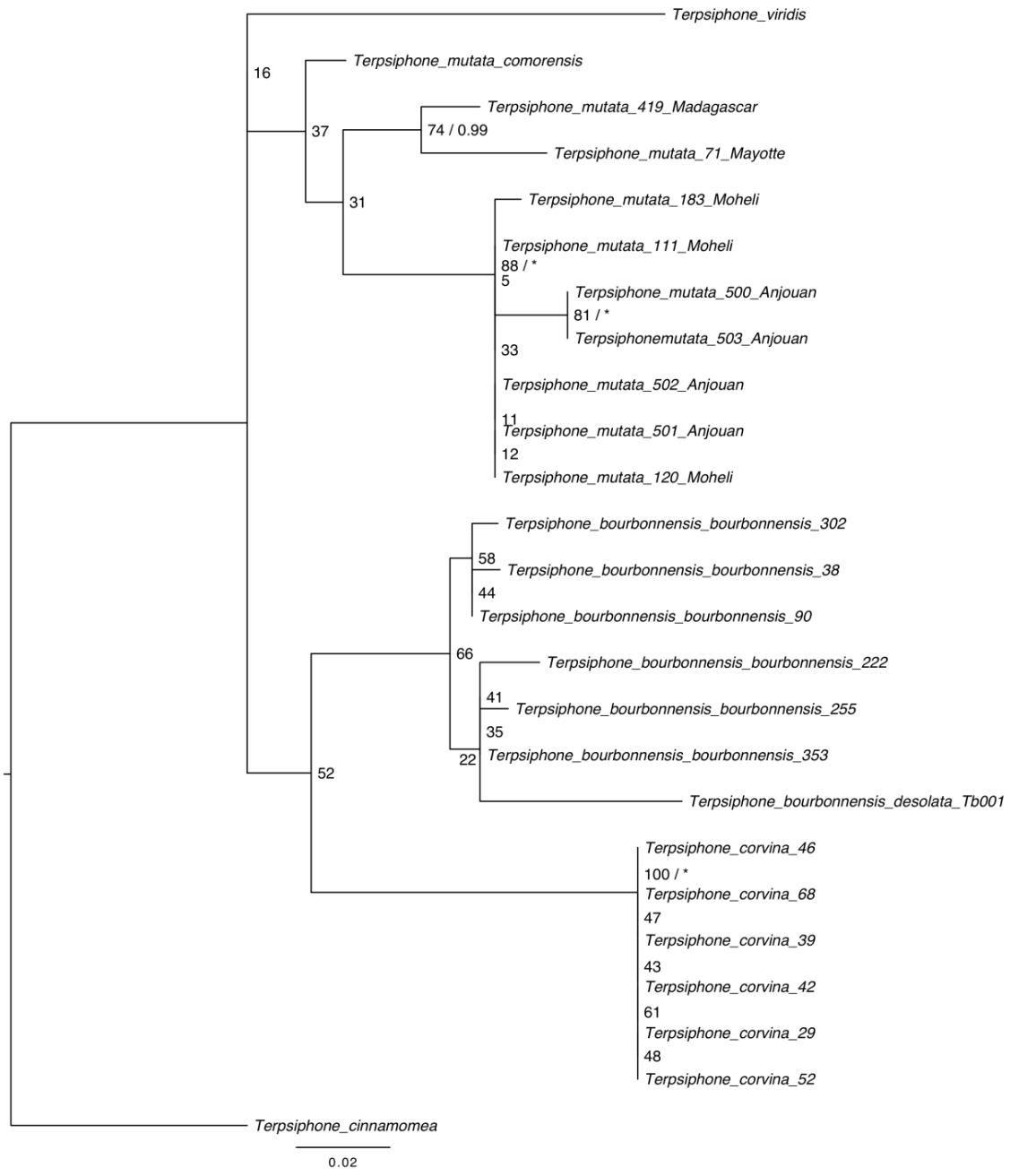
(d) The maximum likelihood tree of the Indian Ocean *Terpsiphone* obtained from the RAxML analysis of *ND2*. Maximum likelihood bootstrap values from 100 replicates are indicated to the right of the nodes. Posterior probabilities of one are indicated by an asterisk (*) to the right. Scale bar indicates the number of nucleotide substitutions per site.



(e) The maximum likelihood tree of the Indian Ocean *Terpsiphone* obtained from the RAxML analysis of *ND3*. Maximum likelihood bootstrap values from 100 replicates are indicated to the right of the nodes. Posterior probabilities of one are indicated by an asterisk (*) to the right. Scale bar indicates the number of nucleotide substitutions per site.



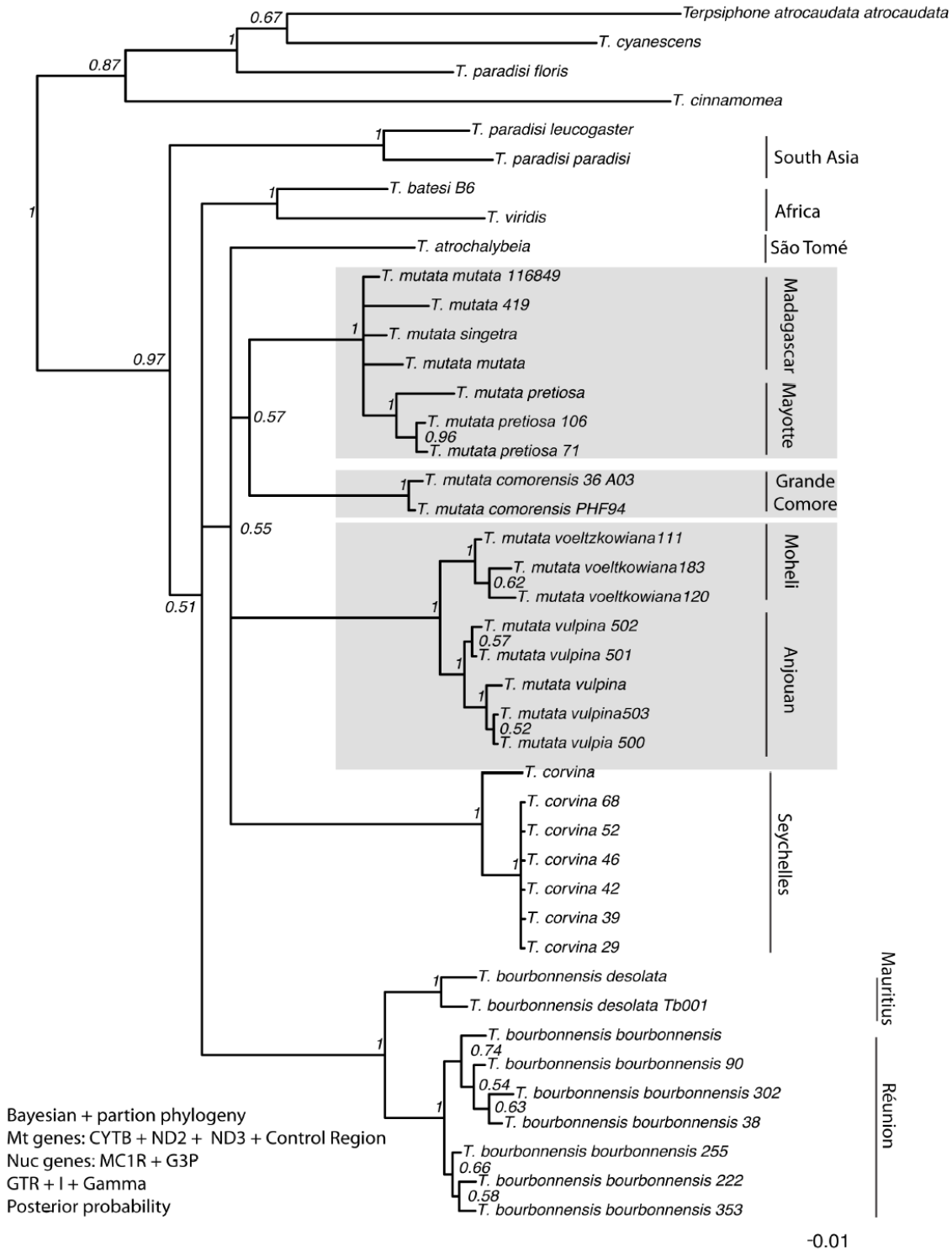
(f) The maximum likelihood tree of the Indian Ocean *Terpsiphone* obtained from the RAxML analysis of the control region. Maximum likelihood bootstrap values from 100 replicates are indicated to the right of the nodes. Posterior probabilities of one are indicated by an asterisk (*) to the right. Scale bar indicates the number of nucleotide substitutions per site.



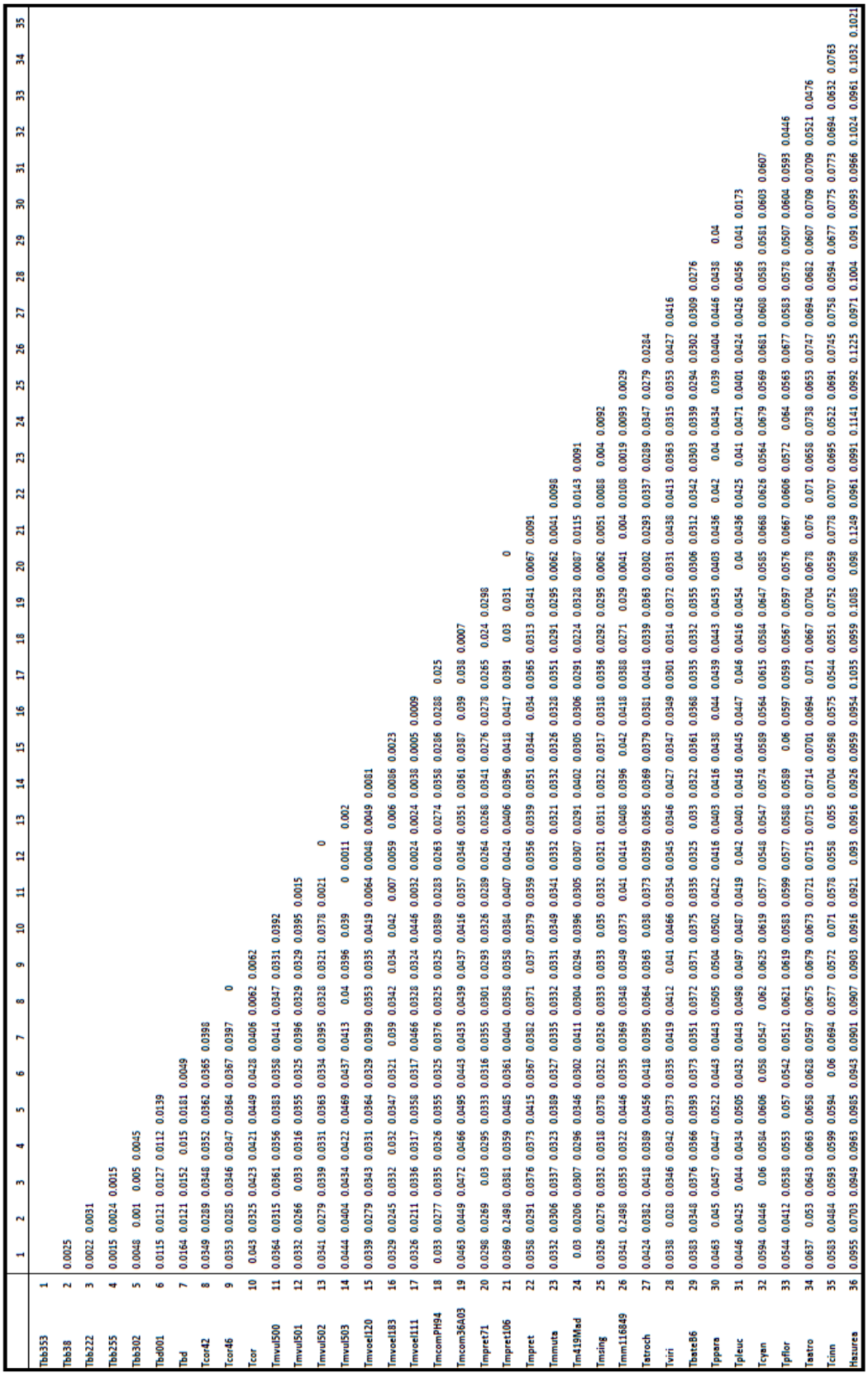
SI 2.2` Results of Approximately Unbiased (AU) and Shimodaira-Hasegawa (SH) tests.

Hypothesis	Delta LnL	AU test	SH test
T. corvina/*T. atrochalybeia* monophyly topology a	-0.0	0.639	0.958
T. corvina/*T. atrochalybeia* monophyly topology a	0.0	0.398	0.884
Indian ocean taxa+*T. atrochalybeia* monophyly topology a	0.4	0.341	0.793
Indian ocean taxa+*T. atrochalybeia* monophyly topology b	0.4	0.344	0.793
Indian ocean taxa+*T. atrochalybeia* monophyly topology c	0.4	0.344	0.793
T. mutata monophyly	1.2	0.451	0.740
T. mutata monophyly	1.2	0.440	0.740
Indian ocean taxa without *T. atrochalybeia* monophyly topology a	194.4	4.00E-15	0
Indian ocean taxa without *T. atrochalybeia* monophyly topology b	194.4	4.00E-15	0

SI 2.3 Partitioned Bayesian analysis topology produced from the mito-nuclear supermatrix analyses. Biogeographic origins are shown on the side of the phylogeny. PP=Posterior probabilities issued from MrBayes. *T. mutata* clades indicated by grey shading.



SI 2.4 Uncorrected pairwise distance matrix for mitochondrial DNA four gene concatenated dataset.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Tbb353	1																																		
Tbb38	2	0.0025																																	
Tbb222	3	0.0022	0.0031																																
Tbb255	4	0.0015	0.0024	0.0015																															
Tbb302	5	0.0048	0.001	0.005	0.0045																														
Tbd001	6	0.0115	0.0121	0.0127	0.0112	0.0139																													
Tbd	7	0.0164	0.0121	0.0152	0.015	0.0181	0.0049																												
Tcar42	8	0.0349	0.0289	0.0348	0.0352	0.0362	0.0365	0.0398																											
Tcar46	9	0.0353	0.0285	0.0346	0.0347	0.0364	0.0367	0.0397	0																										
Tcar	10	0.043	0.0325	0.0423	0.0421	0.0449	0.0428	0.0406	0.0062	0.0062																									
Tmvul500	11	0.0364	0.0315	0.0361	0.0356	0.0383	0.0358	0.0414	0.0347	0.0331	0.0392																								
Tmvul501	12	0.0332	0.0266	0.033	0.0316	0.0355	0.0325	0.0396	0.0329	0.0329	0.0395	0.0015																							
Tmvul502	13	0.0341	0.0279	0.0339	0.0331	0.0363	0.0334	0.0395	0.0326	0.0321	0.0378	0.0021	0																						
Tmvul503	14	0.0444	0.0404	0.0434	0.0422	0.0469	0.0437	0.0413	0.04	0.0396	0.039	0	0.0011	0.002																					
Tmvoel120	15	0.0339	0.0279	0.0343	0.0331	0.0364	0.0329	0.0399	0.0353	0.0335	0.0419	0.0064	0.0048	0.0049	0.0081																				
Tmvoel183	16	0.0329	0.0245	0.0332	0.032	0.0347	0.0321	0.039	0.0342	0.034	0.042	0.007	0.0059	0.006	0.0086	0.0023																			
Tmvoel111	17	0.0326	0.0211	0.0336	0.0317	0.0358	0.0317	0.0466	0.0326	0.0324	0.0446	0.0032	0.0024	0.0024	0.0038	0.0005	0.0009																		
TmcomPH94	18	0.033	0.0277	0.0335	0.0326	0.0355	0.0325	0.0376	0.0325	0.0325	0.0389	0.0283	0.0263	0.0274	0.0358	0.0266	0.0288	0.025																	
Tmcom36A03	19	0.0463	0.0449	0.0472	0.0466	0.0495	0.0443	0.0433	0.0439	0.0437	0.0416	0.0357	0.0346	0.0351	0.0361	0.0387	0.039	0.038	0.0007																
Tmpret71	20	0.0298	0.0269	0.03	0.0295	0.0333	0.0316	0.0355	0.0301	0.0293	0.0326	0.0289	0.0264	0.0268	0.0341	0.0276	0.0278	0.0265	0.024	0.0298															
Tmpret106	21	0.0369	0.2498	0.0361	0.0359	0.0405	0.0361	0.0404	0.0358	0.0358	0.0384	0.0407	0.0424	0.0406	0.0396	0.0418	0.0417	0.0391	0.03	0.031	0														
Tmpret	22	0.0358	0.0291	0.0376	0.0373	0.0415	0.0367	0.0382	0.0371	0.037	0.0379	0.0359	0.0356	0.0339	0.0351	0.0344	0.034	0.0365	0.0313	0.0341	0.0067	0.0091													
Tmmuta	23	0.0332	0.0306	0.0337	0.0323	0.0389	0.0327	0.0335	0.0332	0.0331	0.0349	0.0341	0.0332	0.0321	0.0332	0.0326	0.0328	0.0351	0.0291	0.0295	0.0062	0.0041	0.0098											
Tm419Mad	24	0.03	0.0206	0.0307	0.0296	0.0346	0.0302	0.0411	0.0304	0.0294	0.0396	0.0305	0.0307	0.0291	0.0402	0.0305	0.0306	0.0291	0.0224	0.0328	0.0087	0.0115	0.0143	0.0091										
Tmsing	25	0.0326	0.0276	0.0332	0.0318	0.0378	0.0322	0.0326	0.0333	0.0333	0.035	0.0332	0.0321	0.0311	0.0322	0.0317	0.0318	0.0356	0.0292	0.0295	0.0062	0.0051	0.0088	0.004	0.0092									
Tmm116849	26	0.0341	0.2498	0.0353	0.0322	0.0446	0.0335	0.0369	0.0348	0.0349	0.0373	0.041	0.0414	0.0408	0.0396	0.042	0.0418	0.0388	0.0271	0.029	0.0041	0.004	0.0108	0.0019	0.0093	0.0029								
Tatroch	27	0.0424	0.0382	0.0418	0.0389	0.0456	0.0418	0.0395	0.0364	0.0363	0.038	0.0373	0.0359	0.0365	0.0369	0.0379	0.0381	0.0418	0.0339	0.0363	0.0302	0.0293	0.0337	0.0289	0.0347	0.0279	0.0284							
Tviri	28	0.0338	0.028	0.0346	0.0342	0.0373	0.0335	0.0419	0.0412	0.041	0.0466	0.0354	0.0345	0.0346	0.0427	0.0347	0.0349	0.0301	0.0314	0.0372	0.0331	0.0438	0.0413	0.0363	0.0315	0.0353	0.0427	0.0416						
Tbate86	29	0.0383	0.0348	0.0376	0.0366	0.0393	0.0373	0.0351	0.0372	0.0371	0.0375	0.0335	0.0325	0.033	0.0322	0.0361	0.0368	0.0335	0.0332	0.0355	0.0306	0.0312	0.0342	0.0303	0.0339	0.0294	0.0302	0.0309	0.0276					
Tppara	30	0.0463	0.045	0.0457	0.0447	0.0522	0.0443	0.0443	0.0505	0.0504	0.0502	0.0422	0.0416	0.0403	0.0416	0.0438	0.044	0.0439	0.0443	0.0453	0.0403	0.0436	0.042	0.04	0.0434	0.039	0.0404	0.0446	0.0438	0.04				
Tpleuc	31	0.0446	0.0425	0.044	0.0434	0.0505	0.0432	0.0443	0.0498	0.0497	0.0487	0.0419	0.042	0.0401	0.0416	0.0445	0.0447	0.046	0.0416	0.0454	0.04	0.0436	0.0425	0.041	0.0471	0.0401	0.0424	0.0426	0.0456	0.041	0.0173			
Tcyan	32	0.0594	0.0446	0.06	0.0584	0.0606	0.058	0.0547	0.062	0.0625	0.0619	0.0577	0.0548	0.0547	0.0574	0.0589	0.0564	0.0615	0.0584	0.0647	0.0585	0.0668	0.0626	0.0564	0.0679	0.0569	0.0681	0.0608	0.0583	0.0581	0.0603	0.0607		
Tpflor	33	0.0544	0.0412	0.0538	0.0553	0.057	0.0542	0.0512	0.0621	0.0619	0.0583	0.0599	0.0577	0.0588	0.0589	0.06	0.0597	0.0593	0.0567	0.0597	0.0576	0.0667	0.0606	0.0572	0.064	0.0563	0.0677	0.0583	0.0578	0.0507	0.0604	0.0593	0.0446	
Tastro	34	0.0637	0.053	0.0643	0.0663	0.0658	0.0628	0.0597	0.0675	0.0679	0.0673	0.0721	0.0715	0.0715	0.0714	0.0701	0.0694	0.071	0.0667	0.0704	0.0678	0.076	0.071	0.0658	0.0738	0.0653	0.0747	0.0694	0.0682	0.0607	0.0709	0.0709	0.0521	0.0476
Tcinn	35	0.0583	0.0484	0.0593	0.0599	0.0594	0.06	0.0694	0.0577	0.0572	0.071	0.0578	0.0558	0.055	0.0704	0.0598	0.0575	0.0544	0.0551	0.0752	0.0559	0.0778	0.0707	0.0695	0.0522	0.0691	0.0745	0.0758	0.0594	0.0677	0.0775	0.0773	0.0694	0.0632	0.0763
Htaurea	36	0.0955	0.0703	0.0949	0.0963	0.0985	0.0943	0.0901	0.0907	0.0903	0.0916	0.0921	0.093	0.0916	0.0926	0.0959	0.0954	0.1035	0.0959	0.1085	0.098	0.1249	0.0961	0.0991	0.1141	0.0992	0.1225	0.0971	0.1004	0.091	0.0993	0.0966	0.1024	0.0961	0.1032	0.1021

Chapter 3. Comparison of historical bottleneck effects and genetic consequences of reintroduction in a Critically Endangered island passerine

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Key words: bottleneck, microsatellite, reintroduction, conservation, *Terpsiphone corvina*

Running title: Historical versus reintroduction bottlenecks

ABSTRACT

Reintroduction is an important tool for recovering endangered species; however the magnitude of genetic consequences for reintroduced populations remains largely unknown, in particular the relative impacts of historical population bottlenecks compared to those induced by conservation management.

We characterise 14 microsatellite loci developed for the Seychelles paradise flycatcher and use them to quantify temporal and spatial measures of genetic variation across a 134-year timeframe encompassing a historical bottleneck that reduced the species to ~28 individuals in the 1960s, through the initial stages of recovery and across a second contemporary conservation-introduction-induced bottleneck. We then evaluate the relative impacts of the two bottlenecks, and finally apply our findings to inform broader reintroduction strategy.

We find a temporal trend of significant decrease in standard measures of genetic diversity across the historical bottleneck, but only a non-significant downward trend in number of alleles across the contemporary bottleneck. However accounting for the different timescales of the two bottlenecks (~40 historical generations versus <1 contemporary generation) the loss of genetic diversity per generation is greater across the contemporary bottleneck. Historically the flycatcher population was genetically structured; however extinction on four of five islands has resulted in a homogeneous contemporary population.

We conclude that severe historical bottlenecks can leave a large footprint in terms of sheer quantity of genetic diversity lost. However, severely depleted genetic diversity does not render a species immune to further genetic erosion upon reintroduction. In some cases the loss of genetic diversity per generation can, initially at least, be greater across reintroduction-induced bottlenecks.

3.1 INTRODUCTION

Reintroductions and conservation introductions (referred to as 'reintroductions' from hereon) are important conservation tools for recovering endangered species. Despite the fact that failure rates are high, these techniques are likely to remain popular as long as intensive population management remains a key part of endangered species recovery (Fischer & Lindenmayer 2000; Armstrong & Seddon 2008). Alongside the low success of species reintroductions, attention has increasingly focused on the genetic consequences of reintroductions. In particular, there is interest in how reintroduced populations founded from very few individuals may be compromised by the same genetic problems that are associated with bottlenecked populations, such as loss of genetic variation and inbreeding depression, genetic attributes which are widely accepted to increase extinction risk in natural populations (Saccheri et al. 1998; Bijlsma et al. 2000; Keller & Waller 2002; Frankham 2005).

Despite the widespread popularity of reintroduction as a conservation tool and the large investment of resources required, the magnitude of genetic consequences for reintroduced populations remains largely unknown (Armstrong & Seddon 2008; Groombridge et al. 2012). This knowledge gap is surprising given that notable parallels exist between genetic characteristics of small populations and factors believed to be important determinants of reintroduction success (Leberg & Firmin 2008). For example, reintroducing wild-caught rather than captive-reared stock, and releasing more rather than fewer individuals, has been demonstrated to increase the probability of reintroduction success (Fischer & Lindenmayer, 2000).

3.1.1 The relevance of historical genetic profiles

A reintroduced population's adaptive ability depends upon the amount and type of genetic diversity within the released individuals, a quantity governed by levels of diversity in the source population. Importantly however, the extent of genetic diversity at source will already have been influenced by the species' population history. Historical population size and the pattern and extent of a population's decline (bottleneck 'shape') are widely documented, in both experimental and natural populations, to have a considerable influence on levels of genetic diversity and inbreeding in post-bottleneck/contemporary populations (e.g. Bouzat et al. 1998; Westemeier et al. 1998; England et al. 2003; Taylor et al. 2007; Groombridge et al. 2009). Low levels of historical genetic diversity would indicate a long protracted bottleneck resulting in a contemporary population with reduced genetic diversity, higher levels of homozygosity and an accumulation of inbreeding, and therefore more likely to express deleterious genetic load (inbreeding depression). In contrast a shorter, sharper bottleneck from historically high genetic diversity would suggest less time for inbreeding and a loss of heterozygosity to accumulate and therefore produce a contemporary population more robust to effects of genetic load (Groombridge et al. 2012). Some of these predictions have been demonstrated

experimentally in *Drosophila* populations (Bijlsma et al. 2000; Montgomery et al. 2000; England et al. 2003). Knowledge of historical bottleneck shape can also help clarify whether a lack of evidence for inbreeding depression is due to low statistical power or a true lack of inbreeding depression (Keller et al. 2012). A severe historical bottleneck might reduce genetic variation to such an extent that subsequent bottlenecks through reintroduction leave no appreciable genetic signature, whereas a less-severe historical decline might leave sufficient diversity for reintroduction effects to be detectable. Therefore, a species' historical profile is an important, but frequently overlooked, component when evaluating the genetic consequences of reintroduction. Indeed, many reintroduced populations originate from captivity and therefore experience two managed bottlenecks, one at founding of the captive population and another upon release of individuals. Both bottlenecks will influence the subsequent rate of inbreeding and loss of genetic diversity experienced by the reintroduced population. However, a historical bottleneck imposes background levels of inbreeding and genetic diversity loss depending on its severity and duration, characteristics which can enable prediction of the relative magnitude of subsequent bottlenecks linked to conservation management.

Numerous studies of reintroductions have provided insight into founder and inbreeding effects since the onset of recovery programmes (Robichaux et al. 1997; Stephen et al. 2005; Jamieson et al. 2007; Brekke et al. 2011; Jamieson 2011). However, any conclusions drawn from them remain decoupled from historical bottleneck effects, masking historical genetic signature and potentially overestimating the genetic consequences for reintroduction. Consequently, there is a need to examine temporal measures of genetic diversity within an historical and contemporary context.

In this study we apply a novel set of microsatellite DNA markers developed for the Critically Endangered Seychelles paradise flycatcher (*Terpsiphone corvina*), an endemic island species whose historical decline and population crash is well documented, in order to quantify in detail historical and contemporary measures of genetic diversity across a 134-year timeframe that extends from the historical pre-bottleneck population to the F1 offspring produced from a recent conservation introduction carried out in November 2008. We evaluate the relative impacts of the historical bottleneck on measures of genetic diversity alongside the equivalent impacts imposed by the conservation introduction programme. In addition, the historical range of the flycatcher extended across multiple islands in the Seychelles archipelago (Newton 1867; Diamond 1984), raising the question of whether restricted gene flow between islands resulted in a genetically structured historical population. We examine the genetic data obtained from 53 museum specimens collected from the species' historical distribution to look for evidence of historical genetic structure. We then apply our findings to make management recommendations.

3.2 METHODS

3.2.1 Study species and history

The Seychelles paradise flycatcher is a behaviourally monogamous, sexually dimorphic passerine endemic to the Seychelles (Newton 1867). Adult mortality on La Digue is 21% (Currie et al. 2005), individuals ringed as juveniles have lived to a maximum of 10 years old though most die younger, and mean generation time is ~2 years (RM Bristol unpublished data). Historically recorded on five islands (Diamond 1984) (Figure 3.1), the species declined dramatically in range and numbers in the late 19th-20th century primarily due to habitat loss and introduced predators. The species had disappeared from Aride (68 ha), Félicité (268 ha) and Marianne (9.5ha) by the early 1900s (Nicoll 1906; Vesey-Fitzgerald 1940; Diamond 1984) and from Praslin (2750 ha) by the 1980s (Gerlach 1997), leaving it restricted to La Digue (1000 ha) where the population declined to its lowest estimate in 1965 of 28 individuals, restricted to 300 ha of coastal plateau on La Digue (Gaymer et al. 1969). The population subsequently increased, relatively unassisted, to ~250 individuals by 2000 but remained restricted to La Digue, where scope for further population growth and habitat restoration is limited (Currie et al. 2003c). In November 2008, 23 flycatchers (13 males, 10 females) were introduced to Denis Island, a 140ha island with a predicted carrying capacity of ~40 pairs (Bristol & Groombridge 2007; author's unpublished data), as a first step to increase the species' range and numbers. Denis Island was selected as the site for the first conservation introduction of this species, because although outside the known historical distribution of the species, it is the only island currently considered to have a sufficient area of suitable habitat and an absence of known predators (Currie et al. 2003c; Bristol & Groombridge 2007). Breeding success and survival were intensively monitored in the introduced population for 22 months post release. Founder individuals began breeding within two months of release; by August 2010 the population comprised 24 individuals (author's unpublished data) and by December 2011 (36 months post release) the population was estimated at between 30-33 individuals (Henriette & Laboudallon 2011).

3.2.2 Sample collection and DNA extraction

The Seychelles paradise flycatcher population has been genetically sampled periodically since the 1870s across the historical bottleneck, during the species' initial population recovery and before, during and after the reintroduction itself.

Modern samples

Adult flycatchers were captured for blood-sampling by mist-netting and young were sampled at the nest. Blood samples were collected by puncturing the brachial wing vein using a sterile 25 gauge insulin needle and transferring approximately 50 µl of blood, using a capillary tube, into a rubber-

sealed screw top micro-centrifuge tube containing 1ml of absolute ethanol. The tube was immediately inverted several times to mix the suspension and stored at room temperature prior to long-term storage at -20^{o} Celsius until the genomic DNA was extracted. Blood-sampled individuals were ringed with unique colour ring combinations for future identification and to avoid resampling. A total of 331 individuals were sampled on La Digue (171 from 1999-2001, and 160 from 2007-2010), and all 23 flycatchers introduced to Denis Island were blood sampled and genotyped as were the first seven F1 offspring.

In order to minimise oversampling of related individuals we restricted our genotype dataset to adults (*n*=186), but included the F1 offspring of the reintroduced population. Genomic DNA was extracted from each blood sample using the ammonium acetate method following Richardson et al. (2001). DNA was quantified using a NanoDrop 8000 (Thermo Scientific, USA) and diluted to a concentration of c.10ng/µl using DNA grade water for downstream PCR amplification.

Museum samples

A total of 53 footpad samples were obtained from Seychelles paradise flycatcher specimens held in collections at the Natural History Museum at Tring (*n*=20) and the World Museum Liverpool (*n*=9) in the UK, and the American Museum of Natural History in New York, US (*n*=24). The museum specimens were labelled as collected from Praslin (*n*=21), La Digue *(n*=22), Aride (*n*=3), Ile aux Fous (*n*=2) and unknown (labelled only as collected in Seychelles, *n*=5). The two individuals labelled as collected on Ile aux Fous are certainly mislabelled; Ile aux Fous is a tiny 0.5 ha rock located approximately half-way between Praslin and Aride (Figure 3.1), unable to support Seychelles paradise flycatchers and additionally they have never been recorded there. Genomic DNA was extracted from museum samples using QIAamp DNA Micro kits (Qiagen, UK) and following the manufacturer's protocol for isolation of genomic DNA from forensic case work samples, with an extended overnight step of incubation with Proteinase *K* to ensure complete digestion of the sample material. Negative extraction controls were included and all work with museum samples was carried out in a dedicated museum DNA laboratory where no contemporary avian DNA has been present. The laboratory work on museum samples was carried out in UV-irradiated fume hoods to destroy any contaminant DNA. Details of the museum specimens sampled for this study are provided as Supporting Information (SI 3.2).

3.2.3 Marker development, testing and genotyping

Microsatellite loci were isolated from a microsatellite-enriched genomic library produced using genomic DNA from a single female Seychelles paradise flycatcher sampled on La Digue in 2008 (see Supporting Information, SI 3.1 for details). Thirty microsatellite-containing sequences were

selected for the design of PCR primer sets using PRIMER3 software (Rozen & Skaletsky 2000), labelled with a fluorescent dye (6-FAM or HEX) and initially tested for amplification and polymorphism in a panel of eight flycatcher individuals sampled from across the species' contemporary range. Fragments were amplified in 2µl PCR reactions containing 1x Qiagen multiplex PCR master mix (Qiagen, UK), 0.2 µM of each primer and c.10ng of template DNA following Kenta et al. (2008). Each locus was amplified separately under mineral oil using the following PCR profile: 95°C for 15 minutes followed by 30 cycles of 94°C for 30 seconds, 60°C for 90 seconds and 72°C for 60 seconds, prior to a final period of 60°C for 30 minutes. Fluoro-labelled PCR products were separated on an ABI 3730 48 well capillary DNA Analyser (Applied Biosystems, USA) and genotypes were scored using GeneMapper software (Applied Biosystems, USA). Loci that failed to amplify, were unscorable or were monomorphic were discarded. The remaining loci were amplified in 25 individuals from La Digue. These new loci were checked for sex linkage by genotyping individuals of known sex (12 males and 12 females). Observed and expected heterozygosities and predicted null allele frequencies were calculated using CERVUS version 2.0 (Marshall et al. 1998). Tests for departures from Hardy-Weinberg and linkage equilibrium were conducted using a Markov chain method implemented in GenePop version 3.4 (Raymond & Rousset 1995) and linkage equilibrium was corrected for multiple comparisons using the False Discovery Rate technique (Verhoeven et al. 2005).

Fourteen loci displaying between two and seven alleles in the initial 25 tested individuals comprised the final marker set used to genotype the modern and historical DNA samples for this study. PCRs for all modern samples were conducted in 2µl multiplex reactions designed using Multiplex Manager 1.0 (Holleley & Geerts 2009), and using the PCR profile described for loci testing above. For all museum samples, the loci were amplified individually (single-plexed) in 4µl PCR reactions containing 2µl of QIAGEN multiplex PCR mix, 0.4 µM of each primer and 1µl of DNA, using the same PCR profile as the modern samples except that 46 cycles were run (cf. 30 for modern samples). PCR products from museum samples were genotyped individually for each locus using the same ABI 3730 DNA Analyser used for the modern samples. Genotypes from all samples were scored using GeneMapper software. All PCRs contained negative and positive controls which were genotyped and analysed alongside the samples. Genomic DNA was re-extracted for 10% of the modern individuals and genotyped a second time to check for consistency of allele-calling and identify PCR dropout or adenylation. The low quality and quantity of DNA extracted from museum specimens is known to increase the frequency of genotyping errors, predominantly due to the failure of one allele to amplify at a heterozygous locus (allelic dropout) producing false homozygotes (Taberlet et al. 1996; Gagneux et al. 1997; Sefc et al. 2003; Hoffman & Amos 2005; Pompanon et al. 2005). To ensure the genotypes of the museum samples were as accurate as possible and that both alleles had amplified, PCR amplification and genotyping was replicated a minimum of three times for each sample at each locus. Genotyping error rates were quantified per allele and locus following Pompanon et al. (2005).

3.2.4 Summary statistics

We partitioned our dataset into time periods based on individual sample collection dates in order to test for temporal changes in genetic diversity. The time periods sampled were 1877-1888 ('1880s', *n*=21); 1904-1907 ('1900s', *n*=22); 1940-1955 ('1940s', *n*=6); 1999-2001 (2000, *n*=83); and 2007-2010 (LD, *n*=80). Additionally, we partitioned the contemporary dataset into LD (as above), DI (2008, *n*=23) and F1 (2010, *n*=7) in order to test for any differences between the La Digue source population (LD), the translocated Denis Island population (DI) and the F1 offspring on Denis (F1). All museum samples (1880s, 1900s, and 1940s above) are considered historical samples, and all modern samples (2000, LD, DI and F1 above) are considered contemporary samples. For the purposes of comparisons between pre and post historical bottleneck we consider the oldest samples (1880s above) to be pre-bottleneck and the contemporary La Digue population (LD) to be post bottleneck. For each time period/population we calculated mean number of alleles per locus (N_A), mean number of effective alleles per locus (N_E), observed heterozygosity (H_O) and expected heterozygosity (H_E) using GenAlEx (Peakall & Smouse 2006). The programme FSTAT (Goudet 2001) was used to calculate allelic richness (A_R) standardised to the smallest sample size per locus per time-period and population. We used the programme Microsatellite Analyser (Dieringer & Schlötterer 2003) to calculate pairwise Nei's genetic distance (corrected for population size) and F_{ST} between time periods and contemporary populations. Shapiro-Wilk tests were run to test for normality of data distributions and one-way ANOVA's and *post hoc* Tukey tests were carried out in R version 2.15.1 (R Core Team 2012) to test for differences between each of these standard parameters of genetic diversity across the different time periods and contemporary populations.

3.2.5 Measurement of historical and contemporary population structure

We used the programme STRUCTURE version 2.3.3 (Pritchard et al. 2000) to determine whether any genetic structure was present historically between different island populations and within or between the contemporary La Digue and Denis Island populations. STRUCTURE implements a Bayesian approach to estimate the most likely number of population clusters (*K*) based upon the genotypes of the individuals included in the analysis. STRUCTURE allows the input of predefined populations to enable easier comparison with the allele frequency based structure, but it does not use this information as a prior. We pre-defined populations based on sampling location.

All STRUCTURE analyses were run for 500 000 Markov chain Monte Carlo (MCMC) iterations after a burn-in period of 100 000 iterations, using a model specifying non-informative priors and assuming admixture, whereby a proportion of the genome of each individual is probabilistically assigned to each cluster according to allele frequency by minimising deviations from Hardy-Weinberg

equilibrium. We used the delta$K$ (ΔK) statistic, which is based on the rate of change between successive K values, to infer the uppermost level of structure in the data following Evanno et al. (2005) and plotted the log-likelihood values for K for all runs. We then critically assessed the estimates of ΔK and K in relation to individual sample location and collection date in order to aid interpretation of the STRUCTURE outputs.

For the contemporary analyses ten independent runs of K=1-4 were performed using a model specifying correlated allele frequencies. For the historical analyses ten independent runs of K=1-7 were performed. Given that it may not be biologically realistic to assume that separate island populations have correlated allele frequencies, we carried out an initial analysis specifying a model with correlated allele frequencies, and a second analysis specifying a model with uncorrelated allele frequencies.

Further STRUCTURE analyses were run, under a correlated allele frequency model, on clusters obtained from the initial historical full dataset analyses to test whether there was further population substructure as recommended by Evanno et al. (2005).

3.2.6 Assessment of proportion of total genetic diversity captured by translocation

We implemented a simulation in R version 2.15.1 in order to determine the proportion of alleles that could theoretically be transferred to Denis Island with differing numbers of founder individuals. The model randomly selected different numbers of founder individuals (5-160) from the source population dataset and ran 1000 replicates for each number of individuals in order to determine the proportion of alleles that could be captured in differing numbers of founder individuals chosen at random from the source population.

3.2.7 Estimation of temporal change in effective population size

We applied a Bayesian method developed by Beaumont (2003) and implemented using the programme *tmvp* to our historical and contemporary genotypes in order to estimate changes in effective population size (N_e) across the sampling time period. *Tmvp* samples independent genealogical histories using importance sampling with MCMC in a Bayesian framework in order to estimate recent changes in N_e from temporally distributed allele frequency data. This method assumes that the sampling period is sufficiently short that the effects of mutation are negligible. *Tmvp* makes explicit use of collection dates for all individuals and combines historical and contemporary data to give a posterior distribution of N_e at the time of the oldest sample and at the most recent sample (N_A and N_0 respectively). The programme is able to account for unequal sample sizes across both sampling period and loci. We specified a mean generation time of two years based on field observations of

flycatchers, and rectangular priors of 0-1000 for historical and contemporary N_e. Values of α=0.3-0.7 were stipulated for alpha, the smoothing parameter, with α=0.3 used for the final analysis.

The majority of the historical samples from La Digue were collected during the 1880s, whilst most of the samples from Praslin were collected during the early 1900s, representing a temporal and spatial skew in sampling. Given that a significant signal of genetic structure was detected between the different island subpopulations of the historical flycatcher population with numerous island specific private alleles present (this manuscript), and in order to avoid any bias the skewed sampling might cause, we ran separate *tmvp* analyses for the Praslin and La Digue island populations.

In order to ground truth our genetic estimates of N_e we compared them to estimates of census population size for the different time periods. We obtained estimates of census population size since the 1960s from published sources (Gaymer et al. 1969; Beamish 1972; Watson 1991; Rocamora 1997; Neufeld 1998; Currie et al. 2003a) and we calculated historical population size by relating island area to known territory size. Seychelles paradise flycatchers are a lowland forest dwelling species with an average territory size of 1.04ha (Currie et al. 2003b). The area of coastal plateau forest habitat available to the historical population would have been approximately 1245 ha (La Digue, 300ha; Praslin, 887 ha; Félicité, 35 ha; Marianne, 18 ha; Aride, 5 ha), providing sufficient area for approximately 2400 breeding individuals.

3.2.8 Assessment of the potential effect of genotyping errors on analyses

Finally we investigated how genotyping error rates may affect the results from some of our analyses. In order to test the potential effects of genotyping errors in our historical dataset on the population genetic structure results we ran STRUCTURE analysis on the historical samples with a reduced dataset of five loci, after removing loci with mean genotyping error rates of more than 0.14 per allele (0.27 per locus). The potential effects of genotyping errors on the *tmvp* estimates of historical and current effective population sizes were also considered and the results are interpreted in light of such potential effects.

3.3 RESULTS

3.3.1 Marker characteristics

A set of 14 unique microsatellite markers that amplified easily scorable PCR products were developed and validated using modern samples. All loci were autosomal based on the amplification of heterozygotes in both sexes. Table 3.1 provides details of the 14 loci characterised in this study that were subsequently used to genotype the contemporary and historical samples. The three loci with the

largest allele sizes (*Tcor-003*, 249-305bp; *Tcor-006*, 228-256bp; *Tcor-011*, 217-224bp) failed to amplify in the majority of museum samples (41/53, 43/53 and 44/53 respectively) so were excluded from all analyses involving museum specimens. Failure to reliably amplify products larger than 200bp from degraded museum DNA is not unusual (see Nielsen et al. 1999; Pääbo et al. 2004). Across the 11 remaining loci, the average genotyping success rate was 86.1% (58.5-100%) for the museum samples (n=53) and 99.2% (98-100%) for the contemporary samples (n=193). No genotyping error was detected in the contemporary genotypes; all repeat genotypes were identical. Genotyping error was detected within the genotypes obtained from museum specimens; mean genotyping error rate per allele was 0.14 and per locus 0.28 (see Table 3.3).

3.3.2 Temporal changes in levels of genetic diversity

Figure 3.2 provides box and whisker plots of measures of genetic diversity (N_A, N_E, A_R, H_O, H_E) across 134 years from 1877 to 2010 and shows an overall trend of significant decrease in all measures across the timeframe of this study except H_O which has remained stable. Across 11 loci a total of 79 alleles were present in the museum samples, 32 of which are still present in the contemporary populations, representing a 59.5% loss of alleles across the full extent of the bottleneck. All alleles present in the contemporary population were also present in the museum samples. Mean allelic richness (A_R) decreased by 44.1 % from pre-bottleneck (1880s) levels of 4.7 to contemporary (LD) levels of 2.6, N_E decreased by 43% from pre-bottleneck levels of 4.0 to contemporary levels of 2.3 and H_E decreased by 25.8% from pre-bottleneck levels of 0.7 to contemporary levels of 0.5 while H_O remained stable at just over 0.52. When put into temporal context these losses of genetic diversity represent ~1.19% loss of alleles per generation and a ~0.52% loss of H_E per generation across the ~40 generation historical decline.

Table 3.2 provides a matrix of pair-wise values of genetic distance between the different temporal populations. Nei's genetic distance is lowest between contemporary populations, indicating negligible genetic differentiation between the contemporary source population on La Digue and the reintroduced population on Denis, compared to much greater differentiation between more temporally-spaced samples. Genetic distances become progressively greater as the time-period between pairwise comparisons increases. Comparison of F_{ST} values shows a similar pattern, with little differentiation between the contemporary La Digue and Denis populations, but significant differences in temporal F_{ST} between historical and contemporary populations, indicating substantial temporal genetic differentiation across the full extent of the bottleneck.

An uneven distribution of historical samples across time and space may have influenced our observed temporal patterns of genetic diversity. For example, 80% (16/21) of the samples collected between 1877-1888 were from La Digue, whereas 77% (17/22) of those collected between 1904-1907

were from Praslin and none were from La Digue. Consequently, we calculated the same measures through time for La Digue only, to test whether the temporal patterns of declining genetic diversity and increasing temporal genetic differentiation we observe for the species as a whole holds for this single island population. The results for La Digue mirrored the full dataset, showing a significant decrease in all measures of genetic diversity across the 134 year dataset apart from H_O. The La Digue population experienced a 49% reduction in alleles (from 63 historical to 32 contemporary alleles), a 41.4% (4.5-2.6) reduction in A_R, a 38.4% (3.7-2.3) reduction in N_E and a 22.1% (0.69-0.54) reduction in H_E from the historical pre-bottleneck levels to the contemporary levels (see Supporting Information, SI 3.3.) The pattern of increasing genetic distances and F_{ST} values as the time-period between pairwise comparisons increased also mirrored the full dataset (see Supporting Information, SI 3.4).

3.3.3 Temporal and spatial population genetic structure

Given that the historical populations (and the sampled museum specimens) were distributed across multiple Seychelles islands, the temporal decline in genetic diversity revealed in this study may have occurred against a background of underlying spatial genetic structure. The STRUCTURE analysis of genotypes obtained from the 53 historical museum specimens indicated that the historical flycatcher population was indeed genetically structured between different islands. After running both correlated and uncorrelated allele frequency models, the uncorrelated allele frequency model produced more biologically realistic results in terms of plausible spatial and temporal patterns and was therefore chosen as our final model.

The uncorrelated allele frequency model gives a clear result of ΔK =2 (Figure 3.3). The posterior estimated log-likelihood values indicate K=3 as the most probable number of clusters (Figure 3.3). However, examination of the assignment of individuals to the three inferred clusters shows that samples from Praslin, Aride and 'Ile aux Fous' form a single cluster whereas samples from La Digue form two additional clusters (Figure 3.4). The two La Digue clusters in fact reflect temporal structure; samples collected prior to 1890 are assigned to one cluster and samples collected 1940-1955 are assigned to the other (Figure 3.4). The samples in Figure 3.4 have been ordered by collection date within each pre-defined collection location in order to show this pattern. Each bar represents an individual and the last six individuals collected on La Digue were collected in between 1940-1955 Therefore the actual number of spatially distinct historical clusters is K=2, referring to La Digue and the combined islands of Praslin and Aride in agreement with ΔK=2. Separate analyses of the Praslin cluster and the La Digue cluster showed no population substructure, and the temporal split of samples from La Digue obtained in the initial STRUCTURE run was no longer evident (Figure 3.3.)

As a result of the STRUCTURE analysis, four of the five individuals of unknown collection location (labelled only as coming from 'Seychelles') could be assigned with high probability (0.86-

0.93) to one of the two clusters. Only one specimen could not be assigned with confidence, most likely because the sample from this specimen only amplified at 3 loci. The individuals labelled as collected on Ile aux Fous were assigned to the Praslin/Aride cluster with high probability (0.97-0.98), and were therefore almost certainly collected on one of these two islands. The STRUCTURE assignment clusters and values for each individual are given in Supporting Information, SI 3.2.

Analysis of population structure in the historical dataset with a reduced data set of five loci showing the lowest mean genotyping error rates (<0.14 per allele) detected the same number of population clusters (ΔK=2; K=2) and very similar assignment of individuals to population clusters as the STRUCTURE analysis that used the full dataset of 11 loci (see SI 5, Figure 3.3 and Figure 3.4) indicating negligible effects of genotyping error rates on our STRUCTURE analyses.

Analysis of structure within the contemporary Seychelles paradise flycatcher population on La Digue and between the source La Digue population and the recently reintroduced Denis Island population revealed no detectable signal of structure (Figure 3.3).

3.3.4 Estimates of historical and contemporary N_e

Figure 3.5 gives the posterior distribution of the temporal change in historical and contemporary effective population size (N_e) from *tmvp* analyses for the populations on La Digue and Praslin. The density of points is proportional to the probability density of population size at the time of the oldest sample and the most recent sample. An off diagonal distribution therefore indicates a change in N_e. The resulting output for La Digue provides strong evidence for a severe decline in N_e across the past 134 years. The joint mode and 95% higher posterior density (HPD) limits for the marginal from the density estimation are N_e = 142 (95% HPD limits 69-300) for La Digue in 1879 and a contemporary N_e of just 28 individuals (95% HPD limits 16-57) for the same island population in 2010. An equivalent analysis for Praslin also provides evidence for a severe recent decline in N_e with a joint mode of historical N_e=778 (25% HPD limits 395-990) in 1877 and a N_e=179 (25% HPD limits 90-335) in 1907, the age of the most recent sample from Praslin. The temporal distribution of historical specimens from Praslin is such that they span a relatively short period from oldest to most recently-collected specimen (just 30 years, compared to 131 years on La Digue), with the majority of the Praslin samples collected in 1904. This short timeframe and temporal skew in sampling restricts the accuracy of estimates using *tmvp*, as is reflected by the broad probability density and low confidence limits. Consequently, the estimate of historical N_e for Praslin is accompanied by large HPD limits and should therefore be treated with caution. However, in the absence of a wider distribution of historical samples, this result provides the only available estimate of temporal change in historical N_e on Praslin and is one of a downward trajectory that reflects that observed on La Digue. Indeed, the signal of a historical decline in N_e on Praslin-and over such a short timeframe-agrees with the

population's actual trajectory on Praslin of precipitous decline to extirpation by the 1980's (Gerlach 1997).

3.3.5 Contemporary changes in genetic diversity upon reintroduction

In comparison to a significant loss of genetic diversity across the species' historical population bottleneck since 1877, a second bottleneck brought about by translocation of individuals to Denis Island in 2008 has resulted in a proportionately smaller loss of genetic diversity *per se* (Figure 3.2; Table 3.2). Four of 42 alleles present in the La Digue source population were not captured in the founder individuals of the Denis Island population. This loss of diversity represents a 9.5% reduction in the number of alleles across the bottleneck from contemporary source to reintroduced population, compared to a 59.5% reduction across the historical bottleneck. Mean allelic richness (A_R) remained stable at 2.6 in both the source and reintroduced populations, N_E decreased from 2.3 to 2.2 representing a 3.1% decrease from source to reintroduced population, H_E decreased by 3.5% from 0.54 in the source population to 0.53 in the reintroduced population, while Ho remained stable at just over 0.52. However these losses of genetic diversity occurred instantaneously upon selection of source individuals for reintroduction. Additionally this reduction of genetic diversity only represents the initial loss at founding of the translocated population and does not account for the subsequent effects of genetic drift within the newly establishing population. During the 22 months post-release study period, five of the 23 released individuals died without successfully reproducing, resulting in the loss of two further alleles from the Denis Island founder population and therefore a 14.3% loss of alleles (6/42) from the La Digue source population to the Denis founders. This provides a useful minimum estimate of loss of alleles of 5.3% (2/38) across the first generation of the reintroduced population. Though a minimum estimate, it is still a much higher rate of allelic loss than the 0.19% per generation observed across the historical decline

Thirteen of the 23 released individuals successfully produced F1 offspring during the same 22 month intensive monitoring period. Although they only represent a portion of the F1 generation on Denis (we were only able to sample the first seven F1 offspring of a total of 11 produced during the study, and further F1 offspring have been produced after this study ended), they can provide a useful indication of potential future effects and magnitude of drift on genetic diversity on Denis Island. Across 11 loci, four alleles were not transferred from La Digue to Denis Island and a further five were absent from the first seven F1 offspring. The total of 33 alleles amongst the seven F1 individuals sampled (note: representing only 64% of F1 individuals at that time and likely less than half of the complete F1 generation as more F1 offspring were produced after sampling ended) represents a potential loss of up to 21.4% of alleles compared to the La Digue source population and up to 13.2% loss of alleles in the single generation after founding of the population on Denis Island.

Finally the proportion of alleles actually transferred in the 23 founder individuals (90.5%) approximates closely the number expected from random selection. Figure 3.6 shows that selection of individuals for reintroduction based on their genetic make-up would have increased the proportion of alleles represented in the released individuals to 98%.

3.4 DISCUSSION

Our analysis of temporal patterns of genetic diversity in the Seychelles paradise flycatcher across the past 134 years provides an important perspective not only on the loss of genetic diversity that has occurred as a consequence of this species' steep historical decline, but also on the magnitude and nature of loss of genetic variation from the remnant contemporary source to the reintroduced population. Furthermore, the spatial structure detected within the historical samples that represent the historical population not only provides valuable insight into the evolutionary processes which have shaped this endemic island flycatcher, but also provides important information that can be used to guide long-term reintroduction strategy. Together, these results provide a framework in which to consider broader genetic issues in reintroduction biology.

3.4.1. Historical N_e and bottleneck shape

Our *tmvp* estimates of temporal N_e suggest that the Seychelles paradise flycatcher has endured a recent and severe population decline, with a total historical N_e of ~321 in the late 1800s being some ten-fold higher than the estimated contemporary N_e of ~28. The contemporary estimate derived from the genetic data aligns well with the current census population size of 218-290 individuals (Currie et al. 2003a), assuming an N_e/N ratio of 0.1 for most wildlife populations (this assumption is likely to hold true for the flycatcher population due to the recent fluctuations in population size and the species' skewed sex ratio; Frankham 1995). Additionally, the contemporary N_e also aligns closely to the lowest census size of 28 individuals in the 1960s (Gaymer et al. 1969). *Tmvp*-based estimates of N_e should be relatively robust to effects of genotyping errors from allelic dropout because the analysis uses information on alleles present rather than information on individual genotypes. However, if alleles are completely missing from the museum specimen dataset due to allelic dropout, then this could cause historical $N_{e.}$ to be underestimated. However our estimates of historical N_e obtained from *tmvp* align well with field records of historical flycatcher distributions and estimates of historical carrying capacity based on area of suitable habitat within the flycatcher's historical range supporting their authenticity.

The flycatchers have lost over half of their neutral genetic diversity (59.5% loss of alleles) across a population bottleneck driven by anthropogenic habitat changes, primarily forest loss and

introduced predators, which have occurred since human settlement in the 1770s. This historical loss of genetic variation is consistent with records documenting historical range contraction and population decline (Figure 3.2). Historically recorded on five islands, the species had disappeared from Aride, Félicité, and Marianne by the early 1900s and from Praslin by the 1980s (see Currie et al. 2003c) leaving the species restricted to La Digue (1000 ha) where the population had declined to ~28 individuals by 1965 (Gaymer et al. 1969). The shape of the species' bottleneck has therefore been one of steep decline to very low surviving numbers in only a single population, prior to a steady recovery to the current estimate of 218-290 individuals (Currie et al. 2003a).

Consistent with theoretical expectations for a rapid population decline, whereby N_A should decrease faster than H_O due to the preferential loss of rare alleles (Nei et al. 1975; Chakraborty & Nei 1977; Allendorf 1986), N_A fell dramatically across the flycatcher's 134 year bottleneck profile whereas H_O remained unchanged. However the significant levels of allelic dropout recorded in our historical museum genotypes (mean error rate of 0.14 per allele and 0.28 per locus) are likely to have resulted in an underestimation of historical H_O, masking any real decrease in H_O across the historical bottleneck which undoubtedly occurred in tandem with the observed decreases of all other standard measures of genetic diversity.

The estimate of historical N_e for the flycatcher is low when compared with *tmvp* estimates of N_e for other island endemics such as the Seychelles kestrel (*Falco araea*) (historical N_e of 387; Groombridge et al. 2009), Mauritius kestrel (*Falco punctatus*) (historical N_e of 957; Groombridge et al. 2009) and the Mauritius parakeet (*Psittacula echo*) (historical N_e of 964; Raisin 2010). Nevertheless, the modal estimate of N_e for La Digue is unusually well supported, as indicated by the tight HDP limits on both axes (Figure 3.5). The Seychelles kestrel has a much larger home range than the flycatcher; if the flycatcher's historical range matched that of the kestrel then a much higher historical N_e would be expected. One explanation for the observed situation is that the species always existed at a restricted population size/range, an idea supported by historical records which indicate that flycatchers were not widely distributed – for example, they have never been recorded on the largest island of Mahé (15,500 ha). Furthermore, our genetic estimate of historical N_e of ~321 is broadly in line with an historical census size of c.2400 individuals calculated from availability of suitable habitat within flycatcher's recorded historical range, assuming an N_e/N ratio of 0.1 (Frankham 1995). Alternatively, considerable decline may have occurred prior to collection of the historical specimens, meaning our estimate of historical N_e is an under-estimate. Human colonisation of Seychelles began in the 1770s and thereafter forest clearance was fairly rapid for plantation agriculture (Sauer, 1967; Procter 1984), so our estimated historical N_e in 1877 may represent the population in mid-decline. However, the same applies to the Seychelles kestrel, Mauritius kestrel and Mauritius parakeet, all of which experienced similar or lengthier periods of habitat loss (Mauritius was colonised over 100 years

before the Seychelles) prior to collection of historical museum specimens. Consequently, the most likely explanation is that the flycatcher did indeed have a restricted range, reflected in the relatively low historical N_e obtained by this study.

3.4.2. Loss of historical genetic structure

Our STRUCTURE analysis has revealed that the historical flycatcher population exhibited genetic structure between different island populations, but that following substantial population decline and localised extinction this structure has been lost. Each of the two identified clusters, La Digue and Praslin/Aride had 16 private alleles representing 20.3% (16/79) private alleles between the two clusters. Once different time windows were accounted for, there was no historical genetic substructure within islands; the genetic structure detected within the historical samples collected from La Digue was likely due to genetic drift during the minimum of 50 years (25 generations) between the collection dates of the samples within each cluster. No genetic structure exists within the remnant La Digue population or between it and the introduced Denis Island population. Historical genetic structure may have promoted the retention of higher net levels of genetic diversity across the species as a whole than if the population was panmictic. Nichols et al. (2001) attributed the considerable levels of genetic diversity maintained in the historical population of Mauritius kestrel relative to their island population size to the fact that historically the population was structured, with restricted gene flow between subpopulation clusters around mountain ranges in Mauritius. Such patterns of genetic structure across island archipelagos do however expose these populations to the risk of losing high levels of genetic diversity when divergent island populations go extinct, as our study shows. Species-level allelic diversity within the flycatcher was reduced by 20.3% with the extinction of flycatchers from Praslin and Aride.

The fact that we were able to obtain very similar STRUCTURE results with a reduced dataset of the five loci showing the lowest allelic dropout suggests that our results are reasonably robust to the effects of allelic dropout (SI 5, Figure 3.3, Figure 3.4). This finding is consistent with a study on grasshopper population genetic structure by Manrique-Poyato et al. (2013) who found that STRUCTURE analysis based on data from loci with allelic dropout rates higher than 30% gave very similar results for both ΔK and the assignment of individuals to populations, as a second STRUCTURE analysis on a reduced dataset including only markers with low or no allelic dropout, suggesting that allelic dropout has a negligible effect on this type of analysis.

3.4.3. Relative impacts of historical versus reintroduction bottlenecks

Our finding of a loss of 9.5% of neutral genetic diversity from the source to reintroduced population, compared to the much larger 59.5% historical loss of diversity, is consistent with similar

studies restricted to a handful of other species. Taylor et al. (2007) found that contemporary populations of South Island saddlebacks (*Philesturnus c. carunculatus*) had retained only 25% of alleles at neutral microsatellite loci in historical pre-bottleneck populations, while subsequent reintroductions retained 90% of alleles from the source population, concluding that genetic variation may depend more on the source population's genetic past than on reintroduction bottlenecks. Furthermore, Taylor & Jamieson (2008) found that serial 2nd and 3rd order reintroductions of this species caused no further loss of genetic diversity. Biebach & Keller (2009) found that initial reintroduction-induced bottlenecks lost significantly more genetic diversity than subsequent serial reintroductions of alpine ibex (*Capra ibex ibex*). Wisely et al. (2002) found that the black-footed ferret (*Mustela nigripes*) bottleneck that almost exterminated the species in the 1980's caused the loss of c.40% of alleles at microsatellite loci. Additionally Wisely et al. (2008) found that post-bottleneck reintroductions of black-footed ferrets maintained levels of genetic diversity similar to the source population when they grew rapidly or were supplemented with additional released individuals yearly. However when a reintroduced population did not rapidly increase in numbers and was not regularly supplemented with additional releases, it resulted in a further significant loss of genetic diversity (4 of 14 (28%) alleles lost) measured 10 years (c.5 generations) post-release.

Post release, reintroduced populations are subjected to the effects of genetic drift, an insidious mechanism for loss of diversity illustrated by Wisely et al. (2008) and also by our study. Since the release of the 23 flycatchers onto Denis Island, several individuals have died without successfully breeding while other individuals have bred with disproportionate success. Therefore, to examine the potential effects of drift we measured (*i*) a conservative estimate of the amount of genetic diversity lost across the first generation and (*ii*) the amount of genetic diversity present in the first seven F1 offspring produced on Denis Island.

As a minimum estimate of loss of genetic diversity in the first generation, only alleles unique to individuals that died without breeding during our study period are counted as lost from the population which would result in a 5.26% loss of alleles in one generation. This minimum estimate of loss of alleles across one generation is still much higher than the rate of ~1.19% loss of alleles per generation across the historical bottleneck. While the total genetic diversity lost across the historical bottleneck was greater, the rate of loss has been higher across the contemporary bottleneck. If sustained across multiple generations it could result in a loss of genetic diversity of similar magnitude to that observed as a result of the historical bottleneck. However we would not expect the loss of genetic diversity in subsequent generations to be as great per generation as across the first generation because high initial losses are common during reintroductions due to both the stress of the transfer itself and as the new population adjusts to its new environment.

Secondly we measured the amount of genetic diversity present in the first seven F1 offspring produced on Denis Island. Those offspring possess only 79.6% of the neutral genetic diversity of the La Digue source population, representing a potential worst-case scenario of 21.4 % potential loss of diversity compared to the La Digue source population and potentially 13.1% less diversity than contained collectively within the 23 founders. From field surveys of the reintroduced population at that time, those seven sampled offspring represented 64% of the F1 generation and additional offspring were produced after the end of this study, therefore additional diversity will have remained undetected. Therefore, this worst-case estimate of loss across the first generation is certainly an over-estimation. However, it does provide an indication of the potential erosion of genetic diversity across subsequent generations. Indeed, given the overwhelming evidence that genetic diversity is vital for minimising inbreeding and for maximising a species' potential for long term adaptive evolution, appropriate management to minimise loss of genetic diversity in reintroduced populations remains an important consideration for wildlife managers.

This study has measured genetic variation at selectively neutral microsatellite loci, whereas adaptive genetic diversity is likely to be more important for both the short and long term survival of reintroduced populations. In a recent comprehensive meta-analysis quantifying the effects of bottlenecks on MHC polymorphism, Sutton et al. (2011) showed a positive correlation between MHC polymorphism and neutral diversity, with ~15% greater loss of adaptive MHC diversity than neutral genetic diversity across bottlenecks, and concluded that conservation managers could interpret patterns of neutral genetic diversity as conservative estimates of the true loss of functional gene diversity across bottlenecks. Therefore the level of loss of neutral genetic variation we have documented here for the flycatcher, rather than being viewed as a poor surrogate, might cautiously be viewed as a conservative estimate of loss of functional genetic variation.

3.4.4 Considerations for future management of flycatchers

In an ideal reintroduction system, the long-term aim is to produce a self-sustaining population able to maintain sufficient levels of genetic variation to be able to adapt to future environmental change. Given the bottleneck experienced by the flycatcher population to a low of 28 individuals in the 1960's (Gaymer et al. 1969) and the accompanying loss of genetic structure, the current flycatcher population has unprecedentedly low genetic diversity and potentially compromised adaptive potential. In contrast, the historically structured population that once existed between the different Seychelles islands may have promoted a higher net level of genetic diversity than a panmictic population. These findings pose a dilemma for conservation managers; whether to manage the remaining source and reintroduced populations in isolation so as to promote genetic differentiation and the return of genetic structure over time, or to manage them as a single population in order to maximise retention of genetic diversity over the shorter term? Our study indicates that while there was genetic structure between

island populations historically, there was also some gene flow. Separate populations on Aride and Praslin clustered together to form one subpopulation and assignment of individuals to their respective populations showed a minority of individuals to be clearly admixed. Additionally two museum specimen individuals labelled as sampled on La Digue fell into the Praslin cluster with assignment values of >0.75 (see Supporting Information, SI3.2); assuming no error in labelling of these specimens, this indicates that those individuals, or a recent ancestor, originated from Praslin. Additionally, the five islands that comprised the flycatcher's historical range (Figure 3.1) are neighbouring islands with distances of only a few kilometres between them, whereas the contemporary populations on La Digue and Denis are approximately 52 km apart, making natural gene flow unlikely. Therefore, we advocate (i) management of the two current island populations as one in order to maximise retention of genetic diversity in both populations over the shorter-term, (ii) further reintroductions in order to increase both the species' distribution range and population size in the medium-term, and (iii) a longer-term strategy to reduce management and allow this network of reintroduced island populations to undergo the natural processes of gene flow and drift.

3.5 CONCLUDING REMARKS

Our temporal and spatial analysis of the flycatcher across 134 years of population decline, initial recovery and through a subsequent reintroduction-induced bottleneck has highlighted the importance of both historical and contemporary perspectives in guiding threatened species management and reintroduction strategy.

This study has shown (i) that the Seychelles paradise flycatcher has lost substantial genetic diversity and genetic structure across the historical habitat loss-induced bottleneck, (ii) that the flycatcher introduction-induced bottleneck has left a smaller but unignorable reintroduction signature, and (iii) that in fact genetic diversity appears to have been lost at a faster rate per generation in the 22 months post release than estimates of per generation loss during the historical bottleneck. Future genetic studies of the reintroduced population on Denis Island would be insightful in order to quantify the longer-term magnitude and effects of genetic drift on this island population relative to the species' historical loss of genetic diversity.

We conclude that severe historical bottlenecks leave a large footprint in terms of sheer quantity of genetic diversity lost. However, severely depleted genetic diversity following a historical bottleneck does not render a species immune to further genetic erosion upon reintroduction, a valuable perspective for conservation managers tasked with formulating reintroduction strategies. In some cases the loss of genetic diversity per generation can, initially at least, be greater across contemporary

reintroduction-induced bottlenecks than loss per generation sustained across severe historical bottlenecks.

Finally since the genetic consequences of reintroduction-induced bottlenecks are not yet widely documented in natural systems, we advocate more widespread genetic monitoring of wildlife reintroductions alongside characterisation of historical trajectories of genetic diversity.

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3.6 LIST OF FIGURES AND TABLES

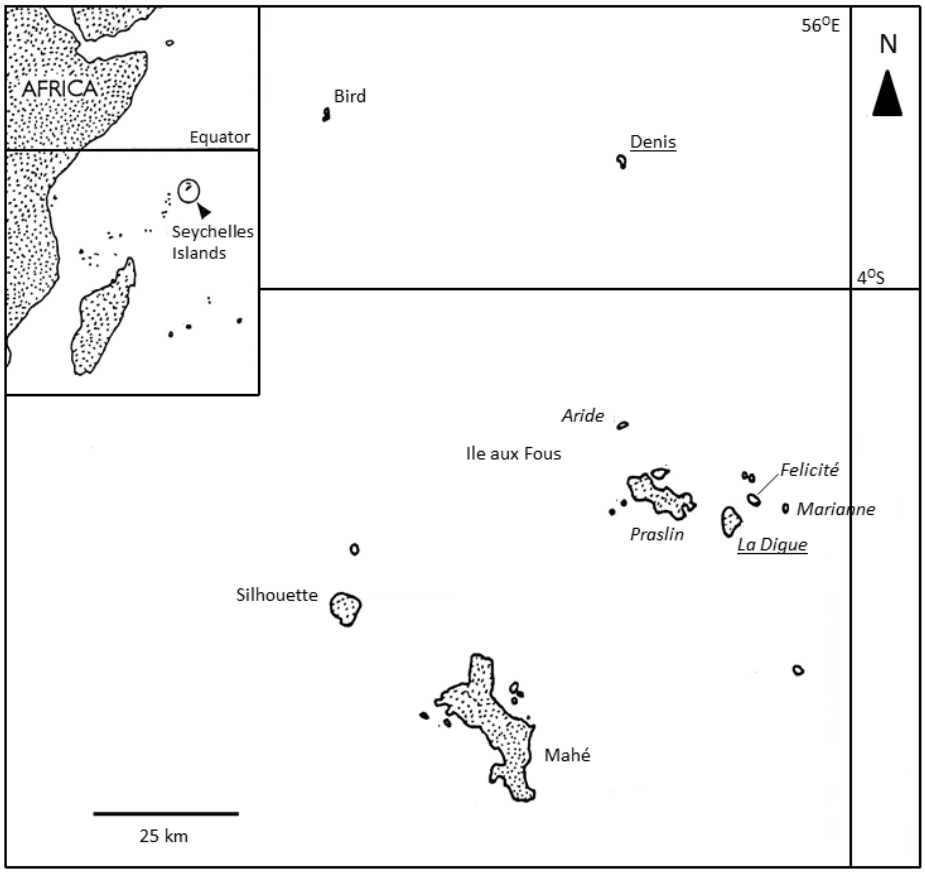

Figure 3.1 Map of the Seychelles showing the historical (*italics*) and current (underlined) distribution of the Seychelles paradise flycatcher.

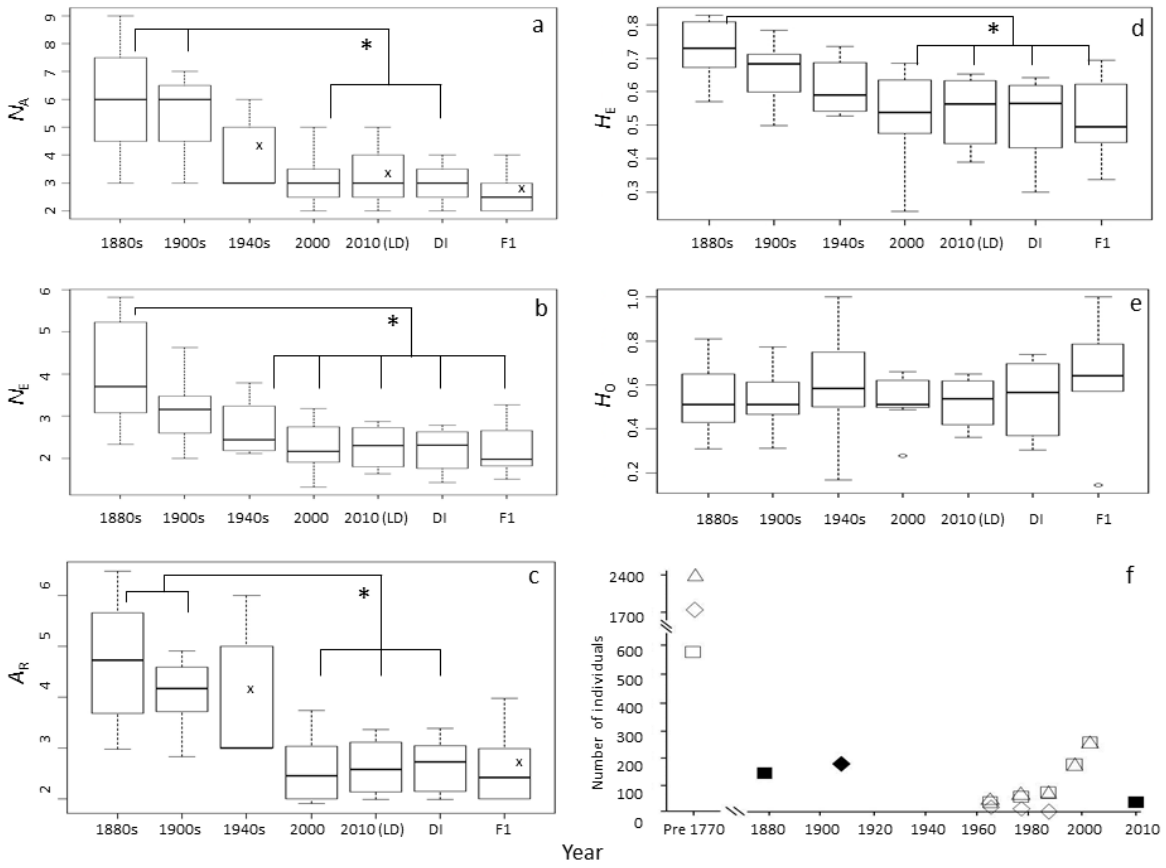

Figure 3.2 Temporal changes in standard measures of genetic diversity and population size.

Temporal changes in; (a) mean number of alleles per locus (N_A), (b) mean number of effective alleles per locus (N_E), (c) mean allelic richness per locus (A_R), (d) mean expected heterozygosity per locus (H_E), (e) Mean observed heterozygosity per locus (H_O), for five different time periods: 1877-1888 ('1880s', *n*=21); 1904-1907 ('1900s', *n*=22); 1940-1955 ('1940s', *n*=6); 1999-2001 (2000, *n*=83); and 2007-2010 (LD, *n*=80), and two contemporary populations; La Digue (LD, as above) and Denis Island (DI), and the first F1 offspring of the Denis Island reintroduced population (F1). x=not included in one-way ANOVA tests of significance as data not normally distributed. Boxplots show median (thicker horizontal line), upper and lower quartiles (top and bottom of box respectively), maximum and minimum values (top and bottom of whiskers respectively, unless outlying values are present-represented by empty circles). The lines indicate significance*; (f) changes in population size of Seychelles paradise flycatchers over time for the total population (triangles), Praslin only (diamonds) and La Digue only (squares); hollow shapes are population census estimates (*N*), solid shapes are genetic estimates of effective population size (N_e).

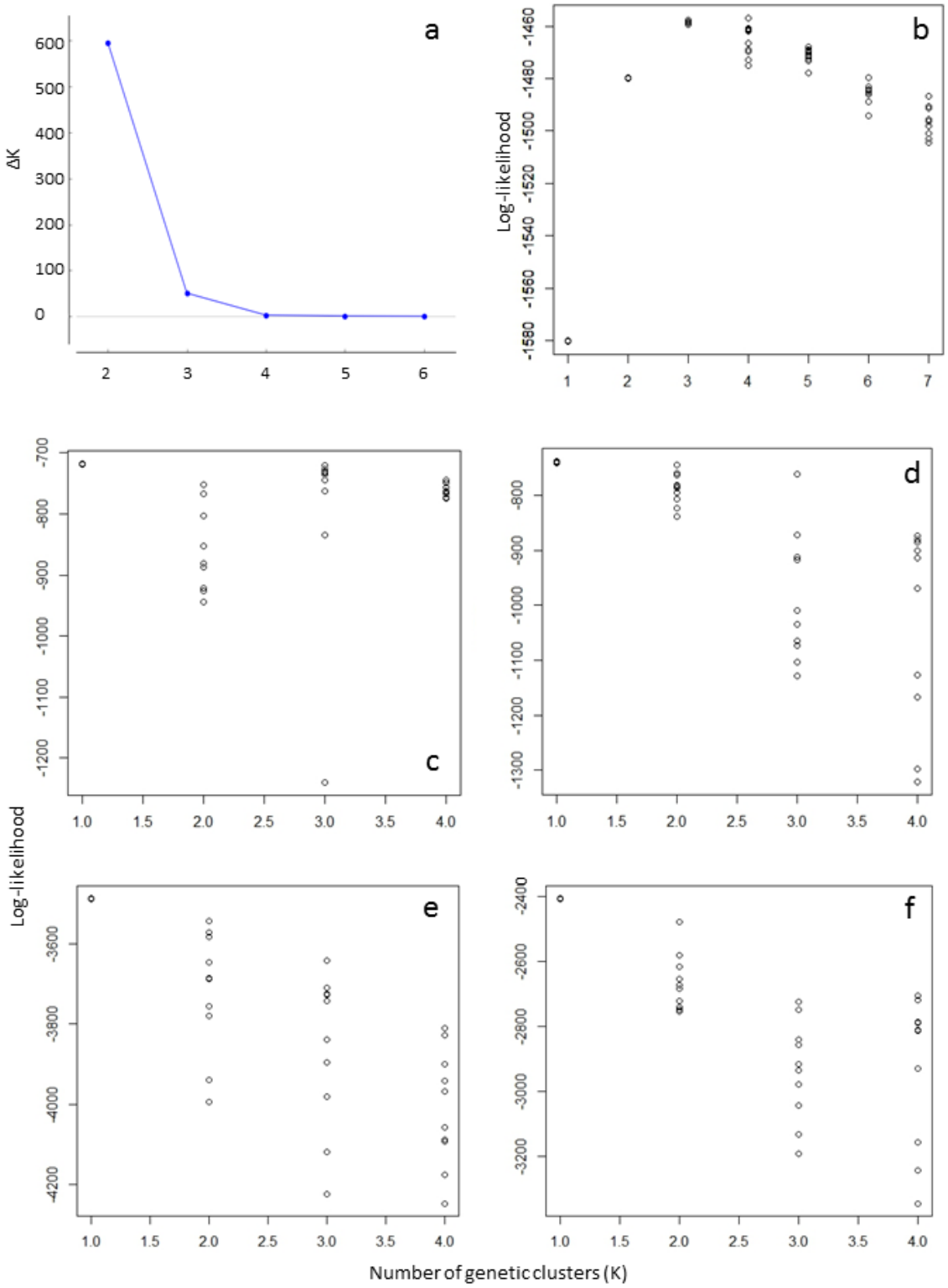

Figure 3.3 Number of inferred genetic clusters within the Seychelles paradise flycatcher historically and contemporaneously based on the STRUCTURE algorithm.

The most probable number of inferred genetic clusters (*K*) for: (a) Evanno ΔK statistic showing the rate of change in the log probability between successive values of *K*, indicating the highest level of genetic structure within the whole historical dataset was ΔK=2; (b) posterior estimates of log-likelihood values (10 independent runs for each value of *K*) for the whole historical sample dataset indicating most probable number of genetic clusters (*K*)=3; (c) posterior estimates of log-likelihood values (10 independent runs) for the historical La Digue genetic cluster indicating no genetic substructure on La Digue historically (most probable value of *K*=1); (d) posterior estimates of log-likelihood values (10 independent runs) for the historical Praslin-Aride genetic cluster indicating no genetic substructure within the Praslin-Aride flycatcher population historically (most probable value of *K*=1); (e) posterior estimates of log-likelihood values (10 independent runs) for the contemporary La Digue flycatcher samples indicating no contemporary genetic structure on La Digue (most probable value of *K*=1); (f) posterior estimates of log-likelihood values (10 independent runs) for the whole contemporary dataset including all samples from both La Digue and Denis indicating no genetic structure within the contemporary flycatcher population between La Digue and the introduced subpopulation on Denis (most probable value of *K*=1).

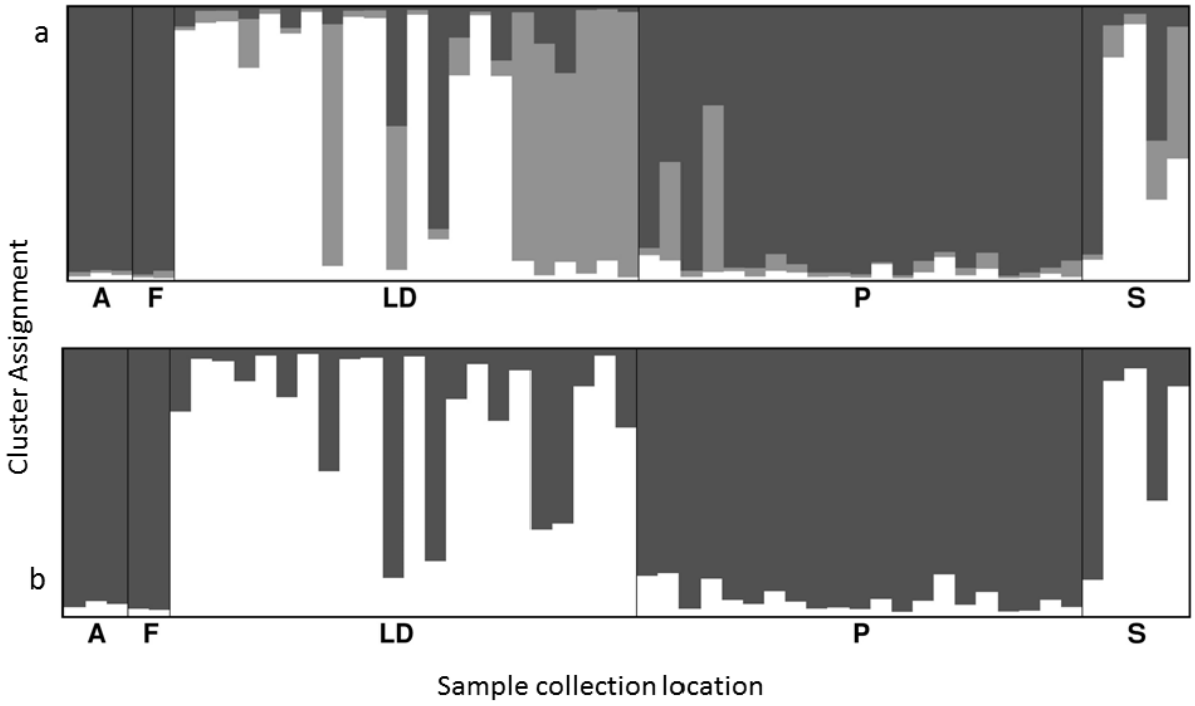

Figure 3.4 Bar plot showing the most probable number of genetic clusters (*K*) within the ancestral population of the Seychelles paradise flycatcher based on the STRUCTURE algorithm.

All historical samples were included in a model assuming admixture and uncorrelated allele frequencies between clusters; (a) barplot of *K*=3 showing the temporal split within the La Digue population (LD), and (b) assignment of individuals when *K*=2, the most realistic number of spatially distinct clusters. Populations were pre-defined based on sampling location Aride (A), Fous (F), La Digue (LD), Praslin (P) and unknown sampling location (S). Samples within each pre-defined population (based on sampling location) are listed in order of collection date. Each bar represents an individual museum sample (*n*=53); the last 6 bars within the La Digue samples are the six individuals sampled between 1940-1955. The proportion of each individual assigned to a genetic cluster is indicated by bar colour.

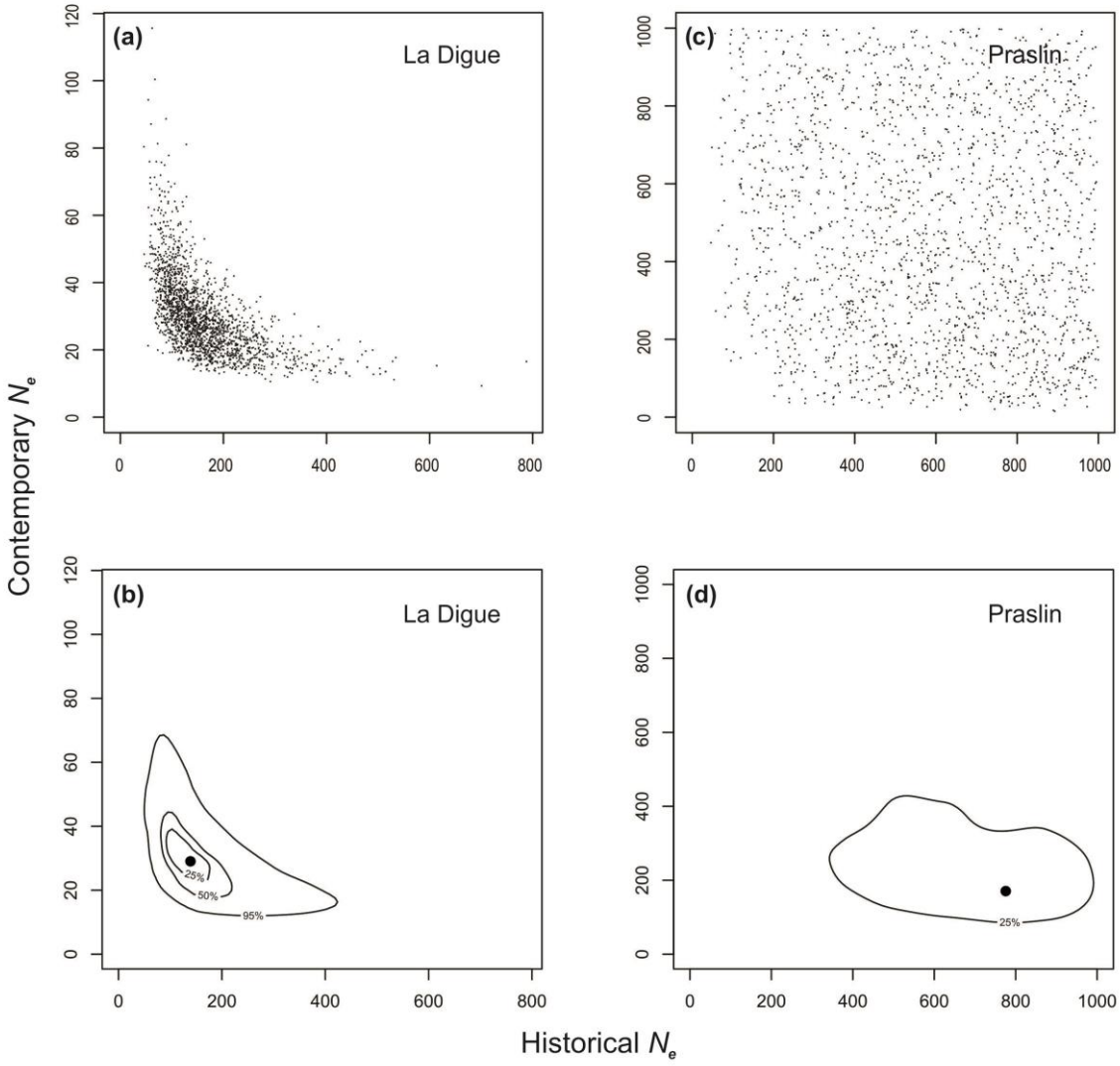

Figure 3.5 *Tmvp* estimates of historical and contemporary effective population sizes.

Tmvp outputs of the posterior distribution of the historical and contemporary effective population size (N_e) for the Praslin and La Digue Seychelles paradise flycatcher populations following the methods of Beaumont (2003). The density of points is proportional to the probability density of population size at the time of the oldest sample and the most recent sample. The joint mode is plotted as a single solid black circle. Circles indicate density limit of posterior distribution: La Digue 25-95%, Praslin 25%.

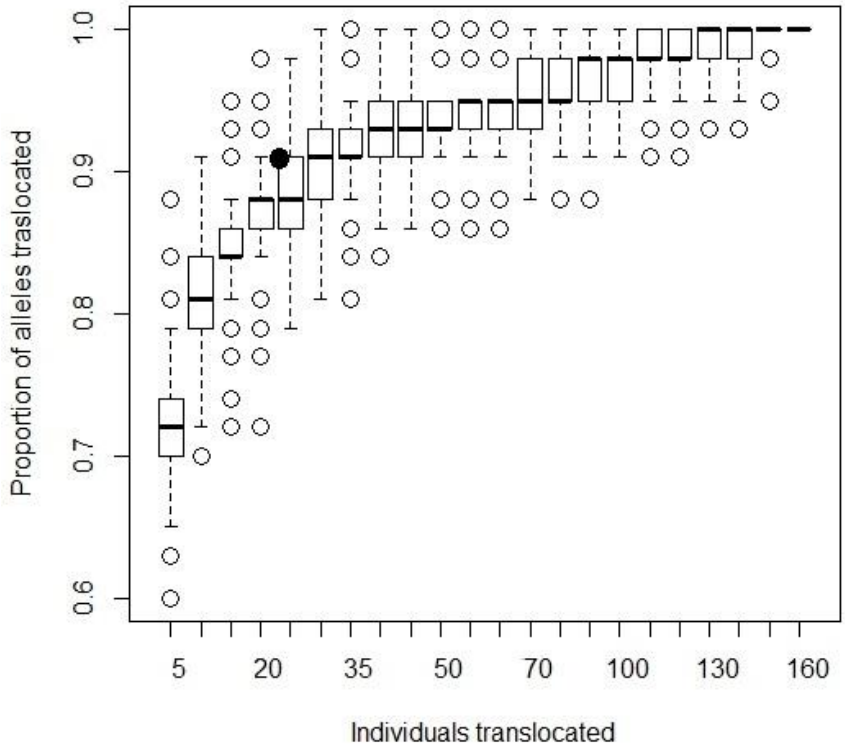

Figure 3.6 Proportion of alleles in the La Digue source population of Seychelles paradise flycatchers that could be captured by translocating different numbers of individuals.

Boxplots show median, 1st and 3rd quartiles and minimum and maximum values obtained in the 1000 replicate runs for each number of individuals translocated. Solid black circle indicates the actual proportion of alleles present in the La Digue source population that were present in the 23 individuals released on Denis Island.

Table 3.1 Characterisation of fourteen autosomal Seychelles paradise flycatcher *Terpsiphone corvina* microsatellite loci‡

‡All loci are autosomal based on the presence of a proportion of heterozygotes in male and female Seychelles paradise flycatchers.

Ŧ chromosome locations were assigned based on a WU-BLAST of the zebra finch genome against the Genomic sequence (hard masked) database using a BLASTN search with the distant homologies settings (http://www.ensembl.org/Multi/blastview). Locations were assigned for sequences with a BLAST hit with an E-value better than E-05 (except for *Tcor-12*) and when the strength of the next nearest hit was E-05 or weaker. *Tcor-006* had an additional hit of similar strength to the zebra finch Z chromosome but when checked in the chicken genome only the chromosome 3 hit was observed and additionally this locus was known to be autosomal based on the presence of heterozygotes in male and female Seychelles paradise flycatchers.

¥, the homology of *Tcor-002* to the unknown chromosome sequence is suspected to be an artefact of the assembly process (based on the identical sequence composition).

Ta, annealing temperature.

Tm, primer melting temperature, primer3 (Rozen & Skaletsky 2000).

n, number of unrelated individuals genotyped from La Digue Island, Seychelles.

*based on the allele sequenced of the individual used to create the microsatellite library (adult female, reference number 42 sampled on La Digue Island in March 2008).

Ho, observed heterozygosity.

He, expected heterozygosity.

pHWE, Hardy–Weinberg equilibrium test *P* value as identified by GenePop version 3.4 (Raymond & Rousset 1995).

Locus	EMBL accession number and clone name	Zebra finch chromosome ₸	Chromosome location (bp) ₸	ENSEMBL WU-BLAST (P)N ₸	Repeat motif	Fluoro-label	Primer sequence (5'-3')	Tm (°C)	Ta used (°C)	n	Expected allele size (bp)*	Observed allele sizes and genotype of individual used to make library (bp)	Ho	He	pHWE	Estimated null allele frequency
Tcor-001	HE971749	Tgu1A	44904778	7.2E-77	TGATA TA TGATG	6-FAM	F: CCACAAACATCATGCACAGAG	60.2	60	24	115	104, 114	0.46	0.51	0.69	0.04
	33_A02				$(TGATA)_{10}$		R: ATCTCAGCCCAGGAAACTCC	60.6				(104, 114)				
Tcor-002	HE971750	Tgu2 &	14146987	2.3E-35	$(GT)_3$ AT $(GT)_3$ GC GT	6-FAM	F: GAACAGAGTGTGTATGTGTGTGC	58.2	60	25	148	127, 136, 148	0.64	0.56	0.90	-0.08
	33_B06	Chr.Unk¥	149325404	7.8E-32	GC $(GT)_{19}$ GA $(GT)_2$		R: TGGGCACTAATAGGAACAGAAC	58.3				(148, 148)				
Tcor-003	HE971751	Tgu2	154982928	1.5E-29	$(TTCC)_{32}$	HEX	F: GAGAGGCTTGCAAGGAAGTATG	60.4	60	25	268	249, 253, 273, 277, 285, 293, 305	0.76	0.66	0.70	-0.10
	33_D08						R: GATCACTCCGCAATTCCATC	60.4				(273, 277)				
Tcor-004	HE971752	Tgu2	95826308	1.6E-45	$(GATA)_{13}$	HEX	F: CTGTGTGAACAGTTGCAGGTC	59.4	60	25	159	149, 162	0.52	0.48	1	-0.05
	33_E11						R: GCACAGCCTTCCATCTCTATG	59.9				(162, 162)				
Tcor-005	HE971753	Tgu8	25430903	4.8E-50	$(TG)_{16}$	6-FAM	F: TCAATGATGCTGATGCTATTG	57.3	60	24	160	162, 164	0.25	0.22	1	-0.06
	33_H04						R: ACTTTGGTTTCTACCTGAATGG	57.3				(162, 162)				
Tcor-006	HE971754	Tgu3 &	98398920	9.4E-23	$(TATC)_{17}$	6-FAM	F: CAGAATGGCATCTTCATTTGG	60.5	60	25	245	228, 240, 244, 248, 252, 256	0.68	0.75	0.72	0.04
	33_H09	TguZ	2123977	8.8E-22			R: CCCAGGCTCAGCAGAATAAG	60.0				(244, 244)				
Tcor-007	HE971755	No hits	Autosomal‡		$(GATA)_{15}$	6-FAM	F: TGGAATGCTAGTTAGGCAAGC	59.5	60	25	174	170, 174, 178	0.76	0.65	0.72	0.09
	34_C02						R: CTGCAGCTGGTACTAGGAAGG	59.2				(170, 174)				
Tcor-008	HE971756	Tgu1A	20529720	9.6E-31	$(TATC)_{11}(TA)_2TATC$	HEX	F: TATTGGGAGCATCAGGAGTTG	60.1	60	25	142	141, 151	0.32	0.33	1	0
	34_C05				$(TA)_2$ $(TC)_3$ $(TATC)_2$		R :CTTGAACCCAAGGTGCTTATTC	60.0				(141, 151)				
Tcor-009	HE971757	Tgu3	59269119	2.5E-23	$(TATC)_2$ TTTC	6-FAM	F: AGGCTGGTTCATCTGTCTGTC	59.3	60	25	138	141, 162, 174	0.56	0.55	0.74	0
	34_D01				$(TATC)_{11}$		R: GCATGTTTGTGGGTATCTGAAG	59.5				(141, 174)				
Tcor-010	HE971758	Tgu13	6533839	8.4E-77	$(TATC)_{17}$	6-FAM	F: CTCCTTGTTCTCCCTCTCTCC	59.4	60	25	125	120, 124, 132, 136	0.72	0.67	0.93	-0.05
	34_E02						R: TTTCTCCCAAATCTGGAAACTC	59.6				(120, 124)				
Tcor-011	HE971759	Tgu1	45364555	4.9E-44	$(TATC)_{13}$	6-FAM	F: CAGCATGAAGTTAAATGAGGAAAG	59.4	60	25	217	217, 224	0.36	0.39	0.64	0.03
	37_B08						R: TGGATATTCGGGTGTCTGTTC	59.8				(217, 217)				
Tcor-012	HE971760	Tgu12	8774113	3.5E-05	$(TAGTG)_{17}$	HEX	F: AGTGTTGGACTGGGCATTG	59.5	60	25	133	82, 129, 134	0.68	0.53	0.16	-0.14
	37_E09						R: CTGCCGTGGACAAGGATAC	59.1				(82, 134)				
Tcor-013	HE971761	Tgu2	73599724	1.8E-21	$(TC)_8$ TT $(TC)_{20}$	HEX	F: TGAAACAAATCTCTTAGCCCTTG	59.8	60	25	123	121, 127	0.44	0.46	1	0.01
	37_F05						R: GCCACTGCCATTTCACAAC	60.1				(121, 121)				
Tcor-014	HE971762	Tgu8	18044505	4.9E-36	$(GT)_{11}$	HEX	F: AGTGCAGAGCAGCTTGTATAGC	59.0	60	25	107	106, 112	0.60	0.51	0.44	0.09
	37_F10						R: CAGGCAGGCAACTCACTG	59.1				(106, 112)				

Table 3.2 Pairwise matrix of Nei's genetic distance (corrected for population size) above of the diagonal and F_{ST} values below the diagonal for five different time-periods and two contemporary populations; La Digue (LD), and Denis Island (DI), and the first F1 offspring of the Denis Island reintroduced population (F1). Significance of pairwise F_{ST} values is indicated by*.

	1880s	1900s	1940s	2000	2010(LD)	DI	F1
1880s		0.281	0.337	0.405	0.407	0.423	0.365
1900s	0.098*		0.397	0.461	0.448	0.470	0.380
1940s	0.105*	0.153*		0.101	0.099	0.082	0.076
2000	0.216*	0.250*	0.086*		0.003	-0.005	0.016
2010(LD)	0.209*	0.238*	0.079*	0.002		-0.011	-0.005
DI	0.202*	0.238*	0.070*	-0.004	-0.010		-0.005
F1	0.149*	0.178*	0.054	0.016	-0.004	-0.001	

Table 3.3 Mean genotyping error rates in the historical museum samples calculated per allele and locus from repeat genotyping of individuals. Shading indicates loci used to test the effects of allelic dropout on historical STRUCTURE analyses.

locus	**error rate per allele**	**error rate per locus**	**observed allele size range**	**proportion of individuals that totally failed to amplify**
Tcor-001	0.10	0.19	93-114	0.06
Tcor-002	0.22	0.43	127-156	0.15
Tcor-004	0.11	0.22	145-162	0.42
Tcor-005	0.13	0.25	155-170	0.34
Tcor-007	0.29	0.58	164-186	0.30
Tcor-008	0.21	0.43	133-159	0.11
Tcor-009	0.14	0.28	141-174	0.08
Tcor-010	0.14	0.27	115-140	0.02
Tcor-012	0.12	0.24	82-134	0.04
Tcor-013	0.03	0.07	116-127	0.00
Tcor-014	0.06	0.13	106-112	0.02
mean	**0.14**	**0.28**		**0.14**

3.7 SUPPORTING INFORMATION

SI 3.1 Seychelles paradise flycatcher microsatellite-enriched genomic library development methods.

Microsatellite loci were isolated from a Seychelles paradise flycatcher microsatellite-enriched genomic library produced by the NERC Biomolecular Analysis Facility (Sheffield, UK) using genomic DNA from a single female Seychelles paradise flycatcher sampled on La Digue in 2008. The library was constructed following the enrichment approach of Armour et al. (1994) using modifications suggested by Gibbs et al. (1997) and enriched for the following seven nucleotide microsatellite motifs: $(GT)_n$, $(TC)_n$, $(TATC)_n$, $(CTTC)_n$, $(AGAT)_n$, $(AACT)_n$, $(TAGTG)_n$ and their complements, which had been denatured and bound to magnetic beads following Glenn and Schable (2005). Enriched fragments were ligated into *Bam*HI-digested, CIP-dephosphorylated pBluescript SK+ (Stratagene) and transformed into *E. coli*. Plasmid DNA was isolated from clones. Fragments were Sanger sequenced in both complementary directions, and a consensus sequence created. Microsatellite sequences were checked for uniqueness using BLASTn v.2.2.4 (Altschul et al. 1997). A total of 245 unique sequences were isolated (EMBL accession numbers HE971749-HE971993). Thirty microsatellite-containing sequences were selected for the design of PCR primer sets using PRIMER3 software (Rozen & Skaletsky 2000) and labelled with a fluorescent dye (6-FAM or HEX).

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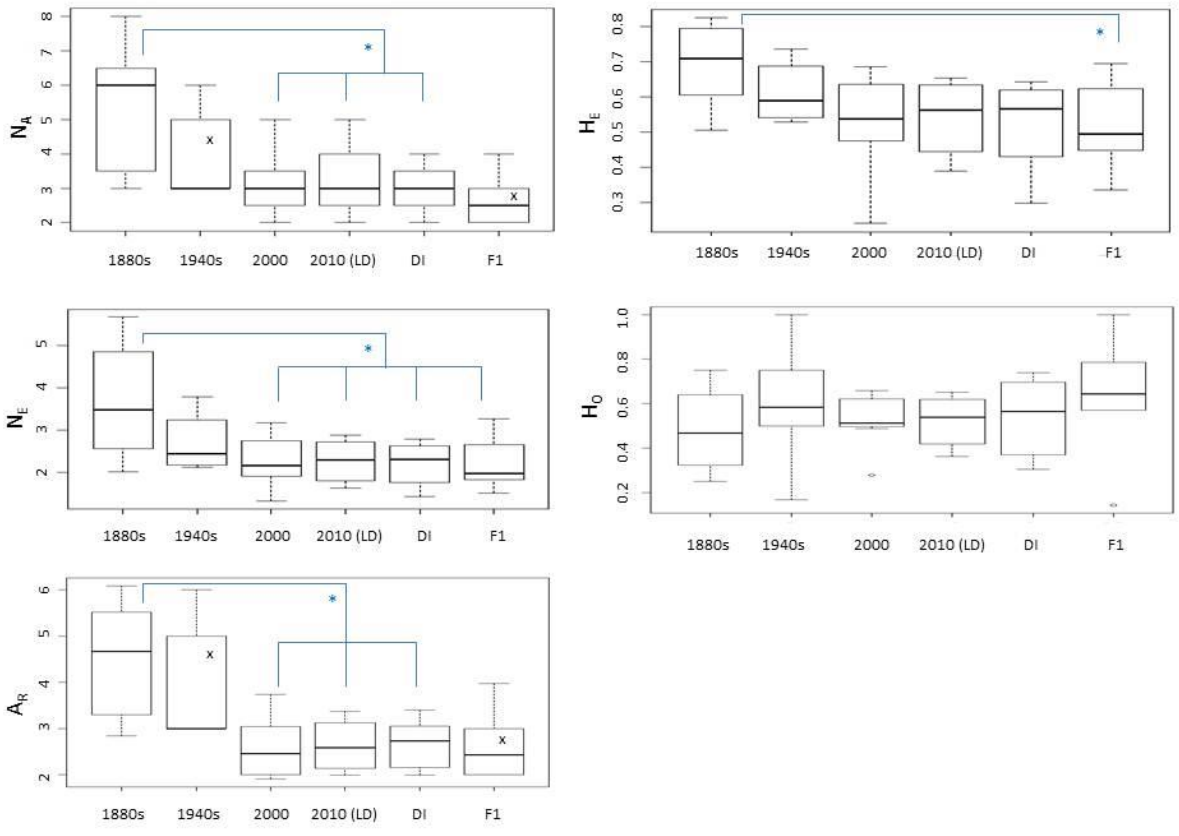
SI 3.2 Details of all museum samples used in this study

Study ID	Museum	Catalogue #	Collector	Year Collected	Collection Location	Sex	STRUCTURE assigned cluster	STRUCTURE assignment value
5001	Liverpool	1980.119				Male	La Digue	0.8809
5002	Liverpool	T3995	Warry	1879	La Digue	Male	La Digue	0.7647
5003	AMNH	652443	Thibault	1904	Praslin	male	Praslin	0.939
5004	AMNH	652438	Thibault	1904	Praslin	male	Praslin	0.9553
5005	NHM Tring	1955.63.2	Ridley & Percy	1955	La Digue	male	La Digue	0.9761
5006	NHM Tring	1946.75.15a	Sapsworth & Goodfellow	1940	La Digue	male	La Digue	0.9203
5007	Liverpool	1980.117				Male	La Digue	0.9288
5008	Liverpool	1980.118a		1880		Male	Praslin	0.8629
5009	AMNH	652461					*Praslin	0.5681
5010	AMNH	652452	Thibault	1905	Praslin	female	Praslin	0.9572
5011	AMNH	652439	Thibault	1904	Praslin	male	Praslin	0.9057
5012	AMNH	652455	Thibault	1907	Aride	male	Praslin	0.9661
5013	NHM Tring	1946.75.13a	Sapsworth & Goodfellow	1940	La Digue	female	*Praslin	0.6745
5014	NHM Tring	1927.12.18.387	Lister	1888	La Digue	male	La Digue	0.812
5015	NHM Tring	1988.21.9	Lantz	1877	Praslin	male	Praslin	0.8476
5016	NHM Tring	1887.12.30.1086	Tristram/Warry	1880	La Digue	male	La Digue	0.8194
5017	NHM Tring	1946.75.12a	Sapsworth & Goodfellow	1940	La Digue	female	*Praslin	0.6528
5018	Liverpool	1980.118	Warry	1880	La Digue	Female	La Digue	0.9831
5019	Liverpool	T3998	Warry	1879	La Digue	male	La Digue	0.9647
5020	AMNH	652459	Thibault	1907	Ile aux Fous	female	Praslin	0.9731
5021	AMNH	652450	Thibault	1904	Praslin	female	Praslin	0.9449
5022	AMNH	652442	Thibault	1905	Praslin	male	Praslin	0.9109
5023	AMNH	652449	Thibault	1904	Praslin	female	Praslin	0.9715
5024	AMNH	652445	Thibault	1905	Praslin	female	Praslin	0.9822
5025	NHM Tring	1881.11.14.1	Tristram/Warry	1880	La Digue	male	*La Digue	0.5459
5026	NHM Tring	1946.75.14a	Sapsworth & Goodfellow	1940	La Digue	female	La Digue	0.8597
5027	NHM Tring	1895.5.1.262	Tristram/Warry	1880	La Digue	male	La Digue	0.9631
5028	Liverpool	T3996	Warry	1879	La Digue	Male	La Digue	0.9557
5029	Liverpool	T3997	Warry	1879	La Digue	Male	La Digue	0.8796
5030	AMNH	652457	Thibault	1907	Aride	female	Praslin	0.9444
5031	AMNH	652440	Thibault	1904	Praslin	male	Praslin	0.9679
5032	AMNH	652454	Thibault	1905	Praslin	female	Praslin	0.98
5033	AMNH	652447	Thibault	1904	Praslin	female	Praslin	0.9734
5034	AMNH	652448	Thibault	1904	Praslin	female	Praslin	0.9345
5035	NHM Tring	1927.12.18.390	Lister	1888	La Digue	female	La Digue	0.9451
5036	NHM Tring	1927.12.18.389	Lister	1888	Praslin	female	Praslin	0.973
5037	NHM Tring	1927.12.18.386	Lister	1888	La Digue	male	La Digue	0.7314
5038	Liverpool	T3999	Warry	1879	La Digue	female	La Digue	0.9762
5039	AMNH	652451	Thibault	1904	Praslin	female	Praslin	0.9836
5040	AMNH	652444	Thibault	1905	Praslin	male	Praslin	0.9381
5041	AMNH	652441	Thibault	1904	Praslin	male	Praslin	0.9421
5042	AMNH	652460					La Digue	0.8606
5043	AMNH	652456	Thibault	1907	Aride	male	Praslin	0.9536
5044	NHM Tring	1878.7.30.4	Lantz	1877	Praslin	male	Praslin	0.8385
5045	NHM Tring	1895.5.1.261	Tristram/Warry	1880	La Digue	male	La Digue	0.9684
5046	NHM Tring	1895.5.1.263	Tristram/Warry	1880	La Digue	female	Praslin	0.8562
5047	NHM Tring	1927.12.18.388	Lister	1888	Praslin	male	Praslin	0.8577

5048	AMNH	652446	Thibault	1904	Praslin	female	Praslin	0.8437
5049	AMNH	652453	Thibault	1907	Praslin	female	Praslin	0.9652
5050	AMNH	652458	Thibault	1907	Ile aux Fous	male	Praslin	0.9761
5051	NHM Tring	1881.11.14.2	Tristram/Warry	1880	La Digue	male	La Digue	0.9735
5052	NHM Tring	1955.63.3	Ridley & Percy	1955	La Digue	female	La Digue	0.7063
5053	NHM Tring	1881.11.14.3	Tristram/Warry	1880	La Digue	female	Praslin	0.7917

Note: STRUCTURE assigned cluster and assignment values averaged across 10 runs for K=2; * low assignment values indicating admixed individuals or individuals with missing genotype.

SI 3.3 Temporal measures of genetic diversity for La Digue Island only. Note the trend of significant decrease in all measures of genetic diversity except H_O s over the past 133 years; x indicates left out of one-way ANOVA tests of significance as not normally distributed; the lines indicate significance *.

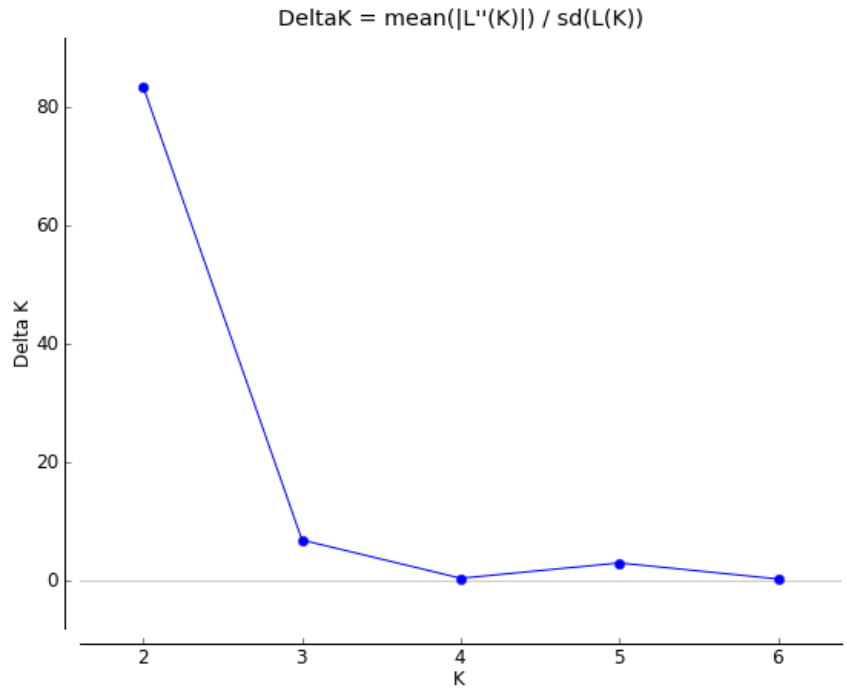


SI 3.4 Pairwise matrix of Nei's genetic distance (corrected for population size) above the diagonal and F_{ST} values below the diagonal for four different time-periods and two contemporary populations; La Digue (LD), and Denis Island (DI), and the first F1 offspring of the Denis Island reintroduced population (F1) using only La Digue historical samples. Significance of pairwise F_{ST} values indicated by*.

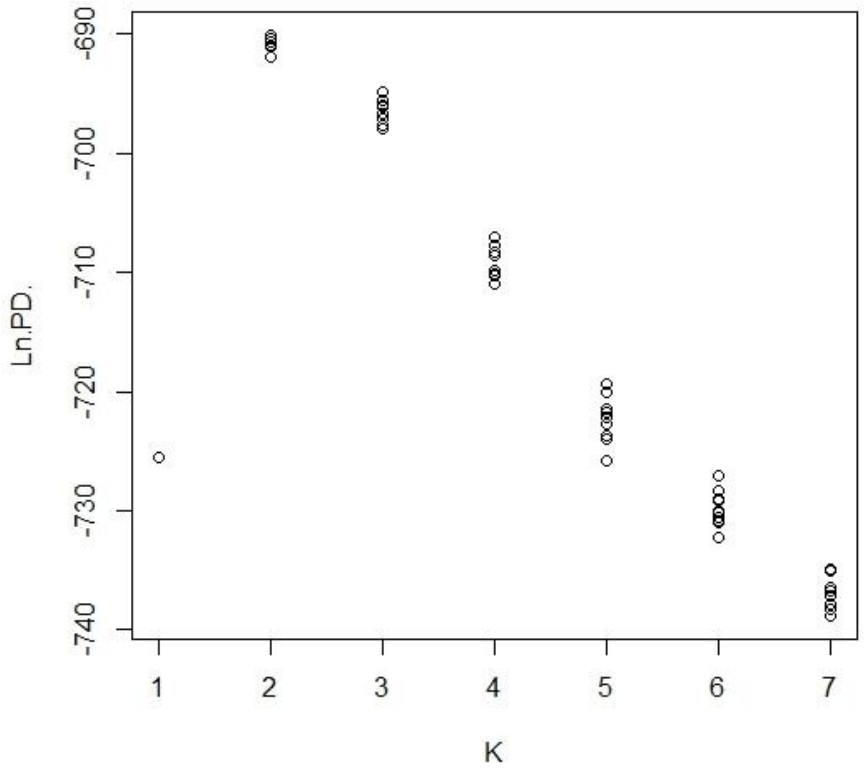
	1880s	1940s	2000	2010(LD)	DI	F1
1880s	0	0.36523	0.44542	0.45086	0.46847	0.40343
1940s	0.121244*	0	0.10137	0.09938	0.08231	0.07601
2000	0.238947*	0.085837*	0	0.00284	-0.00486	0.01613
2010(LD)	0.233332*	0.078855*	0.002334	0	-0.01103	-0.00505
DI	0.229225*	0.069664*	-0.00438	-0.01004	0	-0.0057
F1	0.172402*	0.054179	0.0157	-0.00411	-0.00099	0

SI 3.5 Results of the STRUCTURE analysis using a reduced historical museum sample dataset of five loci showing ≤14% mean genotyping error per allele (≤27% mean genotyping error per locus). Loci with >14% mean genotyping error per allele (which we attribute to allelic dropout) were removed from the dataset.

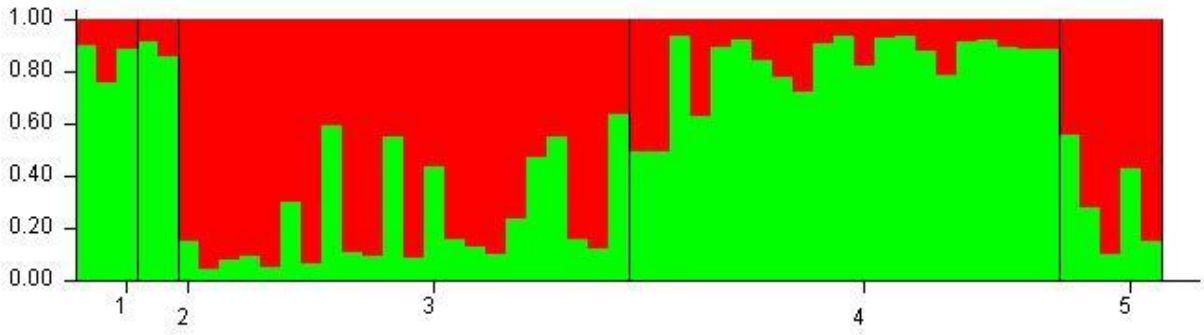
a. Evanno ΔK plot of rate of change between successive log-likelihood values indicating that the most probable number of genetic clusters is two in agreement with the result obtained using the full dataset.



b. Log-likelihood plot of the most probable number of genetic clusters (K) from the STRUCTURE algorithm. K=2 in agreement with results from full museum samples dataset.



c. Bar plot of K=2, the most probable number of clusters obtained with a reduced dataset of five loci.



Pre-assigned sample location: 1= Aride, 2='Iles aux Fous', 3=La Digue, 4=Praslin, 5=unknown collection location. Within each pre-assigned population the samples are ordered according to collection date with the oldest samples first. Assignment values as very similar to those obtained from the full dataset. Samples are in the same order as the analyses using the full dataset so this barplot can be directly compared to those in Figure 3.4.

Chapter 4. Understanding drivers of productivity for source and reintroduced populations of a Critically Endangered endemic island bird species provides guidance for future reintroduction strategy

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Key words: conservation, *Terpsiphone corvina*, productivity, reintroduction strategy

ABSTRACT

Reintroductions are a commonly used tool for rapidly increasing both the range and population size of threatened species, despite the fact they are costly and success rates are generally low. These low success rates have led to a call from the reintroduction community to strategically monitor and evaluate reintroductions in order to understand the drivers of success and failure and to subsequently use this information to improve future success rates.

A conservation introduction of the Critically Endangered Seychelles paradise flycatcher (SPF) has enabled a critical evaluation of the drivers of flycatcher productivity. We quantified differences in levels of productivity of the SPF between three sites; the remnant population on the La Digue plateau, the recently self-colonised population on the La Digue hill, and the population introduced to Denis Island. We then quantified the drivers of SPF productivity within and across these three sites, and lastly used our findings to make recommendations for future reintroductions.

We reveal that productivity was driven by different variables at the three sites. Invertebrate food abundance was the most influential driver of productivity across the three sites, alongside percentage of native vegetation and depredation by the endemic Seychelles bulbul on La Digue, and depredation by the introduced common myna on Denis Island as having substantial negative impacts on productivity. We also reveal that the SPF is more productive in lowland than upland areas. This study also highlighted the importance of looking not only within sites but also among sites in order to gain a fuller understanding of the drivers of productivity.

We provide strong evidence for the importance of protecting the remaining SPF habitat on the La Digue plateau in order to conserve the remnant SPF population. The La Digue plateau seems to be the source of flycatcher production on La Digue and it appears that the hill is a possible sink or buffer zone; without constant reinforcement from the plateau it may not be self-sustaining.

We find that the most important habitat variables to consider when selecting sites for future habitat rehabilitation and reintroductions of SPF are invertebrate food densities, percentage native vegetation, altitude and an absence of common myna and Seychelles bulbuls. We therefore recommend that habitat rehabilitation for SPF is targeted specifically to provide a lowland mixed broad-leaf forest with high invertebrate densities alongside eradication of any Seychelles bulbuls and common mynas in those areas.

4.1 INTRODUCTION

One of the major goals of recovery programmes for restoring populations of threatened species is to reverse declines in population trajectory, distribution and abundance that have been caused directly or indirectly by human activities. For Critically Endangered species in particular, enhancing population size often needs to be achieved as quickly as possible in order to avert extinction, a focus which can lead to longer-term benefits such as maximising retention of genetic diversity and consequently evolutionary potential (Lacy 1994; Tracy et al. 2011; Jamieson & Lacy 2012).

Reintroductions and conservation introductions (hereafter referred to as reintroductions) remain a commonly used tool for rapidly increasing both the range and population size of threatened species despite the fact that reintroduction success rates are generally low (Beck et al. 1994; Griffith et al. 1989; Wolf et al. 1996; Fischer & Lindenmeyer 2000; Armstrong & Seddon 2008; White et al. 2012). Reintroductions are expensive (Kleiman 1989; Lindberg 1992; IUCN 1998; IUCN/SSC 2013) and there is a recognised need to understand and improve reintroduction success rates worldwide (Armstrong & Seddon 2008), in order to maximise return on investment of resources into reintroduction initiatives (IUCN 1998). Avian reintroductions have been shown to have lower success rates than mammals (Griffith et al. 1989; Wolf 1996) and species with a short time to first breeding and which produce larger broods have been shown to be more likely to succeed following reintroduction efforts than species that are slow to mature and have low reproductive rates (Griffith et al.1989). Additionally the release of higher numbers of individuals and wild rather than captive stock (Griffith et al. 1989; Wolf et al. 1996, 1998; Fischer & Lindenmayer 2000; Jule et al. 2008; Aaltonen et al. 2009) have been shown to improve reintroduction success rates. Fundamentally however, reintroduction success rates are most strongly underpinned by the survival rates of the reintroduced founding individuals and their subsequent productivity (Armstrong et al. 1999; Tweed et al. 2003). Indeed, failure to correctly identify and understand the drivers of productivity risks compromising the immediate and long-term management of reintroduced populations. For birds, the factors likely to influence production of fledglings, for example, include the availability of suitable nesting locations, availability of food and the presence/absence of egg and nestling predators (Martin 1987, 1993a; Newton 1998; Armstrong & Seddon 2008; Jones & Merton 2012). Therefore, productivity is a key demographic parameter that needs to be understood and maximised amongst reintroduced individuals in order for reintroduced populations to (i) successfully establish and (ii) increase in size as rapidly as possible to maximise the probability of their long-term persistence.

Aside from the ecological characteristics of the species, choice of reintroduction site is also likely to be an important determinant of reintroduction success. Characteristics of the reintroduction site are likely to influence the survival and productivity of individuals within the establishing

population, for example habitat quality variables such as food abundance, vegetation type and structure, presence or absence of predators, and the available area of suitable habitat. Consequently, site characteristics and levels of productivity, and how those factors interact, are likely to jointly contribute to initial probability of reintroduction success. However, some species may be more robust than others to the array of factors that may affect productivity and subsequent reintroduction success, and across the spectrum of threatened species requiring reintroduction, ecological 'specialists' are likely to be amongst the most sensitive species. For these species correct choice of reintroduction site is of paramount importance because 'specialist' species tend to be those most closely-tied to native habitat and least able to cope with reintroduction into marginal habitats. Indeed, reintroductions of species with a specialist diet have been shown to be less likely to succeed than species with a generalist diet (Wolf 1996). Consequently, insectivorous specialist birds for example, and particularly those that produce small clutches, may be more sensitive than most in terms of the levels of productivity and persistence shown by their reintroduced populations.

In this study we quantify the effects of a variety of biological and environmental factors on productivity in a source and introduced population of the Seychelles paradise flycatcher (*Terpsiphone corvina*; SPF) (Newton 1867), a Critically Endangered insectivorous passerine for which a first conservation introduction was undertaken in 2008 from a worldwide total population of less than 290 individuals restricted to the single 1000 ha island of La Digue (Currie et al. 2003a). Several aspects of the current conservation work for the SPF have meant that the objectives of this study can feed into an adaptive management approach for future restoration efforts. For many species destined for reintroduction efforts, there is considerable choice with respect to reintroduction site; for example species which occupy large geographical areas, or which are capable of occupying mosaics of different habitats. However, for island archipelagos, such as the Seychelles, choice of reintroduction site can be very restricted, particularly when habitats are very degraded, limited in variability and frequently limited in geographical size. Denis Island, a 140 ha island 52 km to the north of La Digue, was selected as the site for the initial reintroduction after 15 potential islands were assessed for suitability to support a SPF population using criteria believed to be important for flycatcher success, based on similarity to the habitat of the remnant population on the La Digue plateau, in particular available area of native broad-leafed plateau forest, invertebrate (food) abundance and presence of freshwater marsh habitat (Hill 2002, Currie et al. 2003c; Bristol & Groombridge 2007). The choice of island for reintroduction amongst candidate islands was further restricted by the presence on several islands of introduced predators. Denis Island was chosen because it was at the time the only island to have sufficient existing high quality broad-leafed native forest habitat and areas of freshwater marsh, whilst also having none of the known predators of flycatchers (native Seychelles bulbuls *Hypsipetes crassirostris*, introduced ship rats *Rattus rattus* and cats *Felis catus*. This study aimed at refining future selection of reintroduction sites by identifying what characteristics enhance productivity.

The Critically Endangered status of the SPF dictated that intervention in the form of a reintroduction was required as soon as possible in order to avert extinction, before drivers of productivity could be studied in detail. Twenty three SPF individuals were introduced to Denis Island in November 2008 (Bristol 2008). Following intensive post-release monitoring from release until August 2010 and during subsequent surveys in 2011 and 2013, the introduced population was confirmed to have grown to at least 40 individuals by July 2013 (Bristol 2013). This first conservation introduction has provided an opportunity to compare productivity in the introduced population to that of the source population alongside known differences between island sites and measured ecological variables so as to understand what factors drive productivity for this species. By quantifying the relative effects of depredation alongside other factors such as habitat quality and food abundance on observed levels of productivity the success of future reintroductions of SPF can be maximised. In this paper, we (i) quantify and assess differences in levels of productivity between the remnant SPF population on the La Digue plateau, the recently self-colonised population on the La Digue hill, and the population introduced to Denis Island, (ii) quantify the different drivers of SPF productivity within and across these sites, and (iii) use the findings to make recommendations for future reintroductions of SPF and of other ecologically similar bird species.

4.2 METHODS

4.2.1 Study species and history

The Seychelles paradise flycatcher is a lowland forest dwelling, behaviourally monogamous, sexually dimorphic passerine endemic to the Seychelles. The species is entirely insectivorous, taking invertebrates both from the surface of leaves (by gleaning, 81%) and by catching them in flight (by hawking, 19%) (Currie et al. 2003b). Flycatchers lay single egg clutches in small open cup-shaped nests woven from coconut fibre, moss, spiders' web and other vegetation, but have multiple nesting attempts per year (range 0-6; average 3.4; Currie et al. 2003b). Successful breeding attempts take c.4 months to complete (c.14 days to build a nest, 17 days of incubation, 15 days for offspring to fledge, followed by c.2-3 months of post-fledging dependence where parents continue to feed their chick). If a breeding attempt fails, pairs often re-nest almost immediately. Breeding occurs throughout the year but peaks in the rainy North-west monsoon season between November-April and troughs during the dry South-east trade wind season from May-October. SPF are relatively long-lived; adults have an annual mortality rate of 21% (Currie et al. 2005) and several ringed individuals have been re-sighted 10 years after ringing (RM Bristol pers. obs.). The SPF is highly territorial maintaining and defending exclusive territories year round. Pairs maintain long-term pair bonds, usually for life; only very rarely have adult territory-holding individuals been observed to move territory (RM Bristol pers. obs.).

Historically recorded on five islands in the Seychelles archipelago (Diamond 1984) the species experienced a dramatic reduction in range and numbers in the late 19th-20th century, attributed to habitat loss through large-scale forest clearance for plantation agriculture, and predation by introduced mammals. The species experienced a severe bottleneck to an estimated low of just 28 individuals in the 1960's, restricted to the relatively wet coastal plateau of La Digue Island (Gaymer et al. 1969). This bottleneck reduced neutral genetic diversity by 59.5% (Bristol et al. 2013; see chapter 3). The species then began an unassisted steady recovery to the current population estimate of 218-290 individuals (Currie et al. 2003a), distributed across the coastal plateau on La Digue (referred to in this study as the 'plateau' population) and with more recent (late 1990's) expansion of the species distribution up onto the mountainside of La Digue (referred to in this study as the 'hill' population; Currie et al. 2003a, RM Bristol pers. obs.).

More than 90% of the coastal plateau on La Digue is private land and under sustained development pressure for both local housing and tourism developments. Consequently, there is little opportunity to increase the amount of suitable habitat on the La Digue coastal plateau, the stronghold of the species, where habitat is already limited and becoming further reduced by continued threat from development. Therefore establishment of additional populations on other suitable islands has long been considered a major priority in order to improve the prospects of long-term survival and reduce the risk of extinction (Watson 1984, 1991; Hambler 1992; Rocamora 1997; Marshall 1997; Currie et al. 2001; Hill 2002; Currie et al. 2003c). Marking the onset of this strategy, twenty three individuals (comprising 13 males and 10 females [7 adult males; 7 adult females; 6 immature males and 3 immature females]) were introduced to Denis Island in November 2008. Following their release, levels of productivity for this newly reintroduced population were intensively monitored for 22 months: all individuals were closely tracked to detect breeding attempts and all breeding attempts were monitored to determine outcome. Alongside the extent of available habitat and the island's predator-free status, Denis Island also presented promising potential for future habitat rehabilitation because the entire island consists of coastal plateau and the island management has a track-record of supporting similar conservation initiatives for endangered bird species.

4.2.2 Study sites

La Digue Island (4° S 55°E) is a 1000 ha island, with an unusually large 220 hectare, relatively moist coastal plateau and a mountainous ridge rising steeply to a maximum elevation of 333m asl. The majority of the flycatcher population are found on the large western coastal plateau where they live in close proximity with the local human population of approximately 2,500 residents and a thriving tourism industry. More recently, flycatchers have colonised the hill on La Digue (Currie et al. 2003a, RM Bristol pers. obs.) which comprised the second study site. The La Digue hill is forested and in contrast to the coastal plateau the hill habitat is not under threat of development.

Denis Island (3°S 55°E), is a 140ha sand cay with a maximum elevation of less than 4m asl (Stoddart & Fosberg 1981). The island is locally owned and hosts a single exclusive holiday resort. The only residents on the island are c.70 resort staff and guests. The resort is restricted to the north–eastern part of the island whereas the rest of the island comprises forest with areas dominated by native broad-leafed forest and areas dominated by abandoned coconut plantation.

All data were collected on La Digue, Republic of Seychelles between January 2008 and May 2010, and on Denis Island, Republic of Seychelles from November 2008 to August 2010. Two study groups were monitored on La Digue, one consisting of 16 territories on the western coastal plateau ('plateau' population; maximum elevation of c.2 m above sea level and a second group consisting of 11 territories on the west-facing hill between 65 and 300 metres above sea level (asl). Following the reintroduction to Denis Island, the resulting population was monitored as a third study group consisting of eight territories.

4.2.3 Data collection

Data were collected on a suite of variables at individual nests, territories and for each study site.

Breeding activity

All study territories were checked every two weeks for breeding activity. We defined breeding activity as nest building, incubating, brooding or feeding dependant young. The female of each known breeding pair was located and followed for between 20 minutes to 1 hour to determine whether the pair was engaged in breeding activity. Previous extensive field monitoring of this species confirmed that such observation periods are known to be entirely adequate to detect any breeding attempt. Once a nest was located it was checked every 2-3 days until either the offspring fledged or the nesting attempt failed. Lay, hatch, fledge and fail dates were recorded for every nesting attempt and confirmed using either a mirror on a pole to check the contents of the nest (for nests lower than 7m, for practical reasons) or by observing from the ground the females behaviour at the nest (for nests higher than 7m). Lay, hatch, fledge and fail dates were assumed to be mid-way between nest checks; for example a nesting attempt was assumed to have failed mid-way between the time when the nest was last active and when it was first noted to have failed. The number of breeding attempts (defined as an egg laid in a nest) per territory ranged from 3-11 (mean=6.76; SD=2.35) over the course of this 30 month study.

Individual nest measures

A number of physical nest site characteristics are documented as having an influence on fledging success/productivity in specialist/insectivorous cup-nesting bird species (Martin & Roper 1988; Currie et al. 2005). The following measures were recorded for every nesting attempt, usually

during incubation: nest location (GPS), nest tree species, nest tree height (metres), nest tree diameter at breast height (DBH), nest height (metres), nest branch direction (pointing up, down or level), nest cover (percentage vegetation cover within 1 foot directly above the nest), distance to nearest road or path (metres) and distance to nearest forest edge (metres).

Territory size and altitude

Territory size was calculated from GPS readings taken each time territory-holding birds were located and followed to monitor breeding activity. These GPS points (between 60-100 points per territory) were used to calculate territory size using the minimum convex polygon method implemented in ArcGIS 9.2 using the HawthsTools package (spatialecology.com). Mean territory size (n=35) was 1.851 ha (SD=0.95). During this study territory sizes and boundaries remained reatively static. Territory height asl *(asl*) at the centre of each territory was estimated from 10m incremental contour lines on a D.O.S. map of La Digue (Series Y851 (D.O.S.20), La Digue, Edition 3.D.O.S. 1984) for all La Digue 'hill' territories. Denis Island and La Digue plateau territories were all estimated to be 2m asl.

Vegetation measures

As the SPF is an insectivorous specialist species that takes the vast majority of its food from the surface of leaves, it is a reasonable assumption that habitat quality, as defined by differences in vegetation parameters, may influence productivity of individual territories and at nest sites within them. Therefore, to account for some of this variability, within each territory a number of vegetation variables were measured in 21 circular plots, each with a radius of five metres (plot area=78.5m^2), spaced a minimum of 20 metres apart and distributed evenly throughout the territory. A GPS reading was taken at the centre of each plot and within each plot the number of trees (tree=DBH$\geq$10cm), tree DBH, and composition of tree canopy species were recorded. At the centre of each plot and 5m north of the plot's centre the following measures were taken in order to measure foliage density (leaf cover) in different height categories from ground level to the canopy: canopy height (metres) ; total leaf cover and percentage leaf cover by species above 3m; total leaf cover and percentage by species above 10m; total leaf cover and percentage by species between 3 and 10m (measured by looking through a 45mm diameter cylinder); and number of times that vegetation touched a pole held vertically in 0-1m, 1-2m and 2-3m height classes as a measure of understory density. From the measures taken in each plot means for each variable were calculated for each territory (e.g. mean canopy height). These data were recorded once for all territories in July 2010. Due to limited time and resources, it was not feasible to carry out further repeated measures within each territory to quantify any temporal variation in these parameters during the study period; however the evergreen nature of the vegetation in the study sites indicated that such temporal differences were likely to be negligible.

Food availability

Food availability is known to have a profound influence on productivity of bird populations (Lack 1954, 1966; Martin 1987). Since flycatchers feed by taking invertebrates from the surface of leaves (gleaning) and also by catching invertebrates in flight (hawking), food abundance was measured at each of the three study sites using two different methods in order to measure invertebrate abundances both in the air and on the surface of leaves.

Malaise flight intercept traps were placed in three different territories within each study site and left in place for five nights in order to collect standardised measures of flying insect abundance. The malaise traps were put in the same nine locations in October 2009, February 2010 and June 2010, in order to measure seasonal variation in flying invertebrate abundance at each site. Catches were stored in 70% ethanol and later identified taxonomically to orderand counted.

The numbers of invertebrates on the undersides of leaves of the most common tree species in each study site (between three and five tree species depending on study site) were counted as a measure leaf invertebrate abundance. Ants and soft bugs were excluded following Currie et al. (2003b) as they do not constitute part of the flycatcher diet, and additionally the recently introduced pest spiralling white fly (*Aleurodicus dispersus*) was excluded as flycatchers do not appear to eat them (RM Bristol pers. obs.). Invertebrates were identified to order, and counted on 300 leaves of each tree species in each study site; a maximum of 20 leaves were sampled per tree. On the La Digue plateau invertebrates were counted on takamaka *(Calophyllum inophyllum)*, badamier (*Terminalia catappa*) and *Allophylus pervillei* leaves, on the La Digue hill invertebrates were counted on takamaka, badamier and cinnamon (*Cinnamomum verum*) leaves and on Denis Island invertebrates were counted on takamaka, badamier, var (*Hibiscus tiliaceus*), *Pisonia grandis* and bwa torti (*Morinda citrifolia*) leaves. The invertebrate leaf counts were conducted at the same times as the malaise trapping in order for the counts to be comparable (October 2009, February 2010, and June 2010). Leaf areas of each species were determined by measuring 20 leaves of each species at each site and using mean leaf area for that species to calculate number of inverts per m^2 for each tree species. Food availability for each nesting attempt was recorded as the leaf invertebrate count and malaise trap count in the month nearest the month the egg was laid.

Predator measures

Depredation of eggs and nestlings at nests is known to have a large impact on productivity of canopy-nesting bird species (Ricklefs 1969; Martin, 1993a, b). Consequently, densities of two species known to depredate flycatcher eggs and nestlings, the Seychelles bulbul and ship rat (Currie et al. 2005) were measured. In addition, two further species that could potentially depredate flycatcher eggs

and nestlings, the introduced common myna (*Acridotheres tristis*) and endemic Seychelles fody (*Foudia sechellarum*) were also measured.

Ship rat abundances were assessed at the two study sites on La Digue during two contrasting time periods, in the north-west monsoon (February-March) and in the south-east trade wind season (August), to gain measures of rat densities in both the wet and dry seasons by removal (extinction) trapping. Trapping was undertaken on the La Digue plateau and hill using 100 big nipper brand rat traps placed on 15x15m grids. On each occasion when abundance was assessed, trapping was continued for 10 days by which time very low numbers of rats were being caught, indicating most rats had been removed from the area. The total number of rats caught across the 10 nights was used as a measure of relative rat density. Rats are absent from Denis Island. Trapping was undertaken on the same grids in the wet and dry seasons to control for potential differences between areas. Rat numbers are known to recover rapidly after removal via immigration and breeding (Innes et al. 1995), especially in such small areas, so the density of rats at a site should not be affected by trapping in the area six months prior. Denis Island is known to be rat-free following their eradication in 2002 and ongoing post-eradication monitoring has not detected any sign of re-invasion (M Naiken pers. comm.).

Seychelles bulbul, common myna and Seychelles fody numbers were measured in each territory. Point counts were undertaken within each flycatcher territory between 7-10am on La Digue and Denis Island throughout the study period. All bulbuls, mynas and fodies were counted within a 25m radius from the observer. The distance and the direction from the observer were also recorded. A minimum of 12 counts were undertaken in each territory and the mean number of bulbuls, mynas and fodies per ha was calculated from these counts. Common mynas are present on both Denis Island and La Digue; Seychelles fodies are present on Denis Island but not on La Digue; Seychelles bulbuls are present on La Digue but absent from Denis Island.

Rainfall

Rainfall was included because it has been shownto affect productivity across a range of bird species (e.g. Moss 1986; Mearns & Newton 1988; Grant et al. 2000; Rodriguez & Bustamante 2003). Monthly rainfall data was obtained from the Seychelles Meteorological Service for weather stations situated on the La Digue plateau, the La Digue hill and Denis Island. The rainfall data for La Digue is complete but the dataset for Denis Island is incomplete due to frequent failure of automated climate recording equipment. For the months of January-May 2009 where rainfall records were incomplete, we used monthly averages based on rainfall recorded for those months in 2006, 2007, 2010 and 2012. In Seychelles, temporal trends in precipitation follow an annual cycle of a rainy season from October to March with peak annual rainfall in December-January, and a dry season from April- September with the lowest annual rainfall in July-August (Walsh 1984). Therefore deriving mean values from previous years is likely to be a sufficiently close approximation for the purposes of this study.

4.2.4 Statistical analyses

(i) Preliminary analyses

All statistical analyses were conducted using R version 2.15.1 (R Core Team 2012). Preliminary analyses tested for differences between the three study sites in number of eggs laid, number hatched and number of fledglings produced per territory-holding adult female. Independent samples t-tests were used where data could be assumed to be normally-distributed. Otherwise Mann-Whitney U tests were used. Binomial proportions tests were used to compare proportions. The results from these initial tests informed the design of our subsequent analyses to identify the factors that underpinned the observed differences in productivity between the three sites.

(ii) Assessment of potential predictor variables

An initial list of potential predictor covariates was examined and critically assessed regarding which ones were the most biologically plausible covariates that could potentially explain/affect fledging success. Data exploration using correlation matrices and Variance Inflation Factors (VIFs) following Zuur et al. (2010) was undertaken to check for collinearity amongst potential explanatory variables. In cases where two covariates had absolute values of correlation $>\pm 0.5$ one of the covariates was removed from further analysis as collinearity of covariates is known to confound GLMM analyses (Zuur et al. 2009, 2010). In addition, high absolute values of correlation ($>\pm 0.8$) between two covariates indicates that one could be substituted for the other and therefore retaining both is unnecessary. Decisions on which covariate to remove from the analyses were made on the basis of minimising redundancy and maximising inclusion of different sources of data.

As a second tier of preliminary analyses, following removal of redundant and highly-collinear variables, we conducted principal components analyses (PCA) to look for obvious patterns of clustering, for example patterns that may be caused by effects of differences between sites. Initially we conducted PCA analyses for La Digue and Denis Island separately as each island had a very different assemblage of potential nest-predator predictor variables. We observed a split in the data (biplot of PCA1 and PCA2) for La Digue; upon closer examination, the two clusters could be largely explained by the nests in the two different study sites on La Digue. Consequently, we also conducted separate analyses for these two study sites (the 'hill' and 'plateau').

(iii) Main analysis

With a reduced set of biologically plausible potential explanatory covariates, we used generalised linear mixed models (GLMMs) in the R package lme4 (Bates et al. 2012) to investigate the probability of a nesting attempt (defined as where an egg was laid) producing a fledgling. GLMMs include

random effects that account for pseudo replication of sampling across time and space, or when multiple responses are measured per individual (Bolker 2009).

In all models, production (=1) or not (=0) of a fledgling from a nesting attempt was the binary response variable and female ID was specified as the random term to control for pseudo-replication due to multiple nesting attempts by females across the study period. A binomial error structure and a logit link function were applied and the models were fitted using the Laplace approximation (Bates et al. 2012).

Model selection and model averaging

We used an information-theoretic (IT) and model averaging approach to model selection (Burnham & Anderson 2002, Burnham et al. 2011; Garamszegi 2011; Symonds & Moussalli 2011) appropriate for complex ecological field investigations such as those reported here (Whittingham *et al.* 2006). Additionally where predictions are what are sought from the analyses, as in this study, Whittingham et al. (2006) recommend the use of model averaging. The IT approach does not depend on a single best model selected by arbitrarily set significance levels (Burnham et al. 2011), but instead uses Akaike's information criterion (AIC) (Akaike 1973) where all models within a candidate set, including the null model fitted with only an intercept, are compared based on model fit and complexity where complexity is penalised (Burnham & Anderson 2002). The model with the lowest AIC value represents the top ranking model with the best fit of the data and the remaining models are ranked according to their relative support using the difference between the model of best fit and the model in question (ΔAIC). For all models we centralised all input predictor variables to a mean of zero and a standard deviation of 0.5 following Gelman (2008) using the R package arm (Gelman et al. 2012) in order to standardise the predictor variables to a common scale as this approach aids interpretation of parameter estimates measured on different scales (Gelman 2008, Greuber et al. 2011).

We used the R package MuMIn (Bartoń 2012) to evaluate all candidate models in a given set and weighted each one based on their AICc (AIC adjusted for small sample sizes) value as in all cases our number of observations to model parameters was <40 (Burnham & Anderson 2002). The threshold for selecting the final model set before model averaging is still somewhat subjective however levels from between ΔAIC<2 to ΔAIC<10 have been suggested as appropriate in order to provide a quantitative estimate of predictor variable relative importance while eliminating implausible models with low weights (Burnham & Anderson 2002, Bolker et al. 2009). We restricted the final model set before model averaging to all models where ΔAICc<4 except where this produced an unreasonably large set of models in which case ΔAICc<2 was used. Model averaging was then applied to the reduced model set to compute the weighted average of parameter estimates (β) and their associated standard errors (SE) and 95% confidence intervals (CI; CIL=lower 95% confidence interval; CIU=upper 95% confidence interval). The relative importances (RI) of explanatory variables were

then calculated by summing the Akaike weights across all models in which the variable was present resulting in an estimate of the probability that the variable of interest features in the best model. Averaged parameter estimates (β), unconditional standard errors (SE), 95% CI's and RIs are presented in model summary results for all GLMMs after model averaging. Explanatory variables are significant where 95% confidence intervals do not cross zero.

All analyses were run for each of the three study sites separately (La Digue hill, La Digue plateau and Denis Island), for each island separately (La Digue and Denis), and for all three study sites together (global analysis) in order to determine factors driving productivity both within and among sites.

Box 1: Summary of model input variable abbreviations used in the text

Abbreviation	Variable	Level variable measured at
nest height	height of nest (metres)	nest
road	distance of nest to road or path (metres)	nest
edge	distance of nest to forest edge (metres)	nest
nest cover	percentage cover within 1 metre above the nest	nest
nest direction	nest branch direction (pointing up, level or pointing down)	nest
nest tree native	nest tree a native or introduced species	nest
asl	territory height above sea level (metres)	territory
native	percentage native trees	territory
dbh	mean tree size (cm)	territory
canopy height	mean canopy height (metres)	territory
canopy cover	percentage canopy cover above 3 metres	territory
leaf3-10	percentage canopy cover in height class 3-10 metres	territory
leaf10	percentage canopy cover above 10 metres	territory
understory	mean understory density between 0-3 metres	territory
tree density	mean number of trees per plot	territory
malaise	mean number of invertebrates caught in malaise traps	site
invert	mean number of invertebrates per m^2 of leaf area	site
bulbul	number of Seychelles bulbuls per hectare	territory
myna	number of common mynas per hectare	territory
fody	number of Seychelles fodies per hectare	territory
rat	rat density per $150m^2$	territory
rain	monthly rainfall (mm)	site
Idf	female ID	nest

Selection of fixed effects (predictor variables)

See Box 1 for a summary of model input variable abbreviations used within the text. Input variables for models varied depending on study site. We used extensive exploratory analyses (Bolker et al. 2009, Zuur et al. 2010, Grueber et al. 2011) including correlation matrices, bivariate tests and graphical inspection in order to identify the most appropriate set of explanatory variables while ensuring the final models were not over-parameterised, but included (retained) the variables of interest, following the general rule of thumb of a minimum of 10 observations per predictor variable

(Lawley & Maxwell 1971; Marascuilo & Levin 1983; Tabachnick & Fidell 2001). Variables with absolute values of correlation of greater than $\pm$0.5 were not included in the same model.

Initially we investigated the effects of individual nest site location on fledging probability. Nest cover (% cover within 1 metre above nest), direction of nest branch (3-level fixed factor up, flat, down), nest tree native species (2 level fixed factor (yes or no)) distance to nearest road/path (metres), distance to forest edge (metres) and nest height (metres) were included as predictor variables. As none of the individual nest site variables had a significant influence on probability of fledging only the highest ranking nest site variable (nest height) was included in subsequent models. Next we investigated whether fledging probability varied between years, but year had no detectable effect on fledging probability and therefore was not included in the final models.

Denis Island

Initially the fixed factors *of nest height, myna, invert, native, rain* and *fody* were included however *rain* and *invert* did not correlate with fledgling (ρ=0.0717, p=0.6519; ρ=0.1392, p=0.3795 respectively) so these factors were removed from the final model in order to reduce the set of input variables as the sample size for number of breeding attempts on Denis Island was small (n=42).

La Digue plateau

Initially *rain, nest height, invert, bulbul, rat, canopy height, native* and *canopy cover* were included as fixed variables. Subsequently, in order to investigate the effect of depredation of eggs and nestlings on fledging probability a second GLMM was run excluding *invert*. Myna and bulbul densities were highly negatively correlated so could not be included in the same model for the La Digue plateau (r=-0.9998, df=111, p< 0.0001).

La Digue hill

A simple model including just *bulbul, native* and *invert* was chosen as the final model in order not to over parameterise as the sample size for the hill was only n=47 nests. *Rain* and *myna* were left out of the model as they did not correlate with fledging (ρ=-0.114, p=0.444; ρ=-0.100, p=0.503 respectively).

La Digue Island

Within the remnant source population on La Digue three potentially important predictor variables of interest (*invert, bulbul* and *native*) were significantly correlated with each other (*invert* & *bulbul* r=-0.587, df=156, p<0.0001; *invert* & *native* r=0.658, df=156, p<0.0001; *bulbul* & *native* r=-0.819, df=156, p<0.0001) and so could not be included in the same model. Therefore three separate

models were run, one including *invert*, one including *bulbul* and one including *native* while all other predictor variables (*canopy cover*, *rain*, *nest height*, *myna* and *rat*) remained the same.

Global analyses including all study sites

First, the fixed variables *myna*, *invert*, *rain*, *canopy height*, *canopy cover*, *nest height* and *native* were included in a model. Then, in order to assess the effects of egg and nestling predators on probability of fledging, *invert* was removed from the model with all other input variables remaining the same.

Finally we removed from the data set nesting attempts where the egg failed to hatch (n=23) and re-ran all analyses looking at the effect of depredation on probability of fledging (as nests that failed to hatch were considered not to have been depredated), however their removal had negligible effect on the results, so these nesting attempts were left in the data set for all results presented.

4.3 RESULTS

4.3.1 Breeding activity

The vast majority of breeding attempts were located during the nest building phase (141/212=67%) or during incubation (55/212=26%) therefore we are confident that the field monitoring detected the majority of breeding attempts. Breeding occurred throughout the year with eggs laid in every month of the study (see Figure 4.1), however the frequency of nesting (defined as an egg being laid in a nest) was higher during the wet North West monsoon season (November-April) than in the dry South East trade wind season (May-October) (χ^2=18.1321, df=1, p <0.0001). There was no difference however in the proportion of nesting attempts successfully fledging in the wet (44.0%) or the dry (36.5%) seasons (χ^2= 0.8522, df = 1, p = 0.3559). The number of chicks fledged per territory across the 30 month study on La Digue ranged from 0-6 (mean=2.556, SD=1.72). The number of chicks fledged during the 18 months that all 3 study sites were monitoried simultaneously ranged from 0-4 (mean=1.667, SD=1.09) (also see Table 4.2).

4.3.2 Differences in productivity between sites

We detected a significant difference between sites in probability of a nesting attempt (defined as an egg laid) producing a fledgling (χ^2=12.5215, df=2, p=0.0019). More eggs fledged on the La Digue plateau than on either the La Digue hill (χ^2=5.7292, df=1, p=0.0167 or on Denis (χ^2=7.9277, df=1, p=0.0049). There was no difference between Denis and the La Digue hill in the proportion of

eggs laid that successfully produced a fledgling (χ^2=0.0309, df=1, p=0.8604) (see Table 4.1 and Figure 4.2).

Adult territory-holding females on Denis and the La Digue plateau laid significantly more eggs than those on the La Digue hill (t=3.5315, df=14, p=0.0033; t=2.1415, df=21, p=0.0441 respectively) while the observed trend in difference in number of eggs laid per adult female between Denis and the La Digue plateau study sites (Figure 4.2) was not significant (t=1.9682, df=19, p=0.0638). However adult territory-holding females on the La Digue plateau fledged significantly more chicks than females on the hill or on Denis Island (plateau & Denis: W=75.5, p=0.0405; plateau & hill: W=111.5, p=0.0018; hill & Denis: W=18.5, p=0.1527), (see Figure 4.2 and Table 4.2).

We observed that 50% of nesting attempts on the La Digue plateau went on to fledge successfully, compared to just 28% on the La Digue hill and 24% on Denis Island. Within both study sites on La Digue aproximately 50% of eggs hatched successfully, while on Denis only 33% hatched- see Table 4.1. On the La Digue plateau 94% of hatchlings went on to fledge successfully compared to 54% on the La Digue hill and 73% on Denis Island (Table 4.1).

4.3.3 Reasons for breeding failure

Out of a total of 212 monitored nesting attempts 39% (n=83) successfully fledged and the remainder 61% (n=129) failed. Field monitoring of nests enabled reasons for nest failure to be documented:11% (n=23) of eggs failed to hatch (these nests were subsequently abandoned by the female some days after their due hatch date); 2% (n=4) of nestlings died in the nest (all <3 days old); 1 nest containing a nestling was destroyed by bad weather; and 42% of nesting attempts were lost due to depredation (n=89), the majority of these (35%; n=75) characterised by disappearance of eggs compared to fewer (7%; n=14) associated with disappearance of nestlings (on a small number of occasions traces of eggshell remains were present in the bottom of the nest cup).

Figure 4.3 and Table 4.3 show the depredation rates for nesting attempts observed at the three study sites. The proportion of nesting attempts depredated differed significantly between sites (χ^2=8.7833, df=2, p=0.0124) with the La Digue hill suffering significantly higher depredation than the plateau (χ^2=7.7501, df=1, p=0.0054), but no significant difference in depredation rates between the hill and Denis Island (χ^2 =1.8103, df=1, p=0.1785) or the plateau and Denis Island (χ^2=0.8046, df=1, p=0.3697). The proportion of eggs depredated was not significantly different between sites (χ^2=0.9271, df=2, p=0.629), but the proportion of nestlings depredated did differ significantly (χ^2=16.6698, df=2, p=0.0002); the La Digue hill population suffered significantly higher nestling depredation than the plateau population (χ^2=13.9128, df=1, p=0.0001) but we found no significant difference in nestling depredation between Denis Island and the two La Digue sites (Denis and hill χ^2=2.2704, df=1, p=0.1319; Denis and plateau χ^2=1.2549, df=1 p=0.2626).

Figure 4.4 shows the proportion of eggs that failed to hatch and were abandoned after their due hatch date ('dud' eggs) at the three study sites. We found a significant difference in number of 'dud' eggs between sites (χ^2=14.4843, df=2, p=0.0007), with a higher proportion on Denis than on either of the La Digue sites (Denis-Hill: χ^2 =7.0416, df=1, p=0.008; Denis-Plateau: χ^2=8.6489, df=1, p=0.0033). However there was no difference in proportion of dud eggs between the two La Digue study sites (χ^2=0.1811, df=1, p=0.6704).

4.3.4 Collinearity amongst potential predictor variables

Both measures of food abundance *invert* (leaf invertebrate density per m^2) and *malaise* (malaise flight intercept trap catches) were very highly positively correlated (r=0.89, df=210, p<0.0001) even though they were measuring different types of insects (flighted versus non-flighted), indicating the suitability of either of these measures to describe food abundance. *Invert* was used as the measure of food abundance in all final models because flycatchers take the majority of their food from the surface of leaves. Refer to Table 4.10 for a summary of leaf invertebrate counts and malaise trapping catches by site and month during this study.

Numerous vegetation variables were highly correlated: proportion of native vegetation (*native*) correlated positively with *dbh* (r=0.77), but negatively with *tree density* (r=-0.67) and *leaf3-10* (r=-0.75) reflecting a trend that native trees tend to be larger and more widely spaced than introduced species. These same vegetation variables (*native, dbh, tree density* and *leaf3-10*) all strongly correlated with our invertebrate abundance variables *malaise* and *invert* (r>$\pm$0.51) showing that higher invertebrate densities were associated with native vegetation. Additionally our measures of leaf cover in different height classes were highly correlated (*leaf3-10* and *leaf10* negatively correlated (r=-0.83) and both measures correlated with *canopy height* (r>$\pm$0.5) reflecting the fact that the majority of forest foliage is located in the crown of trees.

The density of bulbuls (*bulbul*) was strongly negatively correlated with *native* (r=-0.80) and invertebrate abundances (r>-0.57) and highly positively correlated with *asl* (r=0.94).

Asl correlated strongly with many variables indicating a general gradient of habitat change with altitude. *Bulbul, tree density* and *leaf3-10* increased with *asl* (r>0.73), while *native, dbh, invert* and *canopy height* all decreased with increasing *asl* (r>-0.58).

Several individual nest site characteristics were highly correlated: *nest tree height* and *nest tree dbh* were positively correlated (r=0.55) and both these measures were also positively correlated with *nest height* (r>0.80). Additionally *nest direction* was correlated with *nest height* (r=0.62) with higher nests tending to be found on down-hanging branches while low nests were more likely to be on

upright branches or sapling trunks. Additionally distance to the road was positively correlated with distance to the forest edge (r=0.56).

4.3.5 GLMMs

For all sites separately and for the global model that included all three sites, no individual nest site variables were significant in explaining probability of fledging, however *nest height* was the highest ranking individual nest site predictor variable so was included in future models. The results of the GLMMs performed for each site separately and combined together (global model) were similar, therefore for brevity we present here only the result for all sites combined (see Table 4.4). Additionally *year* had no effect on fledging probability (ρ=-0.05, p=0.4884) so was not included in the final models.

GLMM for La Digue plateau site

On the La Digue plateau *invert* was the only variable that significantly predicted probability of fledging. More food increased the probability of a nesting attempt fledging an offspring (see Table 4.5). When *invert* was removed in order to assess the effect of predators on fledging probability, no variables significantly explained fledging probability (see Table 4.5). The intercept-only model had the lowest AICc value representing the best fit of the data to the model, indicating that the fixed variables included in the model had little effect on fledging probability.

GLMM for La Digue hill site

No potential predictor variables explained fledging probability on the La Digue hill (see Table 4.6). The null (intercept only) model had the lowest AICc value indicating the best fit of the data.

GLMM for Denis Island study site

The predictor variable *myna* was the highest ranking variable explaining fledging probability on Denis Island and approached significance (β-1.68, 95% CI-3.60-0.24). Indeed the model with the lowest AICc, representing the best fitting model, included only *myna*. Higher myna densities lowered the probability of a nest successfully fledging an offspring (see Table 4.7).

GLMM for La Digue Island (plateau and hill together)

The results for La Digue Island GLMMs are summarised in Table 4.8. In the model including *invert* more invertebrates significantly increased the probability of a nest successfully fledging a chick (β=1.314, 95% CI=0.59-2.04), and *rain* was the second most influential variable though not significant (β=-0.637, 95% CI=-1.35-0.09), with higher rainfall during a breeding attempt reducing fledging probability. When *invert* was replaced by *bulbul* in the model, *bulbul* significantly explained variation

in fledging probability (β=-0.898, 95% CI=-1.74--0.06) with higher densities of bulbuls reducing the likelihood of fledging. The model including *native* revealed that proportion of native vegetation significantly predicted fledging probability (β=0.878, 95% CI=0.13-1.62), with higher fledging probability at nests in territories containing more native vegetation. All other predictor variables included in the models did not significantly predict fledging probability.

Global analysis with all three sites included

The results of the global analyses including all breeding attempts from all three study sites are summarised in Table 4.9. *Invert* significantly predicted fledging outcome; more invertebrates increased the probability of fledging (β=1.622, 95% CI=0.90-2.34). *Rain* and *nest height* were the second and third most important variables with their influence approaching significance (β=-0.594, 95% CI=-1.28-0.09 and β=0.557, 95% CI=-0.10-1.21 respectively). The other fixed variables included in the model had little influence on fledging probability.

When *invert* was excluded from the model in order to assess the effects of depredation on probability of fledging, *myna*, *native* and *nest height* all significantly influenced fledging probability. Higher myna numbers decreased the probability of fledging (β=-0.973, 95% CI=-1.71--0.24), while more native vegetation (β=0.890, 95% CI=0.18-1.60) and higher nests (β=0.700, 95% CI=0.03-1.37) increased probability of fledging, see Table 4.9.

4.4 DISCUSSION

Our study has revealed a significant difference in SPF productivity (defined as production of a fledging) between sites and that productivity is driven by different variables at different sites. We identified invertebrate food abundance as an important driver of productivity across the three sites, alongside percentage of native vegetation and depredation by the endemic Seychelles bulbul on La Digue, and depredation by the introduced common myna on Denis as having substantial negative impacts on productivity. This study also reveals the importance of looking not only within sites but also across sites in order to gain a fuller understanding of the drivers of productivity.

Measurement of variables

Individual nest variables (nest tree species, nest tree height, nest tree dbh, nest height, nest branch direction, nest cover, distance to forest edge and distance to road) were measured at the individual nest level. Vegetation variables (tree density, tree dbh, percentage native trees, percentage canopy cover, leaf cover between 3-10 metres, leaf cover 10metres+, canopy height, understory density) as well as territory size, territory altitude, and common myna, Seychelles bulbul and

Seychelles fody densities per hectare were measured at the individual territory level. However due to time and feasibility constraints monthly rainfall, seasonal rat densities and tri-annual invertebrate abundances were measured at the site level. This approach is justified given that our main aim was to assess differences in productivity between sites and to identify the drivers of these observed differences between sites (rather than at the individual level within a site), with the ultimate goal of refining criteria for choice of future reintroduction sites. Sites were small and habitat variability within sites was low, therefore variables measured at site level were likely to be sufficiently accurate for the purposes of this study.

4.4.1 Drivers of SPF productivity

Predictors of SPF productivity

La Digue plateau and hill sites

On the La Digue plateau, the site of the main remnant flycatcher population, food abundance was the main variable influencing probability of fledging, with an increase in food abundance increasing fledging success. Given that food abundance was measured at the site level (meaning that within the site all territories had the same food availability for a particular time period) this result shows that temporal or seasonal differences in food availability affected fledging probability. We were unable to determine drivers of fledging probability on the La Digue hill from analysis of the hill dataset alone, likely due to small sample size (n=47 nesting attempts) and homogeneity of each predictor variable between territories at the hill site.

However, when the data for the La Digue plateau and hill sites were combined and analysed together, where all the same predictor variables were present but with more variation at each variable, this analysis enabled a clearer understanding of drivers of productivity across the island of La Digue. Invertebrate food abundance, bulbul density and percentage of native vegetation were all highly correlated so could not be included in the same GLMM, however in separate GLMMs with all other predictor variables unchanged, all three variables significantly predicted fledging probability indicating that all three are important drivers of productivity on La Digue. More food, more native vegetation and lower bulbul densities all increased the probability of fledging.

Both the plateau and hill sites on La Digue suffered equivalent losses of eggs to depredation (c.45%), whilst the plateau experienced almost no depredation of nestlings and the hill lost 41% of nestlings to depredation, resulting in a much lower fledging success on the hill. Bulbul densities were significantly higher in hill territories than in plateau territories, providing further evidence that bulbuls are significant nestling predators on La Digue and appear to have a stronger negative impact on the hill population.

Denis Island site

On Denis Island, myna densities were the main driver influencing fledging probability with higher densities of mynas reducing fledging probability. Although the result was not significant, the lack of significance may be attributable to small sample size (n=42) and homogeneity of myna densities between territories on Denis Island, rather than a small magnitude of their effect. We know depredation is a significant cause of nest failure on Denis Island (and in fact at all three sites; between 43-60% of nests are depredated depending on site, see Table 4.3 and Figure 4.3) however it was not straightforward to tease out drivers of productivity within a site due to the following reason. Study sites are small and within sites the habitat is relatively homogeneous, resulting in little variation between territories within a site for most of the variables measured. This low variation made it difficult to identify drivers of productivity when using data from a single site. For example, myna densities were measured for each territory but on Denis Island mynas were present at relatively high densities in all flycatcher territories, masking any substantial effect that they may have had on fledging probability.

Global analysis of all three study sites combined

Analysing all nesting attempts from the three study sites together enabled us to determine, for variables that are common to all three sites, which were the main drivers of productivity. Food abundance was confirmed as the main driver of productivity. However when *invert* was removed from the input variables in order to look at the effects of depredation, we found that common mynas had a significant negative influence on productivity, in agreement with the separate analysis for Denis Island. We also found that percentage native vegetation had a significant positive influence on productivity. These results are consistent with our finding that *native* and *invert* were positively correlated suggesting that native vegetation harbours higher invertebrate densities than introduced vegetation – in agreement with other studies finding higher invert densities on native vegetation (Hill 2002; Currie et al. 2003b).

Effects of food abundance on productivity

This study provides support within the SPF system for the well-established theory that food limitation is a major driver of productivity (Lack 1954, 1966; Martin 1987; Nagy & Holmes 2005). In addition to simply ensuring that a SPF parent has sufficient food to initiate a breeding attempt and to feed their nestling, higher food abundance may enable parents to spend less time foraging to satisfy their own and their nestling's dietary requirements, leaving them more time to more diligently incubate, brood and nest guard. Such a change in behaviour could lead to both higher hatch rates and lower depredation rates.

It is worth noting however that SPF on Denis Island attempted to breed just as often as those on La Digue (see Figure 4.2) indicating that in terms of food availability the habitat there is sufficient to support almost continuous breeding attempts. This information provides a valuable baseline for level of invertebrate abundance against which to compare other sites for future SPF reintroductions.

Effects of altitude on productivity

Intriguingly food abundance may not be the single main driver of productivity; we detected evidence of an altitudinal effect on productivity. We detected a clear altitudinal effect on flycatcher productivity both on La Digue and across all sites combined. On La Digue, the three significant predictors of fledging probability, *bulbul, invert* and *native* all correlate highly with altitude (*asl*) ($r > \pm 0.82$), showing there is more native vegetation and more invertebrates at lower altitudes and a strong positive correlation between bulbul densities and altitude. Additionally, in the global analyses, more native vegetation (*native)* positively influenced fledging probability and *native* and *asl* were highly correlated ($r=-0.84$, $df=201$, $p<0.0001$), indicating that altitude also has an influence on fledging probability.

Furthermore, at both the hill and Denis Island sites similar insect food abundances were recorded for both leaf dwelling and flying invertebrates, and both sites had lower invertebrate densities than the La Digue plateau. However, SPF on Denis Island (an island entirely below 3 metres asl) bred more frequently (i.e. they laid more eggs per adult female) than those on the La Digue hill (Figure 4.2). SPF on Denis Island attempted to breed year round and laid just as frequently as the flycatchers on the La Digue plateau (Figure 4.1). This evidence indicates an altitudinal effect whereby SPF tend to be more productive in lowland areas.

Effects of individual nest location on productivity

We anticipated that nest cover would be important in enhancing productivity by hiding nests from avian predators, and we predicted that nests with more cover would have a lower chance of depredation and therefore be more likely to successfully fledge, however levels of nest cover had a negligible effect on fledging probability. Currie et al. (2005) found distance to forest edge to be the one nest site variable predicting SPF fledging outcome on the La Digue plateau, but our study did not support this outcome.

Effects of depredation on productivity

On La Digue Island we found clear evidence that egg and nestling depredation by bulbuls significantly negatively influenced productivity (see predictors of SPF productivity, La Digue Island section above). Surprisingly, as the known predators of SPF eggs and nestlings on La Digue (Seychelles bulbuls and ship rats) are absent from Denis, we recorded higher rates of depredation of

both eggs and nestlings on Denis Island than on the La Digue plateau. The only two potential predators on Denis were the common myna and the Seychelles fody. Our GLMM analyses revealed that mynas had the greatest influence on fledging probability of all variables measured on Denis, while fodies had a negligible effect on SPF fledging probability (Table 4.7) indicating that mynas were the cause of reduced fledgling probability on Denis Island. These results are supported by field observations of SPF and other introduced threatened endemic birds (Seychelles warblers and fodies themselves) with gashes and scars on their heads which we attributed to myna attacks. These head scars have never been noted during long-term intensive field monitoring of all three species on other islands, where mynas are present at lower densities or absent. Mynas are present on La Digue, albeit at lower densities than on Denis Island. Previous research into flycatcher egg and nestling predators on the La Digue plateau by Currie et al. (2005) did not confirm mynas as nest predators and this current study found mynas to have negligible influence on fledging probability on La Digue. We do not know if the high density of mynas alone is elevating their depredation rates to problematic and detectable levels, or whether the behaviour of these mynas is unique to the Denis Island population. Whatever the reason, as a result of this study and post-release monitoring of other threatened endemic birds introduced to Denis for conservation, a myna eradication was initiated on Denis Island in mid-2010 and is still to be completed. In January 2010 prior to eradication initiation, myna numbers were estimated at just over 1000 individuals (J van der Woude, unpublished survey data). Trapping substantially reduced myna numbers to a low of c.50-60 individuals by mid-2011 (J van der Woude, unpublished survey data), however the eradication has not been completed and in the absence of consistent trapping pressure myna numbers have rapidly increased again to a current estimate of c.200 individuals (August 2013, J van der Woude, unpublished survey data). The SPF population remained stable at 23-24 individuals from release in November 2008 until the myna eradication commenced, after which time it has risen steadily to the current population estimate of 41 individuals in July 2013 (Bristol 2009; Bristol & Nourrice 2009; French & Bristol 2010; Henriette & Laboudallon 2011; Bristol 2013) in tandem with the decrease in myna numbers on Denis. These survey results provide compelling supportive evidence that mynas were indeed depredating flycatcher eggs and nestlings. This unexpected depredation on Denis highlights the importance of post-release monitoring of reintroduced populations.

Effects of infertile eggs on productivity

We detected a higher incidence of eggs that failed to hatch and were subsequently abandoned some days past due hatch date (infertile eggs and/or early death embryos which we collectively refer to as 'dud' eggs) on Denis Island than at either of the two La Digue sites (see Figure 4.4). It is possible that the stress of translocation resulted in a higher incidence of eggs failing to hatch due perhaps to females not settling and incubating consistently; however we would expect translocation stress to be relatively short-lived and only to affect breeding attempts soon after translocation, whereas we

encountered dud eggs throughout the study period. Small translocated populations can be affected stochastically, and by chance we could have transferred several females who were infertile. Indeed two of eight females on Denis Island failed to hatch any eggs (although some of these eggs were depredated before their due hatch date so we do not know if they were dud eggs or not). However, of the eight breeding females on Denis Island, seven produced at least one dud egg, indicating it is unlikely that chance alone accounts for the high rates of infertility. Likewise it is unlikely that inbreeding depression could account for the higher incidence of 'dud' eggs on Denis as at the time of translocation the individuals on Denis were no more likely to be closely related to each other than individuals on La Digue. This study does not include breeding attempts from individuals born on Denis as it was conducted during the first 22 months post release, whereas levels of inbreeding can only accumulate over time in subsequent generations. Even though we do not know the reason for the higher incidence of dud eggs on Denis Island, this result highlights the need for care in selection of individuals for translocation.

4.4.2 Informing future reintroduction strategy

Source-sink dynamics

This study has shown that the La Digue plateau population is the powerhouse of SPF production on La Digue (a source) and that the hill population is a possible sink or a buffer zone with excess birds residing in lower quality habitat where they are not very productive, and their presence is likely maintained by excess productivity on the La Digue plateau. SPF on the La Digue hill attempt to breed less often than both coastal plateau study sites (Figure 4.2) and their breeding season is also more restricted than the lowland study sites, with no breeding attempts recorded during the driest months of July-September on the hill (see Figure 4.1). Additionally, when hill birds do attempt to breed their eggs and nestlings suffer higher depredation rates than both La Digue plateau and Denis (see Figure 4.3 & Table 4.3). This is important information for planning future flycatcher conservation strategy: it appears that the La Digue hill, where there is a relative abundance of forested habitat that is not threatened with clearance for development, is unfortunately not going to provide a cheap and ready solution to flycatcher conservation. This study lends support to the argument that the remnant SPF population on La Digue was not forced into a corner of suboptimal habitat as sometimes happens with Critically Endangered species, but was residing in the last remaining good-quality habitat on the island, and that SPF are indeed a lowland forest species.

Future approach for quantifying habitat suitability

The high collinearity of *malaise* and *invert* measures of invertebrate abundance indicate that the habitat requirements for both flighted and non-flighted insects are similar and measurement of either one of these variables can provide a sufficient measure of flycatcher food abundance in future

studies. The high collinearity amongst many of the vegetation variables measured indicates that in future a much reduced set of variables could be measured with little loss of information. Collinearity of measures of foliage cover in different height categories, and the correlations between *dbh*, *native* and *tree density* all indicate that measuring just proportion native vegetation and canopy height would be sufficient to capture vegetation variability important for SPF productivity. *Asl* correlated strongly with several variables confirming a general gradient of habitat change with height above sea level with less native vegetation, lower invertebrate numbers and higher Seychelles bulbul numbers with increasing altitude.

Conclusions and recommendations

We found that the SPF is more productive in coastal lowland areas than upland areas therefore future habitat rehabilitation and conservation introductions or reintroductions for SPF should be to lowland plateau areas. Our study found that the most important habitat variables to consider when selecting sites for habitat rehabilitation and translocation of SPF are invertebrate densities, percentage native vegetation and an absence of common mynas and Seychelles bulbuls. We therefore recommend that any habitat rehabilitation for SPF is targeted specifically to provide a forest habitat of mixed broad-leafed native trees promoting high invertebrate densities in lowland areas with an absence of Seychelles bulbuls and common mynas.

Additionally, as highlighted by the unexpected levels of depredation of eggs and nestlings on Denis Island, it may be prudent to avoid translocation to sites with high rat densities as although this study found rats to have negligible influence on flycatcher fledging success, small reintroduced populations are more vulnerable than larger established populations and as highlighted by this study, predator dynamics and behaviour are not necessarily the same in different locations.

In order to streamline data collection of important food and vegetation variables for flycatcher productivity we recommend measuring just proportion native vegetation, canopy height and invertebrate abundance, using either malaise flight intercept traps or leaf counts. However we recommend measuring invertebrate densities more regularly than this study permitted, for example monthly or every two months in order to provide greater clarity on possible temporal/seasonal effects.

Lastly, in order to maximise post–release population growth in future translocations we recommend selection of females of known breeding ability in order to avoid translocating females incapable of reproducing successfully; establishing populations with low numbers of individuals are more susceptible to the influence of individual productivity than reintroductions based on larger numbers of founders.

This study provides additional evidence of the importance of protecting the remaining habitat on the La Digue plateau in order to conserve the remnant SPF population. We found that the La Digue hill is a possible sink and without constant reinforcement from the plateau it may not be self-sustaining. We also recommend future translocations are sourced from La Digue as this population is the largest and most productive population and is therefore most robust to any effects of harvesting.

The first reintroduction of SPF has enabled a critical evaluation of the drivers of SPF productivity. The resulting evidence-based recommendations can be applied through adaptive management to enhance the conservation of the SPF, by guiding both the selection and preparation of future reintroduction sites, and the selection of individuals for future reintroductions.

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4.5 FIGURES AND TABLES

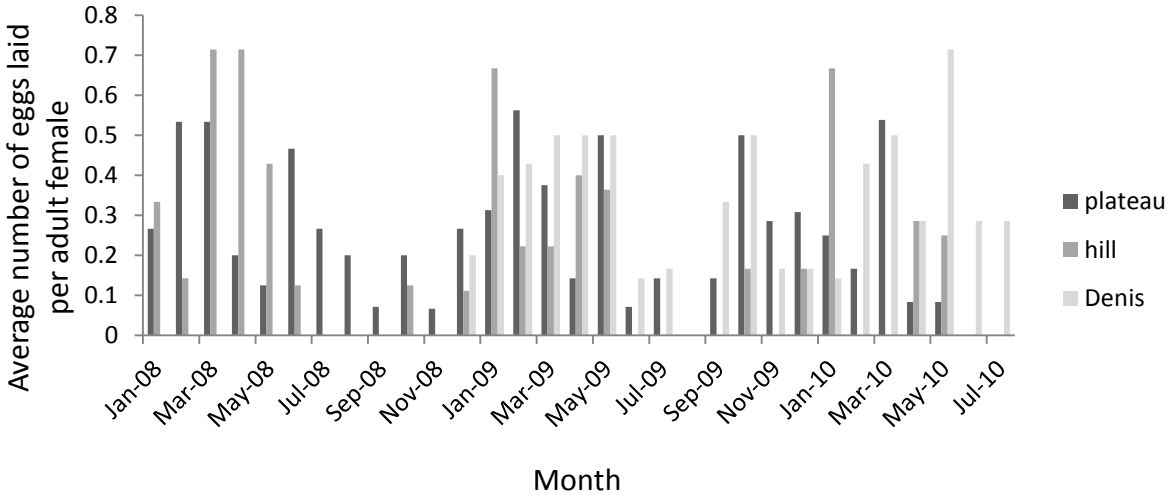

Figure 4.1 Average number of eggs laid per territory holding female per month throughout the study period.

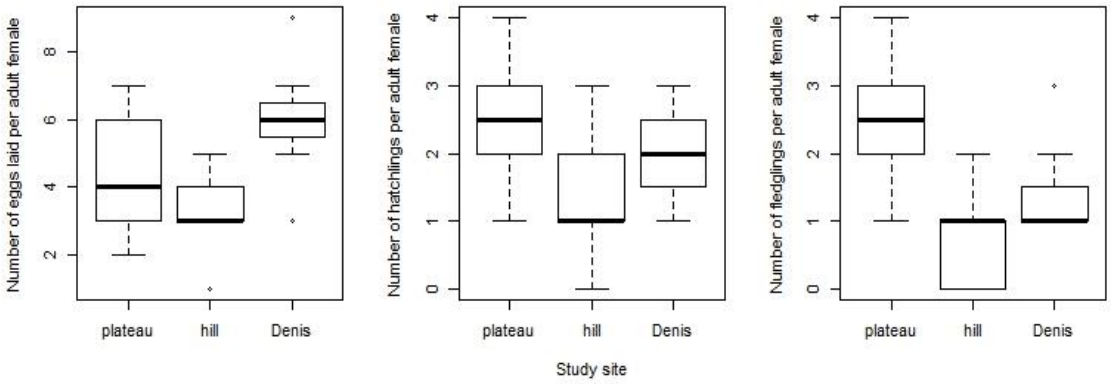

Figure 4.2: Number of eggs laid, hatched and fledglings produced per territory with an adult female for the period January 2009-May 2010 when all three sites were monitored simultaneously.

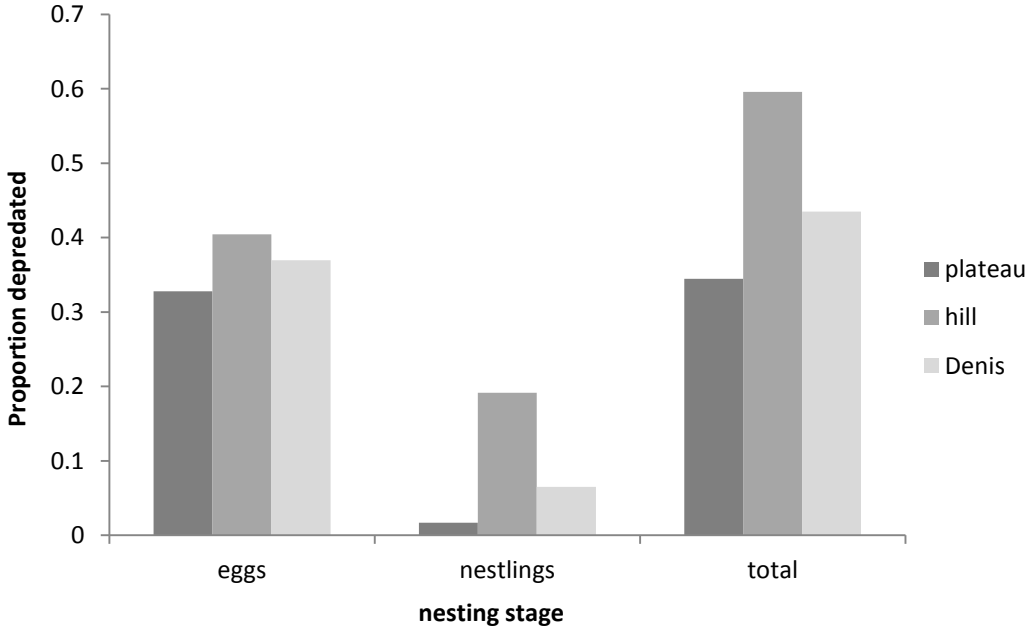

Figure 4.3: Proportion of nesting attempts that were depredated at each of the three study sites.

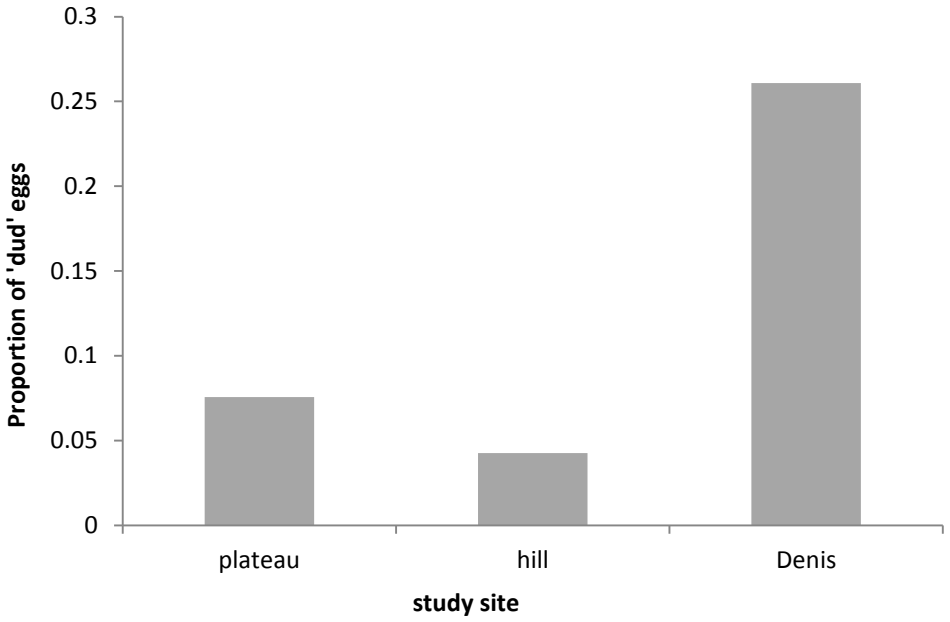

Figure 4.4: Proportion of eggs that failed to hatch and were subsequently abandoned several days past their due hatch date ('dud' eggs) at the three study sites.

Table 4.1: Proportion of eggs that successfully hatched and fledged within the 3 study sites across the whole study period, January 2008-August 2010.

study site	proportion eggs laid that hatched	proportion of nestlings that fledged	proportion of eggs laid that fledged
Plateau (n=119)	0.53 (63/119)	0.94 (59/63)	0.50 (59/119)
Hill (n=47)	0.51 (24/47)	0.54 (13/24)	0.28 (13/47)
Denis (n=46)	0.33 (15/46)	0.73 (11/15)	0.24 (11/46)

Table 4.2: Mean and range of number of eggs laid, hatched and fledged per territory with an adult female at each of the three study sites from January 2009-May 2010, the time that all three sites were monitored simultaneously.

Study site	Number of eggs laid per adult female	Number hatched per adult female	Number fledged per adult female
plateau (n=63)	4.50 (2-7)	2.43 (1-4)	2.36 (1-4)*
hill (n=28)	3.11 (1-5)*	1.44 (0-3)	0.78 (0-2)
Denis (n=42)	6.00 (3-9)	2.00 (1-3)	1.43 (1-3)

mean + (range) shown in brackets per territory with an adult female; *denotes significance

Table 4.3: The proportion of nesting attempts depredated at the egg and nestling phases.

study site	proportion depredated as egg	proportion depredated as nestling	proportion depredated overall
Plateau (n=119)	0.33 (39)	0.02 (2)	0.34 (41)
Hill (n=47)	0.40 (19)	0.19 (9)	0.60 (28)
Denis (n=46)	0.37 (17)	0.07 (3)	0.43 (20)

Table 4.4: Global model: Effects of individual nest site variables on fledging probability for nests from all study sites combined.

Explanatory variable	Estimate (β)	SE	95% CIL	95% CIU	RI
(Intercept)	-0.17	0.28	-0.72	0.38	NA
nest.height	0.82	0.49	-0.14	1.78	0.55
edge	-0.59	0.38	-1.34	0.16	0.51
factor(nest.direction)2	-1.58	1.28	-4.09	0.94	0.38
factor(nest.direction)3	-1.07	0.49	-2.03	-0.10	0.38
nest.cover	0.44	0.35	-0.25	1.13	0.32
nest.tree.native	0.34	0.41	-0.46	1.15	0.10

n=168

Table 4.5: Factors affecting fledging probability on the La Digue plateau.

(a)	Estimate (β)	SE	95% CIL	95% CIU	RI
(Intercept)	0.07	0.21	-0.34	0.48	NA
invert	**0.97**	**0.43**	**0.13**	**1.80**	**1.00**
rain	-0.56	0.43	-1.39	0.28	0.40
native	-0.52	0.42	-1.34	0.30	0.38
nest height	0.40	0.42	-0.41	1.22	0.29

(b)	Estimate (β)	SE	95% CIL	95% CIU	RI
(Intercept)	0.08	0.21	-0.34	0.50	NA
native	-0.55	0.43	-1.40	0.29	0.35
nest height	0.43	0.41	-0.37	1.24	0.29
rain	-0.19	0.38	-0.94	0.56	0.11
bulbul	-0.15	0.41	-0.95	0.66	0.10

n=113, (a) including *invert*, (b) excluding *invert*

Table 4.6: Factors affecting fledging probability at La Digue hill site.

Explanatory variable	Estimate (β)	SE	95% CIL	95% CIU	RI
(Intercept)	**-1.14**	**0.35**	**-1.82**	**-0.45**	**NA**
invert	-0.66	0.66	-1.95	0.64	0.33
native	0.23	0.70	-1.15	1.60	0.20
bulbul	0.18	0.68	-1.16	1.52	0.19

n=45

Table 4.7: Factors affecting probability of fledging on Denis Island.

Explanatory variable	Estimate (β)	SE	95% CIL	95% CIU	RI
(Intercept)	-1.30	0.42	-2.13	-0.47	NA
myna	-1.68	0.98	-3.60	0.24	0.63
native	1.20	0.91	-0.59	2.98	0.37
nest.height	1.17	0.86	-0.52	2.85	0.37
fody	-0.67	0.77	-2.18	0.84	0.07

n=42

Table 4.8: Factors affecting fledging probability on La Digue Island: model (a) includes *invert* but excludes the collinear *bulbul* and *native*, (b) includes *bulbul* but excludes *invert* and *native* and (c) includes *native* but excludes *bulbul* and *invert*; all other predictor variables are the same in the three models.

(a) Explanatory Variable	Estimate (β)	SE	95% CIL	95% CIU	RI
(Intercept)	-0.28	0.17	-0.61	0.06	NA
invert	**1.31**	**0.37**	**0.59**	**2.04**	**1.00**
rain	-0.63	0.37	-1.35	0.09	0.65
nest.height	0.31	0.35	-0.38	0.99	0.29
canopy.cover	-0.16	0.35	-0.84	0.52	0.19
rat	0.17	0.40	-0.61	0.95	0.19
myna	0.08	0.35	-0.60	0.76	0.17

(b) Explanatory Variable	Estimate (β)	SE	95% CIL	95% CIU	RI
(Intercept)	-0.27	0.18	-0.62	0.08	NA
bulbul	**-0.90**	**0.43**	**-1.74**	**-0.06**	**1.00**
nest.height	0.38	0.35	-0.30	1.06	0.20
rain	-0.32	0.33	-0.97	0.33	0.18
rat	0.27	0.36	-0.43	0.97	0.15
canopy.cover	-0.14	0.35	-0.82	0.53	0.13

(c) Explanatory Variable	Estimate (β)	SE	95% CIL	95% CIU	RI
(Intercept)	-0.26	0.18	-0.61	0.10	NA
native	**0.88**	**0.38**	**0.13**	**1.62**	**1.00**
nest.height	0.41	0.35	-0.27	1.09	0.20
rain	-0.30	0.33	-0.96	0.36	0.15
rat	0.25	0.36	-0.46	0.96	0.13
myna	0.14	0.35	-0.55	0.84	0.11
canopy.cover	-0.13	0.35	-0.82	0.56	0.11

n=158, (a) invert, (b) bulbul, c) native

Table 4.9: Factors affecting fledging probability of flycatcher nesting attempts on La Digue and Denis islands combined; (a) full model, (b) excluding *invert.*

(a) Explanatory variable	Estimate (β)	SE	95% CIL	95% CIU	RI
(Intercept)	-0.45	0.16	-0.77	-0.14	NA
invert	**1.62**	**0.37**	**0.90**	**2.34**	**1.00**
rain	-0.59	0.35	-1.28	0.09	0.69
nest.height	0.56	0.33	-0.10	1.21	0.62
myna	-0.34	0.38	-1.09	0.41	0.21
canopy.height	-0.32	0.34	-0.99	0.36	0.21
canopy.cover	-0.24	0.32	-0.87	0.39	0.13

(b) Explanatory variable	Estimate (β)	SE	95% CIL	95% CIU	RI
(Intercept)	-0.45	0.16	-0.77	-0.13	NA
myna	**-0.97**	**0.37**	**-1.71**	**-0.24**	**1.00**
native	**0.89**	**0.36**	**0.18**	**1.60**	**0.95**
nest.height	**0.70**	**0.34**	**0.03**	**1.37**	**0.78**
canopy.height	-0.42	0.36	-1.13	0.29	0.35
rain	-0.15	0.32	-0.78	0.48	0.20
canopy.cover	-0.01	0.32	-0.63	0.61	0.18

n=193, (a) invert included, (b) invert removed

Table 4.10: Summary of malaise trap catches and leaf invertebrate counts by site and month

Study site	October 2009	February 2010	June 2010
hill	10.04 (109.0)	10.76 (73.0)	8.81 (78.0)
plateau	21.01 (156.0)	16.34 (134.0)	11.60 (125.0)
Denis	11.00 (109.5)	8.93 (90.3)	7.25 (86.33)

Number of invertebrates per m^2 (excluding ants and softbugs) counted on leaves of the most common tree species within each study site; Average malaise trap catches during 5 consecutive nights trapping in three territories within each study site are in parentheses.

Chapter 5. Evolutionary and ecological determinants of parental provisioning and survival of juvenile Seychelles paradise flycatchers and implications for management of reintroduced populations

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Key words: reintroduction strategy, *Terpsiphone corvina*, parental provisioning, paternity, offspring sex ratio

ABSTRACT

We quantify parental feeding rate, parental food volume delivery, and offspring fledging weight, survival to independence and extra-pair paternity (EPP) for source and reintroduced populations of the Critically Endangered Seychelles paradise flycatcher. We analyse these variables alongside a suite of territory-specific and site-specific measures of habitat quality to (i) identify biological and ecological predictors of nestling feeding rate, fledging weight, fledgling sex, and survival to independence (ii) quantify levels and effects of EPP, and (iii) examine how an understanding of these processes can help guide future reintroduction strategy for this endemic island species and for other threatened passerines.

First, we reveal a high level of EPP with 71% of offspring (SPF produce single egg clutches) sired by males other than the social male and we interpret this as a potential adaptation to lack of choice of one's social mate. Second, we find that female flycatchers invest significantly more in nestling rearing than males and provide ¾ of nestling feeds. We propose this may be a male response to high probability that the fledgling in the nest is not the male's biological offspring. Third, we find that offspring fledging weights and survival to independence are not predicted by feeding rates, food volume provided to nestlings or by any habitat variables, but there is evidence that female flycatchers may protect offspring from any effects of food shortage in low quality habitat by incurring a personal cost in terms of short-term weight loss and long-term reduction in breeding frequency. Finally, we find that offspring from low quality territories, defined as territories with low invertebrate food abundance, are significantly more likely to be male. We propose that female flycatchers in low quality territories choose to produce male offspring because males have the opportunity to increase their reproductive output in low quality habitat; males can range outside their territory to secure extra pair paternity while females are tied to reproducing within their own territory with the prospect of reduced lifetime productivity in low quality habitat.

Based on our finding that low invertebrate abundance results in an offspring sex ratio bias in favour of males, we recommend that in order to promote a balanced sex ratio (i) habitat rehabilitation at potential future reintroduction sites is focused to promote high invertebrate abundance and (ii) flycatchers are only introduced to sites that, in addition to meeting other criteria demonstrated to be important components of flycatcher habitat (i.e. lowland native forest with an absence of common myna and Seychelles bulbul predators), can demonstrate leaf invertebrate densities higher than Denis Island.

5.1 INTRODUCTION

High quality habitat is often cited as an important determinant of success amongst endangered species reintroduction programmes (Wolf et al. 1996; Armstrong et al. 2002; Cook et al. 2010; Osborne & Seddon 2012). Successful reintroduction therefore relies on identifying suitably high quality habitat and understanding how habitat influences breeding performance. The first breeding cycles by the reintroduced founding individuals are likely to be pivotal in determining how readily a reintroduced population establishes in its new environment and, in particular, the growth and development of resulting offspring and their survival to independence will be a crucial first step. Consequently, understanding the role that habitat and food availability have on juvenile success can help optimise the early phases of reintroduction.

Whilst habitat is likely to be a key factor in reintroduction success, newly reintroduced populations usually consist of relatively small numbers of founding individuals and therefore it is important to maintain a balanced sex ratio, at least in the short–term, in order to maximise the potential number of reproductive pairings in subsequent generations. Amongst birds, females of some species have a high degree of control over the sex of their offspring, (Heinsohn et al. 1997; Komdeur 1997, 2002; Robertson et al. 2006), for example the Seychelles warbler (*Acrocephalus sechellensis*) is able to modify its offspring sex ratio in favour of producing females in high quality habitat (Komdeur 1997, 2002), while female kakapo (*Strigops habroptilus*) in high condition tend to produce more male offspring (Clout et al. 2002; Robertson et al. 2006). Therefore, understanding what ecological factors may influence offspring sex ratios in endangered bird species can clearly be beneficial for short-term reintroduction strategy and long-term conservation management of the resulting established populations.

In many wildlife species, one of the main links between habitat quality within a territory and probability of juvenile success is the extent and quality of parental provisioning. Amongst birds, parental provisioning can take the form of many different strategies. For example, some species confer all the provisioning on the female parent (e.g. kakapo), whereas some other species breed cooperatively whereby helpers (usually related) aid in the provisioning of offspring (e.g. Seychelles warbler), while in other species the pair share the responsibility of providing for their offspring (e.g. Seychelles paradise flycatchers, *Terpsiphone corvina*). However interpreting observed differences in parental investment is not straightforward because extra pair paternity (EPP) is known to be very common in birds, occurring in approximately 90% of avian species (Arnold & Owens 2002; Griffith et al. 2002) with levels of EPP ranging from 0-76% EPP documented for socially monogamous bird species (Petrie et al. 1998), with higher and more variable levels of EPP observed in passerines compared to non-passerine species (Petrie et al. 1998; Griffith et al. 2002). In socially monogamous species EPP refers to offspring sired by males other than the social male. Therefore, there are likely to

be interactions between social and evolutionary dynamics amongst individuals, parental provisioning of offspring and habitat quality, all factors which may have a bearing on reintroduction success. Consequently, in order to be able to optimise future reintroduction strategy for many endangered bird species requires one to not only understand how habitat quality/food availability is transferred via parental provisioning to viability of offspring, but also to assess EPP and sex-specific parental effects that may have a long-term impact on offspring sex ratio and ultimately on evolutionary viability of the reintroduced population.

In this study we quantify parental feeding rate, food volume delivery, offspring fledging weight, offspring survival to independence and genetically confirm EPP for source and reintroduced populations of the Seychelles paradise flycatcher (*Terpsiphone corvina*; SPF) (Newton 1867), a Critically Endangered passerine endemic to the granitic Seychelles islands (IUCN 2013). We analyse these variables alongside a suite of territory-specific and site-specific measures of habitat quality to (i) identify biological and ecological predictors of fledging weight, feeding rate and survival to independence of flycatcher fledglings, (ii) quantify levels of EPP amongst the source and reintroduced populations, (iii) quantify the sex ratio of fledglings for the source and reintroduced population, and (iv) examine how an understanding of these processes can help guide future reintroduction strategy for the SPF and for other threatened passerines.

5.2 METHODS

5.2.1 Study species and history

The Seychelles paradise flycatcher is a lowland forest-dwelling, behaviourally monogamous, sexually dimorphic and dichromatic, insectivorous passerine endemic to the Seychelles (Watson 1981; Currie et al. 2003b). Flycatchers lay single egg clutches in small open cup-shaped nests woven from coconut fibre, moss, spiders' web and other vegetation, but have multiple nesting attempts per year (range 0-6; average 3.4; Currie et al. 2003b). Both parents build the nest, the female incubates and both parents feed the chick. Successful breeding attempts take c.4 months to complete: nest building requires c.7-14 days, incubation lasts c.17 days and the nestling period lasts c.14-15 days, followed by a 2-3 months post-fledging dependency period where the parents continue to feed their juvenile, after which time the juvenile is expelled from the territory (Currie et al. 2005; RM Bristol, unpublished data).

Breeding occurs throughout the year but peaks in the rainy North-west monsoon season between November-April and is at its lowest frequency during the dry South-east trade wind season from May-October (Currie et al. 2005; see chapter 4). SPF are relatively long-lived; adults have an

annual mortality rate of 21% (Currie et al. 2005) and several ringed individuals have been re-sighted 10 years after ringing (RM Bristol pers. obs.). The SPF is highly territorial, maintaining and defending exclusive territories year round. Pairs maintain long-term pair bonds, usually for life; only very rarely have adult territory-holding individuals been observed to move territory (RM Bristol pers. obs.).

Historically recorded on five islands in the Seychelles archipelago (Diamond 1984), the species experienced a dramatic reduction in range and numbers in the late 19^{th}-20^{th} century, attributed to habitat loss through large-scale forest clearance for plantation agriculture, and predation by introduced mammals. The species experienced a severe bottleneck to an estimated low of just 28 individuals in the 1960's, restricted to the relatively wet coastal plateau of La Digue Island (Gaymer et al. 1969). This population bottleneck reduced neutral genetic diversity by 59.5% (Bristol et al. 2013; see chapter 3). The species then began an unassisted steady recovery to the current population estimate of 218-290 individuals (Currie et al. 2003a), distributed across the coastal plateau on La Digue (referred to in this study as the 'plateau' population) and with more recent expansion of the species distribution up onto the mountainside of La Digue (referred to in this study as the 'hill' population; Currie et al. 2003a; RM Bristol pers. obs.).

More than 90% of the coastal plateau on La Digue is private land and under sustained development pressure for both local housing and tourism developments. Consequently, there is little opportunity to increase the amount of suitable habitat on the La Digue coastal plateau, the stronghold of the species' main (source) population. Furthermore, given that the majority of the entire world population of the species resides on one small island, there is a need to establish additional populations on other suitable islands, a need long considered to be a major priority for improving the prospects of the species' long-term survival and reducing the risk of extinction (Watson 1984, 1991; Hambler 1992; Rocamora 1997; Marshall 1997; Currie et al. 2001; Currie et al. 2003c). Marking the onset of this strategy to restore the flycatcher, twenty three individuals (comprising 13 males and 10 females) were introduced to Denis Island in November 2008. Following their release, levels of productivity for this newly reintroduced population were intensively monitored for 22 months.

5.2.2 Study sites

All data was collected on La Digue Island, (4^{o} S 55^{o}E) Republic of Seychelles between January 2008 and May 2010, and on Denis Island (3^{o}S 55^{o}E), Republic of Seychelles from November 2008 to August 2010. Two study groups were monitored on La Digue Island, one consisting of 16 territories on the western coastal plateau (maximum elevation of c.2 m above sea level (asl)) and a second group consisting of 11 territories on the west-facing hill between 65 and 300 metres asl. Following the introduction of flycatchers to Denis Island, the resulting 'reintroduced' population was monitored as a third study group consisting of eight territories.

La Digue Island is a 1000 ha island, with an unusually large 220 hectare, relatively moist coastal plateau and a mountainous ridge rising steeply to a maximum elevation of 333metres asl. The majority of the flycatcher population are found on the large western coastal plateau where they live in close proximity with the local human population of approximately 2,500 residents and a thriving tourism industry. More recently, flycatchers have colonised the hill on La Digue (Currie et al. 2003a; RM Bristol pers. obs.) which comprised the second study site. The La Digue hill is forested and, in contrast to the coastal plateau, the habitat there is not under threat of development.

Denis Island is a 140ha sand cay with a maximum elevation of less than 4m asl (Stoddart & Fosberg 1981). The island is locally owned and hosts a single exclusive holiday resort. The only residents on the island are c.70 resort staff and guests. The resort is restricted to the north–eastern part of the island, whereas the rest of the island comprises forested habitat with areas dominated by native broad-leafed forest and areas dominated by abandoned coconut plantation.

5.2.3 Data collection

Chick fledging weights and survival to independence

All 35 flycatcher study territories were monitored every 2 weeks for breeding activity. Once a nest was located it was monitored every 2-3 days until the attempt either failed or a chick fledged. Nestlings in all accessible nests (52/83) were weighed, measured, ringed and blood sampled at between 11-15 days old (mean$\pm$95% CI 13.42$\pm$0.30 days). Nestlings were ringed with a unique colour ring combination for future identification, weighed to the nearest 0.5g using a pesola spring balance, and their tarsus and wing length were measured to the nearest 0.5mm using a vernier caliper and wing rule respectively. Nests were accessed using a five metre free standing ladder, enabling all nests below 6.5 metres to be reached. Post fledging, juveniles and their parents were located and followed every two weeks to determine juvenile survival to independence and to locate subsequent breeding attempts.

Nestling diet and feeding rates

In order to quantify nestling diet and feeding rates we conducted 60 minute-duration feeding watches (between 2-6 watches per nestling) in the morning between 0730-1000hrs or in the evening between 1600-1800hrs during the second week of the nestling phase when chicks were 8-15 days old. The observer sat quietly c.6-10 metres from the nest, a distance close enough to positively identify prey items using 10x magnification binoculars, but far enough away not to influence parent attendance and behaviour at the nest, and recorded the following information: date, observation start and end times, chick identity (ID), nest ID, female and male parent ID. Every food item fed to the nestling was identified to taxonomic order, size-classed (small <c.5mm, medium c.5-15mm or large >c.15mm), and

the ID of the parent feeding, and the time of each feed was recorded. From the feeding watches a mean feeding rate per hour (*feeding rate)* was calculated.

By eight days old flycatcher nestlings have almost attained their fledging (and adult) weight and are able to consume the same sized prey as adults, therefore feeding rates between different nestlings aged 8-15 days is expected to be comparable. We compared nestling diet (this study) to adult flycatcher diet using published data on adult diet from Currie et al. (2003b). One observer (RM Bristol) undertook the feeding observations in both studies therefore the data are comparable and observer bias is negligible.

Food units

Given that different sized food items provide different volumes of food to nestlings, we calculated *food units* as an estimate of the total food volume provided to nestlings per hour based on the sizes of the food items delivered to nestlings. We considered one small food item to be equivalent to one food unit, a medium sized food item (2-3 times the size of a small food item) to be equivalent to 2.5 food units, and a large food item (4-6 times the size of a small item) to be equivalent to 5 food units. The application of this method enabled the data on food items being delivered to a nest by a male or female parent during a particular period of time to be quantified to reflect the amount of food being delivered rather than just the number of items.

Habitat quality and measurement of habitat variables

In chapter 4 we assessed a suite of habitat variables to determine their importance in driving SPF productivity. We determined that invertebrate food abundance was the most influential driver of productivity, and that lowland native forest with high invertebrate abundance and low numbers of predators (common mynas *Acridotheres tristis* and Seychelles bulbuls *Hypsipetes crassirostris*) constituted "high quality" habitat for flycatchers. Therefore in this chapter we include these measures of habitat quality in our analyses. We also include canopy height because, although it was not found to influence fledging probability (see chapter 4) other evidence exists that canopy height is important for flycatchers; Currie et al. (2003a) found canopy height to be the only significant variable predicting flycatcher presence in an island wide SPF survey of La Digue (RM Bristol also has unpublished survey data that supports this finding). The habitat variables mean canopy height (*canopy.height*), percentage native vegetation (*native*), myna density per hectare (*myna*) and mean height above sea level (*asl*) were measured for each territory following the methods described in detail in chapter 4. Bulbul density per hectare was not included as a variable because bulbuls are absent from Denis Island. Two measures of food abundance were measured at each of the three study sites. Malaise flight intercept traps were used to measure flying insect abundance (*malaise*) and counts of invertebrates on

the undersides of leaves of the 3-5 most common tree species in each study site were undertaken to measure non-flying leaf invertebrate abundances (*invert*) following the methods described in detail in chapter 4. *Malaise* and *invert* estimates of invertebrate abundance were found to be highly positively correlated (see chapter 4), indicating the suitability of either of these measures to describe food abundance. In line with chapter 4, we used *invert* as the measure of food abundance for this study because flycatchers are known to take the majority of their food from the surface of leaves (Currie et al. 2003b).

Blood-sampling and genotyping for paternity analysis

Blood samples were collected from all nestlings when they were ringed and measured prior to fledging. Adult flycatchers were captured in mist nests, ringed with unique colour ring combinations and blood-sampled. Blood samples were collected by puncturing the brachial wing vein using a sterile 25 gauge insulin needle and transferring approximately 50 µl of blood, using a capillary tube, into a rubber-sealed screw top micro-centrifuge tube containing 1ml of absolute ethanol. The tube was immediately inverted several times to mix the suspension and stored at room temperature prior to long-term storage at -20^{o} Celsius until the genomic DNA was extracted. A total of 58 chicks (from a total of 83 fledged during this study) and 102 adult flycatchers, mostly from the study territories, were blood sampled and genotyped during this study. Approximately 76% of territory-holding individuals from the 27 study territories on La Digue, and all 23 flycatchers introduced to Denis Island were ringed, blood-sampled and genotyped. Genomic DNA was extracted from each blood sample using the ammonium acetate method following Richardson et al. (2001). DNA was quantified using a NanoDrop 8000 (Thermo Scientific, USA) and diluted to a concentration of c.10ng/µl using DNA grade water for downstream PCR amplification.

All individuals were genotyped at a set of 11 autosomal polymorphic microsatellite loci isolated from a SPF-specific microsatellite library and characterised in Bristol et al. (2013; chapter 3 of this thesis). PCR conditions follow those detailed in Bristol (2013; chapter 3 of this thesis). Fluoro-labelled PCR products were separated on an ABI 3730 48-well capillary DNA Analyser (Applied Biosystems, USA) and genotypes were scored using GeneMapper software (Applied Biosystems, USA). All PCRs contained negative and positive controls which were genotyped and analysed alongside the samples. Genomic DNA was re-extracted for 10% of the modern individuals and genotyped a second time to check for consistency of allele-calling and to estimate genotyping error. These eleven polymorphic microsatellite loci, with between two and eight alleles per locus, were used to determine whether the social father of each nestling was also the biological father. In order to test the accuracy of our genotypes and consequently our paternal assignment (i.e. whether the social father was or was not the biological father), all female parent genotypes were checked against their nestling's genotype to determine whether our genotype data identified them as the biological female parent.

Sexing of adults and juveniles

Adult flycatchers can be easily sexed in the field as they are highly sexually dimorphic: males have glossy black plumage, electric blue bills and eye rings and elongated central tail streamers, while the females' are tri-colour with black heads, chestnut wings, tails and upper-parts and pale off-white under-parts. Juveniles are harder to sex in the field, but juvenile females have grey throats while juvenile males have black throats like adult females. Nestlings and fledglings cannot be sexed in the field. Therefore, all blood-sampled juveniles were genetically sexed using two different microsatellite sexing typing loci, Z-002A (Dawson 2007) and P2P8 (Griffiths et al. 1998) using the PCR conditions described in Dawson (2007) and Griffiths (1998). Fluoro-labelled PCR products were separated on an ABI 3730 48 well capillary DNA Analyser (Applied Biosystems, USA) and sexing genotypes were scored using GeneMapper software (Applied Biosystems, USA). Juveniles that were not blood-sampled were sexed in the field based on throat colour. We confirmed the accuracy of our field sexing method by comparing our field sexing decisions based on throat colour with the molecular sexing results. Thirty of 33 (90.1%) of our field sexing calls agreed with the molecular sexing assignment. In the three cases where results did not agree we used the molecular sex in preference to the field sexing call. For almost all analyses in this study we included only individuals that were sexed genetically as we required a fledging weight for every individual included in the analyses and for all individuals for which we obtained a fledging weight we also obtained a blood sample from which we genotyped and genetically sexed the individual.

5.2.4 Statistical analyses

All statistical analyses were conducted using R version 2.15.1 (R Core Team 2012). In order to test for differences in offspring fledging weights, nestling feeding rates and food volume delivered between the sexes (male and female offspring), the three study sites and the two seasons (the dry and windy South-East trade wind season (SE) and wet North-west monsoon season (NW), and for differences in parental contribution to nestling feeding, independent samples T-tests, Mann-Whitney U tests, Kruskal Wallis tests, 1-way ANOVAs and binomial tests to compare proportions were used as appropriate. To account for observed differences in fledging weights and feeding rates between male and female nestlings, male and female offspring were analysed separately in subsequent analyses.

To examine predictors of fledging weight we ran separate simple bivariate GLMs fitted with Gaussian error structures and identity link, and with fledge weight as the response variable for each of the following predictor variables; *feeding rate, food units, native, malaise, invert, myna,* female ID (*Idf*), Male ID (*Idm*) and *EPP* (categorised as a 2-level factor [yes or no]).

To examine the predictors of feeding rate and food volume fed to nestlings we ran simple bivariate GLMs fitted with a Gaussian error structure and identity link, and with either *feeding rate* or *food units* as the response variable for each of the following habitat predictor variables; *native, malaise* and *invert.* Food units fed to female nestlings were log-transformed to improve model fit and the GLM fitted with a quasi-error structure to account for non-normal residual errors.

To examine habitat variables predicting offspring sex and survival to independence we used Generalised linear mixed models (GLMMs) in the R package lme4 (Bates et al. 2012). We used an information-theoretic and model averaging approach to model selection (Burnham & Anderson 2002; Whittingham et al. 2006). All input variables were centralised to a mean of zero and a standard deviation of 0.5 following Gelman (2008) using the R package 'arm' (Gelman et al. 2012) in order to standardise predictor variables to a common scale in order to aid comparative interpretation of model averaged coefficients. Before model averaging, we restricted all model sets to $\Delta AIC_c<4$ in order to eliminate potentially implausible models with low AIC weights (Burnham & Anderson 2002; Bolker et al. 2009). Model averaging was then applied to the reduced model set using the R package MuMIn (Bartoń 2012) to compute a weighted average of parameter estimates and their associated standard errors (SE). The relative importance (RI) of explanatory variables was calculated by summing the Akaike weights across all models in which the variable was present resulting in an estimate of probability that the variable of interest features in the best model. All GLMMs were fitted with a binomial error structure, a logit link function and the models fitted using the Laplace approximation. In all models female ID (*Idf*) was included as the random term to account for temporal pseudo-replication. For all GLMMs parameter estimates (β), their standard errors (SE) and 95% confidence intervals (CIL=lower 95% confidence interval, CIU=upper 95% confidence interval) are reported alongside the Relative Importance (RI) of each explanatory variable. Explanatory variables are significant where 95% confidence intervals do not cross zero.

To examine habitat predictors of offspring sex we ran two GLMMs with offspring sex (female=0, male=1) as the binary response variable and including as predictor variables; *native, canopy height, and canopy cover* and either *invert* or *site* as these two variables were seen to be highly correlated ($r=0.76$, $p<0.001$) and therefore it was not considered appropriate for them both to be included in the same model. To examine habitat predictors of offspring survival to independence a GLMM was fitted with survival to independence as the binary response variable (survived=1, died=0) and *native*, *invert*, *canopy.height* and *myna* as the predictor variables.

Due to small and differing sample sizes of individuals for which we had information on feeding rates, total food volume, EPP, fledging weights and survival to independence, we ran bivariate GLMs fitted with a binomial error structure and a logit link function to examine whether nestling

fledging weights, feeding rates, total food volume or extra pair paternity predicted survival to independence.

5.3 RESULTS

5.3.1 Chick fledging weights and differences between sex, site and season

Male flycatcher chicks were significantly heavier than female chicks at fledging (17.63 grams±0.41 95% CI, n=27, versus 16.38 grams±0.41 95%CI, n=25; Mann-Whitney U test W=530.5, p=0.0003). There was no difference in fledging weights between the three study sites for male or female fledglings (ANOVA male fledglings, $F_{2,24}$=1.277, p=0.297; Kruskal-Wallis test female fledglings, χ^2=1.0639, df=2, p=0.5874). There was also no difference in chick weights between the wet and dry seasons, (male fledglings: T-test t=-0.4094, df=25, p=0.6858, n=27; female fledglings: Mann-Whitney U test, W=86, p=0.1621, n=25).

5.3.2 Chick feeding rates and food volume; differences between sex, site and season

The mean feeding rate for flycatcher nestlings aged 8-15 days was 11.55 (±1.03, 95% CI) feeds per hour. Feeding rates (number of feeds/hour) differed significantly for male and female nestlings (Mann-Whitney U test, W=139, p=0.0305) with male nestlings fed at higher rate than females (12.62 (±1.52, 95% CI) feeds per hour versus 9.98 (±1.31, 95% CI) feeds per hour respectively). However there was no significant difference in the number of food units (total food volume per hour) provided to male and female nestlings (W=102, p=0.06713). There was no seasonal effect on either feeding rate or number of food units delivered to nestlings per hour (male nestling data only was used as not enough feeding watches on female chicks in SE season; Mann-Whitney U test, W=357.5, p=0.3331; T-test, t -1.0317, df=17, p=0.3167 respectively) nor any effect of site on either feeding rate or total food units (male nestlings data only as not enough female nestlings produced on the hill or Denis Island; Kruskal-Wallis χ^2=0.4983, df=2, p=0.7795; ANOVA $F_{2,17,}$=0.017, p=0.845 respectively).

5.3.3 Predictors of offspring fledgling weight, feeding rate and food volume provided to nestlings

None of the predictor variables *feeding rate, food units, native, invert, myna, Idf or Idm* predicted fledge weights of male or female fledglings. In addition, feeding rates for both male and female offspring were not predicted by *native, invert* or *myna*. Total food units fed to offspring was not predicted by *native, or invert* for male nestlings, however for female nestlings total food units was significantly predicted by *native* (β=0.017, SE=0.007, t=2.471, p=0.027) but not by *invert*.

5.3.4 Habitat predictors of offspring sex

Food abundance significantly influenced fledgling sex with offspring more likely to be female in territories with higher invertebrate food abundance and more likely to be male in territories with lower invertebrate abundance (β=-1.36, SE=0.53, 95% CI-2.40--0.33) while *native* and *canopy height* did not explain fledgling sex (see Table 5.1). *Invert* and *site* were highly correlated (r=0.76, p<0001) and therefore were not included in the same model; however their significant positive correlation meant both were able to explain variation in offspring sex. Indeed when *site* was substituted for *invert* with all other input variables (*native*, and *canopy height)* unchanged site significantly predicted fledgling sex (β=1.36, SE=0.61, 95% CI 0.17-2.55) while *native* and *canopy heigh*t had negligible influence on fledgling sex (see Table 5.1).

5.3.5 Relative parental contribution to nestling feeding

Both male and female parents fed their nestlings the same invertebrate taxa and in similar proportions (see Table 5.2). However female parents fed their offspring at a significantly higher rate than male parents (8.68±1.03 (mean±95% CI) feeds per hour versus 2.87±0.45 (mean±95% CI) respectively; Mann-Whitney U test, W=12141.5, p<2.2e-16), see Table 5.3. Overall female parents provided 75.1% (877/1167) of all nestling feeds, and fed more prey of all size classes to their offspring than male parents; however of the feeds provided to nestlings by males, they provided proportionately more large prey items and less small prey items than female parents (prop.test small food items χ^2=13.1318, df=1, p=0.0003; medium sized food items χ^2=1.8613, df=1, p=0.1725; large food items χ^2= 4.6704, df=1, p=0.0307). Female nestlings were fed significantly less small prey items than male nestlings (χ^2=5.95, df=1, p=0.0147) and significantly more medium sized prey items than male nestlings (χ^2=5.98, df=1, p=0.0144).

5.3.6 Extra-pair paternity in SPF

All allele calls (genotypes) were consistent i.e. no genotyping error was detected across all 11 loci. 28.8% (15/52) of social fathers were also the biological fathers of the chick in their nest, while 71.2% (37/52) of social fathers were not the biological fathers. There was no difference in proportion EPP between Denis and La Digue (χ^2=1.7635, df=1, p=0.1842). In order to test whether this result could have been effected by genotyping error, we repeated this comparison for the female parents and found that 100% of social mothers (n=50) were also the biological mothers, confirming negligible genotyping errors within the genotype dataset and indicating that the high percentage of EPP is likely to be a true result and not an artefact of genotyping error. A further indication that these results are

authentic is that for those cases where the genotypes of the social father and offspring did not match, their genotypes usually differed at multiple loci rather than at a single locus.

5.3.7 Effect of EPP on nestling feeding rates, food units and fledging weights

There was no difference in fledging weights between chicks produced from EPP versus non-EPP pairings (female EPP mean weight=16.5 grams±0.46 (95% CI), n=18, versus female non-EPP mean weight=16.0 grams±0.40 (95% CI) n=4; Mann-Whitney U test: W=26, p=0.403; male EPP mean weight=17.7 grams±0.41 (95% CI), n=15 versus male non-EPP mean weight=17.6 grams±0.99 (95% CI), n=9; two sample T-test: t=-0.1174, df=22, p=0.9076). Nor was there any difference in feeding rates or total food units provided to EPP versus non-EPP nestlings by either their mother or their father (Mann-Whitney U tests and T-tests as appropriate, all results ns).

5.3.8 Who is siring EPP offspring?

On Denis Island the entire reintroduced population was genetically sampled so we were able to determine with certainly the fathers of all fledglings sampled. In the three cases where the social father was not the biological father, one of the offspring was sired by a male from a neighbouring territory while two were sired by males located at a distance of two territories away.

5.3.9 Predictors of survival to independence

There was no indication that fledging weight influenced likelihood of survival to independence (fledging weight: male fledglings β=0.203, SE=0.595, z=0.340, p=0.734; female fledglings β=0.09291, SE=0.92717, z=0.100, p=0.92). Additionally, of 63 offspring that were monitored post-fledging, 54 successfully reached independence and there was no indication that *native*, *invert*, *myna* or *canopy height* influenced their likelihood of surviving to independence (see Table 5.4). Table 5.5 shows the fledgling weights of male and female fledglings that survived until independence versus those that did not.

5.3.10 Nestling diet and a comparison to adult diet

Table 5.2 shows identified items within nestling diet. Orthoptera was the most commonly consumed food type comprising 30.3% of nestling diet. The second most important food group for nestlings was Diptera, comprising 17.2% of their diet. The other three taxonomic groups each comprising over 10% of nestling diet were Blattodea, Lepidoptera and Araneae. This study of flycatcher nestling diet added three new taxonomic groups to our knowledge of flycatcher diet (Gastropoda, Hemiptera, Coleoptera), though all three new groups were consumed at very low frequencies and therefore are clearly not important components of flycatcher diet.

Table 5.1 provides a summary of adult and nestling diet including taxonomic groups consumed and their relative importance for adults and nestlings. Orthoptera was the most important food type for both age groups comprising 30.3% of nestling diet and 41.1% of adult diet. Nestling diet contained a far higher proportion of medium and large sized prey items than the adult diet; chick diet comprised 43.6% (481/1103) small-sized prey items, 39.7% (438) medium items and 16.7% (184) large items, whereas adult comprised 88.5% (2869/3241) small-sized prey items, 8.4% medium sized items (271) and 3.2% (101) large items.

5.4 DISCUSSION

Female flycatchers invested significantly more than males in rearing of nestlings and provided ¾ of feeds and 70% of total food volume to nestlings. We determined that the SPF exhibits a very high level of EPP with 71% of offspring being sired by males other than the social male. Surprisingly however we found no evidence that either offspring fledging weights or survival to independence were predicted by feeding rates, food volume provided to nestlings, EPP or any habitat variables including food abundance, predator densities or vegetation measures. However food abundance significantly influenced fledgling sex with offspring more likely to be female in territories with higher invertebrate food abundance and more likely to be male in territories with lower invertebrate abundance. Together, these findings suggest that important evolutionary mechanisms are occurring within the population. We discuss the implications of these mechanisms within an evolutionary context then use our findings to provide guidance for future reintroduction strategy.

5.4.1 Effects of offspring and parent gender

Flycatchers are sexually dimorphic, with males slightly larger and heavier than females, and this size difference is apparent in fledglings with males shown to be 7% heavier than females. Male offspring were observed to be fed at a higher rate than female offspring during the nestling period; however interestingly they did not receive significantly more total food volume than female offspring as female offspring are fed proportionately less small prey and proportionately more medium-sized prey than male offspring. This result is most likely explained by the fact that female offspring are generally produced in territories with a higher invertebrate food abundance (discussed below), where presumably parents have more choice of food items and therefore they can preferentially select larger prey items thereby providing in less feeds the energy requirements required by their nestling.

Female parents invest significantly more in nestling production than male parents; they feed their nestlings significantly more than males by providing 75% of feeds and 70% of total food volume

to nestlings. Other *Terpsiphone* paradise flycatchers where offspring provisioning has been reported do not exhibit this imbalance; *T. mutata* parents both contribute evenly to offspring provisioning (Mizuta 2005) as do *T. viridis* parents (Byron 1961). However SPF male parents seem to redress this imbalance to some extent; of the 25% feeds provided by male parents proportionately more are of large size. This result may be because male flycatchers are slightly larger than females and are therefore more able to capture larger prey items, however given the relatively small amount of size dimorphism between the flycatcher sexes, it is equally plausible that this result is simply due to the fact that male parents provide far fewer feeds than females, and therefore can afford to be more selective about food items they chose to bring to the nest.

5.4.2 Impacts of habitat, food and feeding on fledglings

Effect of habitat on fledgling sex

We found that lower invertebrate food abundance (i.e. lower quality territories; see chapter 4) predicted that fledglings would be male, and that higher invertebrate food abundance (i.e. higher quality territories; see chapter 4) predicted that offspring would be female.

Given that no significant difference in the number of food units provided to male and female offspring was observed, we can assume that male and female offspring 'cost' the same to parentally raise in terms of energy. Therefore, all other things being equal, application of sex allocation theory would predict a 50:50 sex ratio regardless of habitat quality. Trivers & Willard (1973) hypothesised that females should adjust their offspring sex ratio in response to available resources in order to optimise their fitness. We propose it is possible that female parents holding lower quality territories chose to produce males rather than females because male flycatchers are likely to have a higher chance of successfully reproducing in lower quality habitat as they can travel to disparate territories and thereby enhance their reproductive output through extra pair paternity (EPP) whereas females are tied to reproducing in their own territory, regardless of its quality.

In addition, *site* correlated highly with invertebrate abundance and was also therefore able to explain fledgling sex. The La Digue plateau, which has higher levels of invertebrates than both the La Digue hill and Denis Island, had a relatively non-biased sex ratio of fledglings (29 female fledglings versus 23 male) whereas the hill and Denis Island sites showed a bias towards male fledglings and both sites produced very few female fledglings (hill: 2 female versus 7 male fledglings; Denis Island: 2 female versus 8 male fledglings). These sex-ratio biases may be due to chance as our sample sizes for the hill and Denis Island were small; however given that our analysis, showing how lower quality habitat produced more male offspring and higher quality habitat produced more female offspring, was conducted on the full data set of fledglings from all three sites, it is likely this result is not due to

chance, meaning that there are likely to be important implications for how reintroduced populations are managed.

5.4.3 Impacts of habitat and food delivery on fledging weights

Surprisingly, once sex was accounted for by analysing male and female nestlings separately, fledging weights were not predicted by feeding rates or total food volume provided to nestlings, nor by any of the habitat variables investigated including food abundance, percentage native vegetation, myna density and canopy height. Hour-long feeding watches may not provide an accurate estimate of overall feeding rates, or food units provided to nestlings, though 2-6 feeding watches were conducted per nestling and an average hourly rate calculated for each nestling which ought to control for variable feeding rates. It is also possible that for tropical bird species, particularly tropical island species such as the SPF that tend to have a longer lifespan and breed at a slower rate than temperate species (Murton & Westwood 1977; Covas 2011), parents may always be able to provide enough food to their single nestling by incurring a cost to themselves in poorer quality habitat. Therefore food availability and other habitat variables may not reflect fledgling weight (Rodenhouse & Holmes 1992; Nagy & Holmes 2005). Female SPF do tend to lose weight during breeding attempts (RM Bristol unpublished data), providing support for the argument that females will provide for their nestling at a personal cost. Food shortage may not affect the weight of nestlings, but may impact on productivity in other ways, for example by reducing nesting frequency. Indeed we found that SPF pairs on the La Digue hill, a site with relatively low food availability, attempted to breed less frequently than plateau birds (see chapter 4). In a large experimental study of the effects of low food abundance Rodenhouse & Holmes (1992) found that nestling growth rates, mass and fledging success per brood of Black-throated blue warblers (*Dendroica caerulescens*) were no different between control sites and experimental sites where caterpillar food was reduced by insecticide application to forest blocks. However where caterpillar food was experimentally reduced pairs made significantly fewer nesting attempts. In a similar study using supplementary feeding Nagy & Holmes (2005) found that supplementary-fed and control females did not differ in the number or mass of offspring fledged, however supplementary-fed females re-nested at a significantly higher frequency than control females. Both studies concluded that the greatest reduction in productivity due to food limitation may occur due to a reduced number of nesting attempts, rather than due to lowered growth rates or survival of individual nestlings, a conclusion supported by our findings.

5.4.4 Extra pair paternity

We detected a high rate of EPP in the SPF of 71% (published range for socially monogamous species 0-76%; Petrie et al. 1998). Adult males regularly foray into other pairs' territories–sometimes several territories away from their own and groups of up to six males are regularly observed chasing

after fertile females, i.e. females that are nest building or have just completed nest building and are about to lay. Intrusions by neighbouring males into territories with females around laying stage (i.e. fertile) has also been observed in the Madagascar paradise flycatcher *Terpsiphone mutata* and taken as circumstantial evidence suggesting males were seeking extra pair copulations (EPC) (Mizuta 2000). However EPCs are hard to quantify as SPF copulation is secretive and rarely observed. Based on thousands of hours of behavioural field observations by RM Bristol, adult territory holding female SPF do not foray outside their territories, and do not appear to actively EPCs.

It appears that all the effort put into chasing other males' mates does result in a high level of EPP. Potentially this high observed rate of EPP could be a mechanism for selecting a preferred mate. It is vitally important for SPF to gain a territory as they reach adulthood in order to survive, and once a territory is obtained birds remain there for life (only very rarely have adult SPF been observed to move territory, and in the very rare occasions where this behaviour has been observed it is to a neighbouring territory). Therefore, the importance for survival of gaining a territory may mean that individuals cannot be too choosy about their social mate and that a mechanism which SPF have evolved to counter this lack of choice of social mate is to indulge in high levels of EPP.

We found that extra pair paternity had no influence on fledging weight, feeding rates or food units provided to offspring by either parent. Males rearing nestlings that were the result of EPP and were not their biological offspring did not feed these nestlings any differently than nestlings that were their own biological offspring. Just as EPP is very variable among bird species, so are males' responses to EPP in their brood. Some studies, in agreement with ours, have found no evidence that cuckolded males reduced their levels of parental care (e.g. house martins *Delichon urbica*, Whittingham & Lifjeld 1995) while other studies have found that males invest less in broods with higher proportions of EPP (e.g. reed buntings *Emberiza schoeniclus*, Dixon et al. 1994; dunnocks *Prunella modularis*, Burke et al. 1989). However males cannot recognise their own offspring as they do not preferentially feed their own biological offspring compared to EPP offspring in the same brood, and they still invest in broods where 100% of the offspring have been sired by EPP (Burke et al. 1989; Westneat et al. 1995; Kempenaers & Sheldon 1996). Studies that have found evidence of males adjusting their level of parental care in relation to levels of EPP conclude that males do so based on their perception of the likelihood they have been cuckolded (e.g. Burke et al. 1989; Dixon et al. 1994).

One possible interpretation is that male SPF do assess the likelihood of EPP in their brood and their response to such a high level of EPP is to assume all their nestlings are probably not their biological offspring, which would explain why male SPF consistently invest significantly less than females in both incubation and chick rearing; in contrast to males, the female can be certain that the offspring she is investing in is her own biological offspring, whereas males have a 71% chance that their social nestling is not their biological offspring.

5.4.5 Predictors of survival to independence

We detected no evidence that SPF fledging weight influenced survival to independence. Our sample size was too small to adequately address this question, particularly as the vast majority of fledglings survived to independence. Of the 63 fledglings for which we have data for survival to independence, 54 (86%) went on to reach independence. However we have fledging weights for only 38 of these individuals. This study suffers from small sample sizes, a problem inherent to studies of many Critically Endangered species. In a study of SPF on the La Digue plateau, Currie et al. (2005) found that individual SPF recruited into the population 9-10 months after fledging were generally heavier than those not seen off their natal territory. Additionally, no habitat variables including measures of food abundance, vegetation and predator densities predicted probability of survival to independence. Finally whether a chick was the product of EPP or not also did not influence survival to independence. The lack of any predictors of juvenile survival to independence could be due to small sample sizes of our data set, or it could be for the same reasons that we found no predictors of chick fledging weight, i.e. the SPF parents, particularly females, may be able to protect their juvenile from the negative effects of any food shortage by incurring a cost to herself and potentially to her future productivity.

5.4.6 Chick diet versus adult diet

Chick and adult diet are very similar both in terms of the taxonomic groups of prey items and also in terms of the relative importance of each taxonomic group in the diet. The main difference between chick and adult diet is that chicks consume a higher proportion of medium (40% versus 8%) and large (17% versus 3%) prey items, and a correspondingly lower proportion of small prey items (44% versus 89%) than adults. SPF parents must preferentially select larger prey items for their nestlings. This makes energetic sense in terms of providing more calories and nutrition to nestlings at a time of rapid growth and development as medium and large sized prey are c.2-6 times larger than small prey and assumedly also contain 2-6 times more food value per item.

5.4.7 Relevance to reintroduction strategy

Our finding that offspring sex is influenced by habitat quality (defined by food abundance), and that more male offspring are produced in lower quality habitat, has important management implications for reintroduced populations. If the SPF are to be introduced to islands comprising low quality habitat, a scenario not inconceivable given the severe degradation of habitats on many of the Seychelles islands, the resulting populations may end up with biased sex ratios in favour of males with both short- and long-term implications. In a small establishing population this prediction has important implications for initial population growth since fewer females' means fewer potential breeding pairs and a reduced ability for population growth as each pair can only produce a maximum of one offspring

per breeding attempt. In the longer term an uneven sex ratio caused by production of predominantly males could also have implications for evolutionary viability of the population.

The La Digue hill and Denis Island sites both had lower invertebrate densities than the La Digue plateau and both sites produced predominantly male offspring. For the La Digue hill the effects of offspring sex ratio bias are unlikely to affect the population as it appears to be maintained by excess production on the La Digue plateau (see chapter 4). However for the small reintroduced population on Denis Island, where immigration from other sites cannot occur naturally, the effects of an offspring sex ratio bias could be more serious. In a recent survey of the Denis Island SPF population 4½ years post reintroduction, the population does indeed have more males than females, but the bias is not extreme (22 males versus 17 females) (Bristol 2013) therefore it appears that, for now at least, adequate females are being produced. However Denis Island was selected as the recipient site for reintroduction not only because of its rat free and Seychelles bulbul free status but also because it was considered to have high quality flycatcher habitat including sufficient invertebrate food abundance. Care will need to be taken to ensure future reintroduction sites have adequate habitat quality in terms of food availability to ensure production of both male and female offspring in similar proportions. Therefore, based on these observed habitat-related effects on offspring sex ratio we would recommend reintroducing flycatchers only to additional islands where invertebrate surveys demonstrate there to be higher levels of invertebrate food abundance compared to Denis Island.

Currently several islands in the Seychelles are the focus of restoration activities to rehabilitate heavily degraded habitat by eradicating introduced invasive rats (*Rattus* spp.) and common mynas, and by re-planting native vegetation particularly in very degraded coastal plateau areas. Two of these islands, North Island and potentially Félicité (see Figure 1.1 in chapter 1), may become suitable candidates for flycatcher introductions in the future if, in addition to meeting the other criteria found to be important components of flycatcher habitat quality (e.g. lowland native forest with an absence of common myna and Seychelles bulbul predators; see chapter 4), their invertebrate abundances can be increased by re-planting of appropriate lowland native tree species. Our finding that adequate levels of invertebrate food is necessary not only to ensure sufficient SPF breeding *per se* but importantly to ensure adequate production of female offspring, will be incorporated into the on-going habitat rehabilitation programmes on these islands. Managers on both of these islands have expressed a desire to introduce flycatchers, and we are now in a good position to guide habitat rehabilitation to maximise flycatcher invertebrate food abundance on these islands based on sound scientific data, a broader need widely recognised within the conservation community (Armstrong & Seddon 2008).

This study uncovers interesting relationships between evolutionary (extra pair paternity) and ecological (habitat quality) determinants of productivity. The relationship between habitat quality and

productivity is not manifested in fledgling weights, but rather in offspring sex and in the overall frequency of breeding. SPF provide an example of another bird species that appears to have some control over the sex of their offspring as has been documented for other birds (e.g. Heinsohn et al. 1997; Komdeur 1997, 2002; Robertson et al. 2006), which is presumably adaptive by enabling production of the sex (males in SPF) that have the opportunity to increase their reproductive output in poor quality habitat more than females. Males can range outside their territory to secure extra pair paternity while females are tied to reproducing within their own territory with the prospect of reduced lifetime productivity in poor compared to high quality habitat. This study therefore provides guidance for reintroduction strategy not only for SPF but also for other threatened passerines whose productivity may also be impacted in complex ways by both evolutionary and ecological variables.

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5.5 FIGURES AND TABLES

Table 5.1: Factors explaining fledgling sex (a) including invert but excluding site; (b) including site and excluding site.

(a) Explanatory variable	Estimate (β)	SE	CIL	CIU	RI
(Intercept)	0.21	0.25	-0.29	0.70	NA
invert	**-1.36**	**0.53**	**-2.40**	**-0.33**	**1.00**
canopy height	0.23	0.56	-0.86	1.32	0.21
native	-0.03	0.61	-1.23	1.17	0.19
(b)	Estimate (β)	SE	CIL	CIU	RI
(Intercept)	0.20	0.26	-0.30	0.70	NA
site	**1.36**	**0.61**	**0.17**	**2.55**	**1.00**
canopy height	0.24	0.55	-0.84	1.33	0.21
native	-0.14	0.61	-1.33	1.05	0.20

n=71, (a) including invert, (b) including site.

Table 5.2: Flycatcher nestling diet by taxa, frequency and parent.

Taxa	frequency in nestling diet	% in nestling diet (n=727)	% fed by mother (n=543)	% fed by father (n=184)	% adult diet (n=341)*
Orthoptera	220	30.3	29.1	33.2	41.1
Diptera	125	17.2	19.0	11.4	8.2
Blattodea	93	12.8	12.5	13.6	7.9
Lepidoptea	90	12.4	12.7	12.5	25.8
Araneae	85	11.7	10.9	14.1	10.6
Hymenoptera	59	8.1	7.6	9.8	2.3
Neuroptera	24	3.3	3.9	1.1	1.5
Odonata	17	2.3	2.8	1.1	2.3
Coleoptera	11	1.5	1.5	2.2	0
Phasmatodia	1	0.1	0.0	0.5	0.3
Hemiptera	1	0.1	0.0	0.5	0
Gastropoda	1	0.1	0.2	0.0	0

* adult diet data from Currie et al. (2003b)

Table 5.3: Feeding rates and number of food items fed to nestlings by size and parent.

	total	small	medium	large	unknown size
all feeds	1167	451 (38.6%)	410 (35.1%)	170 (14.6%)	136 (11.7%)
feeding rate per hour	*11.55(1.03)*	*4.46 (0.85)*	*4.06 (0.47)*	*1.68 (0.30)*	*1.35 (0.34)*
mother	877	367 (41.8%)	298 (34.0%)	116 (13.2%)	96 (11.0%)
feeding rate per hour	*8.68 (0.84)*	*3.63 (0.78)*	*2.95 (0.38)*	*1.15 (0.23)*	*0.95 (0.25)*
father	290	86 (29.8%)	112 (38.5%)	54 (18.7%)	38 (13.1%)
feeding rate per hour	*2.87 (0.45)*	*0.86 (0.24)*	*1.10 (0.24)*	*0.54 (0.16)*	*0.37 (0.15)*

Number of food items fed to nestlings aged 8-15 days by size class (small, medium, large and unknown size) and by parent (mother and father) with percentages in parenthesis; mean feeding rate per 60 minutes in *italics* with ±95% confidence intervals in parentheses.

Table 5.4: Factors influencing survival to independence.

Explanatory variable	Estimate (β)	SE	CIL	CIU	RI
(Intercept)	2.99	0.72	1.57	4.41	NA
canopy height	1.76	1.12	-0.44	3.96	0.59
invert	-1.34	1.18	-3.64	0.97	0.39
myna	2.01	2.14	-2.19	6.20	0.39
native	-0.68	1.38	-3.38	2.03	0.06

n=63

Table 5.5: Mean fledging weights (grams) with 95% confidence intervals for male and female offspring that survived to independence and for those that did not survive to independence, and weights of all male and female fledglings, regardless of whether they survived to independence of not, for comparison.

	male fledglings		female fledglings		all fledglings	
	survived	died	survived	died	female	male
mean weight	17.43	17.30	16.56	16.50	16.38	17.63
95% CI's	±0.49	±1.17	±0.44	±0.98	±0.41	±0.41
n	15	4	16	2	25	27

n=number of observations; mean weight (grams); CI=confidence interval.

Chapter 6. Synopsis

6.1 SUMMARY OF KEY FINDINGS

In chapter 2 I construct a molecular phylogeny of *Terpsiphone* flycatchers of the Indian Ocean and use it to investigate their evolutionary relationships. Colonisation of the western Indian Ocean has been within the last two million years and greatly postdates the formation of the older islands of the region. A minimum of two independent continent-island colonisation events must have taken place in order to explain the current distribution and phylogenetic placement of *Terpsiphone* in this region. Five well-diverged Indian Ocean clades are detected; however the relationship between them is unclear. Short intermodal branches are indicative of rapid range expansion across the region, masking exact routes and chronology of colonisation. The Indian Ocean *Terpsiphone* taxa fall into five well supported clades, two of which (the Seychelles paradise flycatcher and the Mascarene paradise flycatcher) correspond with currently recognised species, whilst a further three (within the Madagascar paradise flycatcher) are not entirely predicted by taxonomy, and are neither consistent with distance-based nor island age-based models of colonisation. I identify the four non-Mascarene clades as evolutionarily significant units (ESUs), while the Mascarene paradise flycatcher contains two ESUs corresponding to the Mauritius and Réunion subspecies. All six ESUs are sufficiently diverged to be worthy of management as if they were separate species.

In chapter 3 I characterise 14 microsatellite loci developed for the SPF and use them to quantify temporal and spatial measures of genetic variation across a 134-year timeframe encompassing a historical bottleneck that reduced the species to ~28 individuals in the 1960s, through the initial stages of recovery and across a second contemporary conservation-introduction-induced bottleneck. I find a temporal trend of significant decrease in standard measures of genetic diversity across the historical bottleneck, but only a non-significant downward trend in number of alleles across the contemporary bottleneck. However accounting for the different timescales of the two bottlenecks (~40 historical generations versus <1 contemporary generation) the loss of genetic diversity per generation is greater across the contemporary bottleneck. Historically the flycatcher population was genetically structured; however extinction on four of five islands has resulted in a homogeneous contemporary population.

In chapter 4 I **c**ritically evaluate the drivers of SPF productivity. I reveal that productivity is driven by different variables at the three study sites; the La Digue plateau, the La Digue hill and Denis Island. Invertebrate food abundance is the most influential driver of productivity across the three sites, alongside percentage of native vegetation and depredation by the endemic Seychelles bulbul on La Digue, and depredation by the introduced common myna on Denis Island as having substantial

negative impacts on productivity. I also find that the SPF is more productive in lowland than upland areas and provide strong evidence for the importance of protecting the remaining SPF habitat on the La Digue plateau in order to conserve the remnant SPF population.

In chapter 5 I quantify parental feeding rate, parental food volume delivery, and offspring fledging weight, survival to independence and extra-pair paternity (EPP) for source and reintroduced populations of the SPF. First, I reveal a high level of EPP with 71% of offspring sired by males other than the social male and interpret this as a potential adaptation to lack of choice of one's social mate. Second, I find that female flycatchers invest significantly more in nestling rearing than males and provide ¾ of nestling feeds. I propose this may be a male response to high probability that the fledgling in his nest is not his biological offspring. Third, I find that offspring fledging weights and survival to independence are not predicted by feeding rates, food volume provided to nestlings or by any habitat variables, but there is evidence that female flycatchers may protect offspring from any effects of food shortage in low quality habitat by incurring a personal cost in terms of short-term weight loss and long-term reduction in breeding frequency (chapters 4 and 5). Finally, I find that offspring from low quality territories, defined as territories with low invertebrate food abundance, are significantly more likely to be male. I propose that female flycatchers in low quality territories choose to produce male offspring because males have the opportunity to increase their reproductive output in low quality habitat; males can range outside their territory to secure extra pair paternity while females are tied to reproducing within their own territory with the prospect of reduced lifetime productivity in low quality habitat.

6.2 CONTRIBUTIONS TO THE CONSERVATION MANAGEMENT OF THE SPF AND OTHER THREATENED PASSERINES

6.2.1 Phylogenetic contributions

My phylogenetic reconstruction (chapter 2) highlights the importance of subspecific molecular phylogenetic reconstructions in complex island archipelago settings in clarifying phylogenetic history and ESUs that may otherwise be overlooked and inadvertently lost. I used the Pons et al. (2006) GMYC method to objectively delimit species units. This method, based on DNA sequence data, makes it a powerful tool to assist conservation planners with the difficult task of objective allocation of finite conservation resources. My findings have enabled a re-evaluation of conservation priorities within the *Terpsiphone* flycatchers of the Indian Ocean.

My phylogenetic reconstruction of *Terpsiphone* in the western Indian Ocean uncovered six cryptic ESUs that do not all align with current taxonomy, however all are more diverged than sister

species of some other passerines that have full-species status (see for example Lovette & Birmingham 1999; Johnson & Cicero2004) and I therefore consider that all six ESUs are sufficiently diverged to be worthy of management as if they are separate species. The Seychelles paradise flycatcher *T. corvina* is highly evolutionarily distinct and forms its own monophyletic clade dating back to the early Pleistocene. Within *T. bourbonnensis* on the Mascarenes, my findings suggest that the two island lineages on Mauritius and Reunion are sufficiently diverged to warrant management as separate ESUs. Remarkably my analyses revealed a high degree of divergence within the Madagascar paradise flycatcher *T. mutata* species found on Madagascar and the Comoros. Given these levels of genetic differentiation I observe within *T. mutata*, my findings suggest that three island lineages within *T. mutata* (Madagascar+Mayotte; Anjouan+Moheli; and Grande Comore) should be considered as separate ESUs and that they should be managed separately for conservation.

This information is likely to be important because the population of *T. b .desolata* on Mauritius, consisting of 100-223 pairs, is considered to be under threat from habitat degradation, fragmentation and impacts of invasive species. Currently, due to the subspecific status afforded to the flycatcher population on Mauritius, and the fact that the Réunion population is still fairly widespread and common, the population on Mauritius has struggled to attract conservation resources, despite local efforts to obtain funds for basic survey and ecological studies of this island form. Our findings may help to improve the conservation attention that this island population receives. Likewise our finding *T. mutata* forms 3 ESUs, that are not predicted by current taxonomy or by distance-based or island age-based models of colonisation, can help prioritise conservation efforts on the Comoro islands. Little conservation work is currently undertaken on their *T. mutata* subspecies due to the species' wide range and healthy overall numbers. Knowledge that there are three highly diverged lineages amongst these nearby islands may encourage baseline survey work to determine in more detail population sizes and distributions of these unique lineages and allow this novel phylogenetic diversity to be conserved. Given the critical conservation status of *T. corvina* (IUCN 2011), the current conservation efforts to improve the species' long-term survival prospects are supported by our findings and should be continued.

6.2.2 Population genetic contributions

Threatened species management has often taken the stand that threatened species have little genetic diversity left to lose, therefore genetic monitoring and management has been low on the list of priorities for their management. My finding that severely depleted genetic diversity following a historical bottleneck does not render a species immune to further genetic erosion upon reintroduction (chapter 3) has important implications for both future reintroduction strategy for the SPF and is also equally relevant for reintroductions of other threatened species. It provides novel evidence of the importance of incorporating genetic management into reintroduction programmes in order to

maximise retention of available genetic diversity and to avoid inadvertent loss of further genetic diversity.

I show that the Seychelles paradise flycatcher has lost substantial genetic diversity and genetic structure across the historical habitat loss-induced bottleneck, but also that the flycatcher introduction-induced bottleneck has left a smaller but unignorable reintroduction signature, and that in fact genetic diversity appears to have been lost at a faster rate per generation in the 22 months post release than estimates of per generation loss during the historical bottleneck.

Given the bottleneck experienced by the flycatcher population to a low of 28 individuals in the 1960's and the accompanying loss of genetic structure, the current SPF population has unprecedentedly low genetic diversity and potentially compromised adaptive potential. I also found that while the historical SPF population was genetically structured, there was also some gene flow between island populations. I therefore advocate (i) management of the two current island populations as one in order to maximise retention of genetic diversity in both populations over the shorter-term, (ii) further reintroductions in order to increase both the species' distribution range and population size in the medium-term, and (iii) a longer-term strategy to reduce management and allow this network of reintroduced island populations to undergo the natural processes of gene flow and drift.

In a wider conservation context my finding that threatened species with low genetic diversity are still susceptible to further loss as a result of reintroduction is likely to be highly relevant for the majority of threatened species. I therefore advocate genetic management of reintroductions should become the norm rather than the exception, and should be routinely incorporated into the planning, monitoring and management of all reintroductions.

6.2.3 Demographic and evolutionary contributions

The first reintroduction of SPF enabled a critical evaluation of the drivers of SPF productivity (chapter 4). My analyses revealed the importance of looking across multiple sites (source and reintroduced in the case of the SPF), in order to provide a more comprehensive understanding of the drivers of productivity. This finding will be equally relevant for other species, where reintroductions and habitat rehabilitation are undertaken as part of recovery programmes, particularly as managers often do not know if the remnant population of a particular threatened species is living in the best quality habitat for that species, or whether they are clinging on in suboptimal habitat. Therefore strategic monitoring of reintroductions can provide valuable information with which to fine-tune future reintroduction site selection and preparation.

Fine tuning SPF habitat assessment and recommendations for future reintroductions

My assessment of the drivers of SPF productivity (chapter 4) revealed that the main drivers of SPF productivity are invertebrate food abundance, native vegetation, altitude and the Seychelles bulbul and common myna. Higher invertebrate densities and higher percentage of native vegetation positively influence productivity, while higher altitudes and higher densities of SPF egg and nestling predators the common myna and the Seychelles bulbul negatively affect productivity. Therefore the most important habitat variables to consider when selecting sites for future habitat rehabilitation and reintroductions of SPF are invertebrate food densities, percentage native vegetation, altitude and an absence of the common myna and the Seychelles bulbul. The importance of invertebrate food abundance to flycatcher productivity is further emphasised by my finding that offspring sex is influenced by habitat quality (defined by food abundance), and that more male offspring are produced in lower quality habitat (chapter 5).

These findings together have important management implications for reintroduced populations. If the SPF are to be introduced to islands comprising low quality habitat, a scenario not inconceivable given the severe degradation of habitats on many of the Seychelles islands, the resulting populations may end up with biased sex ratios in favour of males with both short and long-term implications. In a small establishing population this prediction has important implications for initial population growth since fewer females' means fewer potential breeding pairs and a reduced ability for population growth as each pair can only produce a maximum of one offspring per breeding attempt. In the longer term an uneven sex ratio caused by production of predominantly males could also have implications for evolutionary viability of the population.

These findings have enabled me to fine-tune the factors important for flycatcher habitat rehabilitation and reintroduction. I recommend that habitat rehabilitation for SPF is targeted specifically to provide a lowland mixed broad-leaf forest promoting high invertebrate densities alongside eradication of any Seychelles bulbuls and common mynas, and that in order to promote an even sex ratio flycatchers are only introduced to sites that can demonstrate leaf invertebrate densities higher than Denis Island.

In order to streamline data collection of important food and vegetation variables for flycatcher productivity I recommend measuring just proportion native vegetation, canopy height and invertebrate abundance, using either malaise flight intercept traps or leaf counts. However I recommend measuring invertebrate densities more regularly than this study permitted, for example monthly or every two months in order to provide greater clarity on possible temporal/seasonal effects.

Lastly, given the relatively high level of 'dud' eggs observed in the reintroduced population on Denis Island compared to the source La Digue population, in order to maximise post-release

population growth in future translocations I recommend selection of females of known breeding ability in order to avoid translocating females incapable of reproducing successfully; establishing populations with low numbers of individuals are more susceptible to the influence of individual productivity than reintroductions based on larger numbers of founders.

My finding that female flycatchers have a degree of control over the sex of their offspring, and that they show a pattern of offspring sex allocation in relation to habitat quality, adds another species to the growing list of species to show control over the sex of their offspring in relation to habitat quality or to female condition (which is a proxy for habitat quality). For example: female kakapo produce more male offspring when they are in good condition, which for many years had a negative effect on efforts to recover the species as *ad libitum* supplementary feeding was a central component of the kakapo recovery programme. The supplementary feeding did increase kakapo productivity; however the vast majority of offspring were male (Clout et al. 2002). When the relationship between female condition (female weight) and offspring sex allocation was discovered, the supplementary feeding programme was revised to manage the weights of females resulting in elimination of the offspring sex ratio bias (Robertson et al. 2006). Komdeur et al. (1997, 2002) also showed that female Seychelles warblers have a high degree of control over the sex of their offspring and skew the sex ratio of their offspring in favour of females in good quality habitat. Offspring sex ratio skews in relation to female and habitat condition have also been shown in other taxa (e.g. mammals; Trivers & Willard 1973; Hoefs & Nowlan 1994). The finding that numerous species have control over the sex of their offspring certainly has important implications for the conservation management and reintroduction of threatened species in general, as its effects can render conservation efforts to recover a species ineffective if the evolutionary and ecological drivers of sex allocation are not understood. However as is demonstrated by the kakapo recovery programme if the drivers of offspring sex allocation are understood, they can be used to the advantage of species recovery.

The importance of the remnant La Digue SPF population

This study provides additional evidence of the importance of protecting the remaining habitat on the La Digue plateau in order to conserve the remnant SPF population. My research indicates La Digue plateau is the source of flycatcher production on La Digue and it appears that the hill is a possible sink or buffer zone that without constant reinforcement from the plateau may not be self-sustaining. However protecting the remaining coastal plateau habitat on La Digue is a major challenge. Over 90% of the western plateau is privately owned and subjected to contuinual habitat loss for domestic housing and tourist developments. The two main canopy forming native broadleaved trees on the western plateau Takamaka (*Calophyllum inpohyllum*) and Badamier (*Terminalia catappa*) are both protected under Seychelles law and can only be felled under licence, however permissions are granted to fell for construction (Seychellois need a place to live) and enforcement is also a major problem.

Finally I recommend that future translocations are sourced from La Digue as this population harbours the greatest genetic diversity and is the largest and most productive population, and is therefore the most robust to any effects of harvesting.

Choice of islands for future SPF reintroductions

Creation of additional populations of SPF is the highest priority action listed in the SPF species Action Plan (Currie et al. 2001) in order to increase the number of breeding populations. Currently there are no islands suitable for conservation introduction or reintroduction of SPF. However two islands stand out as potentially suitable for future flycatcher reintroductions; North and Félicité (see Figure 1.1 in chapter 1). North Island (201 hectares) has been of the focus of restoration activities to rehabilitate heavily degraded habitat since c.2000 in association with construction and development a resort on the island. Ship rats (*Rattus rattus*) were eradicated in 2005, a myna (*Acridotheres tristis*) eradication is currently underway and replanting native vegetation, particularly in degraded coastal plateau areas is ongoing. North has a large area of coastal plateau (c.67 hectares), the majority of which is still grassland and gardens, however there is a small area of Takamaka dominated broadleaved forest. Island owners are supportive of conservation activities and are committed to increasing the area of lowland forest. Seychelles White-eyes have already been successfully introduced to the island following the rat eradication. Significant replanting is required before North would be suitable for flycatchers, however the habitat rehabilitation work, funded by the resort is underway. North Island, like Denis, is outside the known historical range of flycatchers, however with limited choice of potential islands I do not think this fact should be given too much weight.

Félicité is a 267 hectare island situated 3.2km from La Digue and within the known historical range of SPF. Much of the island is high and rocky, however there is a significant area of aproximately 35 ha of coastal plateau. Habitat for SPF on Félicité is currently quite degraded and considerable habitat rehabilitation work is required before the island would be suitlable for reintroduction. However in 2009 a consoirtium started a large hotel resort development on Félicité and as part of this development they are planning to undertake significant habitat rehabilitation. If this habitat rehabilitation can be guided to provide good SPF habitat (which would involve rat and myna eradications and considerable replanting of native broadleaved woodland in lowland areas) then Félicité would be high on the list of islands to support a SPF reintroduction. The people of La Digue would be much happier to see ‘their’ flycatcher reintroduced to Félicité than to an island further from La Digue, a consideration that the Seychelles Ministry of Environment do take into account. However with only 35 hectares of coastal plateau, Felicite will not likely hold a large population which also must be taken into consideration. Managers on both North and Félicité have expressed a desire to

introduce flycatchers, and we are now in a good position to guide habitat rehabilitation to maximise flycatcher invertebrate food abundance on these islands based on sound scientific data.

Closing remarks

My research shows the importance of targeted monitoring of reintroductions and measuring their genetic diversity, their productivity and their survival against source populations in order to inform future management strategy. Using this research-by-management type approach to threatened species management where management actions are strategically monitored and the findings used to inform future management action is the way forward for threatened species conservation and reintroduction biology.

6.3 FUTURE RESEARCH

All the research I have undertaken to determine drivers of productivity and what constitutes good quality flycatcher habitat has been based within flycatcher territories i.e. based on where flycatchers were. As discussed in chapter 4, many habitat variables were quite homogenous within my study sites, which could result in an underestimation of their importance in my analyses of factors important to flycatcher productivity. Our understanding of what constitutes high quality SPF habitat would therefore benefit from a comparison of habitat variables in areas where flycatchers are not with areas where they are.

Given the high level of extra pair paternity (EPP) I observed in the SPF (chapter 5) further research to determine which males are securing EPP, and what variables correlate with success in gaining EPP would be very interesting. Variables such as male heterozygosity, male tail length (male flycatchers have very long tails of up to 33cm long, and tail length is very variable between males) and degree of engorgement of male fleshy eye ring, for example, could be quantified alongside paternity of offspring.

Future genetic studies of the reintroduced population on Denis Island would be insightful in order to quantify the longer-term magnitude and effects of genetic drift on this reintroduced island population relative to the species' historical loss of genetic diversity and to the source population on La Digue.

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Appendices

Appendix 1 Island assessment report

Assessment of island suitability to support self-sustaining flycatcher *Terpsiphone corvina* populations

This document has been prepared under the Darwin Initiative funded project "Investing in island biodiversity; restoring the Seychelles paradise flycatcher" by the Project Officer Rachel Bristol and the Project Leader Dr Jim Groombridge at the request of the Principal Secretary of the Ministry of Environment, Dr Rolph Payet.

Introduction

Human colonisation of Seychelles in the 1770's followed by forest clearance for timber, agricultural development and guano extraction resulted in severe environmental degradation and the extinctions of endemic bird populations on many islands. Records of historical distributions of endemic birds are sketchy and incomplete prior to 1865 (Rocamora & Skerrett, 2001).The Seychelles paradise flycatcher was historically recorded on a minimum of 5 inner islands, see table 1 for details. The flycatcher experienced a marked reduction in range following human colonisation attributed to forest clearance on coastal plateau areas and the accompanying introduction and establishment of alien mammal predators.

Table 1: The documented historical range of flycatchers

Island	Year	Population estimate	Reference	Comments
Felicite	1906 (extinct)	-	Nicoll (1906)	(sporadic reports of solitary individuals 1970's-1990's)
Marianne	1936 1998 2000	- c.3 c. 1-2	Vesey-Fitzgerald (1940) Parr and Shah (1988) Hill (2001)	(sporadic reports of solitary individuals 1970's-1990's)
Aride	1907 (extinct)	-	Diamond (1984)	
Praslin	1978 1989 (extinct)	c.3 c.3	Watson (1984) Gerlach (1997)	
La Digue	1965 1971 1977 1988 1996-97 2001	28 50-90 66 73 138 (150-200) (218-290)	Gaymer et al. (1969) Beamish (1972) Watson (1981) Watson (1988) Rocamora (1997) Currie et al. (2003)	

The only viable population of flycatchers occurs on one island and is therefore potentially vulnerable to extinction. Population Viability Analysis modelling of the flycatcher population using all the available real data on breeding success, juvenile recruitment and adult survivorship predicts the La Digue population has an almost 100% probability of being extinct in within 30 years. Establishment of additional populations on other suitable islands is considered a major priority in improving its chances of long-term survival and decreasing the risk of extinction (Currie et al. 2001; Currie et al. 2003; Hill 2002; Watson 1984, 1991; Hambler 1992; Rocamora 1997; Marshall 1997).

In addition current and future levels of development on La Digue, especially in the western plateau (the strong-hold of the species) are incompatible with any significant future improvement in the flycatcher's conservation status, due to the limitations that development -compounded by multiple ownership - imposes on expansion of the flycatcher population. The most feasible option to increase flycatcher range and numbers in the short-medium term is establishment of additional populations by translocation of individuals to other islands with suitable habitat.

A flycatcher Species Action (management) Plan was produced following a number of stakeholder workshops in 2001. The Objective of the Species Action Plan for flycatchers is to increase the number of breeding populations to at least 3 by 2006 (Currie et al. 2001) by establishment of additional populations on other suitable islands. However at the time of writing in 2001 no islands were suitable to support flycatcher populations because they either lacked sufficient broad leafed native plateau forest habitat or had alien predators, specifically *Rattus rattus* and cats *Felis catus.*

The Seychelles Paradise flycatcher is currently considered the most threatened bird in Seychelles. Research assessing habitat requirements of the Flycatcher was conducted on La Digue from 1999-2001 (BirdLife Seychelles). The work was conducted in parallel with assessment and ranking of suitability of medium sized islands within the inner Seychelles archipelago for avian ecosystem (including flycatcher) restoration using biological, geographical and anthropogenic criteria (Hill 2002). This document updates the previous work by combining data on recent successful predator eradications and current progress or future potential for habitat restoration, allowing a reassessment of the translocation options for the Seychelles Paradise Flycatcher. Specifically, this review uses both published and more recent unpublished data to re-assess fifteen inner Seychelles islands for their suitability to support flycatcher populations using criteria known to be important for flycatchers.

Summary of Flycatcher habitat requirements

1. Food

The flycatcher appears to be exclusively insectivorous (Watson 1988, 1991; Currie et al. 2003a). The diet includes Orthoptera, Lepidoptera, Coleoptera, Araneae and Diptera (Watson 1991; Gerlach 1997;

Currie et al. 2003a) although numerous small unidentifiable prey items are also taken. Flycatchers feed throughout the vegetation strata, though mostly mid-canopy, predominantly by gleaning prey items from the surface of leaves (either on the wing or from a perched position) and to a lesser extent taking aerial insects in flight (Watson 1988, 1991; Currie et al. 2003a).

2. Vegetation

All research into flycatcher habitat requirements highlights the importance of native broad-leafed plateau woodland (Watson 1981, 1988, 1991; Currie et al. 2003a). Field studies show that flycatcher territories contain significantly more native tree species than predicted by their availability on the plateau. Furthermore, flycatchers use native tree species significantly more for foraging and nesting than predicted by their availability within territories, and there is an inverse correlation between density of native tree species and territory size (Currie et al. 2003a). On the La Digue plateau, flycatcher territories comprise predominantly native broad-leaved woodland and range in size from 0.4- 2.5ha (mean 1.04ha; Currie et al. 2003a).

3. Presence of water/ marsh

The relationship between flycatchers and fresh water sources appears to have been over-emphasised in the past. An island wide survey in 2001 indicated that flycatchers are not necessarily associated with wetland. For instance, in a study by Currie et al. (2003c), presence of high canopy native forest was the only significant factor associated with flycatcher distribution. In addition 66% of prey items were not associated with water and less than 10 % of identified prey was dependant on water at any stage in their life cycle (Currie et al. 2003a). However, tall canopy is often associated with water/dampness, and it would therefore be unwise at this stage to translocate flycatchers to islands that do not contain any marshland areas.

4. Altitude

There is no data available for flycatcher breeding success, recruitment or mortality for territories on the hill on La Digue. The island wide survey in 2001 indicated that flycatchers are not necessarily associated with coastal plateau. Whilst we do know that there are flycatcher territories on the hill and that successful breeding does occur (RM Bristol pers. obs.), the majority of flycatcher territories (>70% in 2001) are found on the plateau. Indeed, there are significantly more birds per unit area on the plateau than hill areas (Currie et al. 2003c) and all areas with broad leaf woodland on the plateau house flycatchers, whereas flycatchers are not associated with all areas of broad leaved native forest on the hill. Given this strong habitat preference for plateau broad-leafed woodland, flycatchers should not be translocated to islands lacking sufficient coastal plateau broad-leafed woodland areas.

5. Predators

Flycatchers have been lost from at least four other Seychelles islands within their historic range, and it is likely that predation contributed significantly to this loss. Indeed, rats (*Rattus rattus, R. norvegicus*), cats (*Felis catus*) and Seychelles bulbuls (*Hypsipetes crassirostris*) are all known predators of flycatchers (Currie et al. 2005; JU Bristol pers. comm.). Although the flycatcher population on La Digue has so far persisted in the presence of rats and cats, perhaps due to this population choosing to locate its nests on the ends of long, thin down-hanging branches making them less vulnerable to predation (Currie et al. 2003b), a small translocated founder population will be extremely vulnerable to impacts of predation. An iron rule developed by conservationists recovering island species in New Zealand and elsewhere is that eradication of predators is an essential prerequisite prior to any reintroductions, and is a crucial component for restoring island ecosystems. It is therefore not appropriate to translocate flycatchers to any island with rats, cats or bulbuls.

6. Threats

The major threat to the flycatcher on La Digue is habitat loss through deforestation for housing, tourist development, clearance for agriculture and more recently the emergence of Takamaka wilt disease *Leptographium calophylli*. Currently the vast majority (>90%) of flycatcher pairs occur outside the 21 ha Veuve Special Reserve, and mainly on privately owned land. This distribution makes their territories more vulnerable to loss or degradation through development. Furthermore, the majority (>70%) of the flycatcher population live on the large western plateau where they are in close contact with the human populace.

Island assessment

Rationale regarding island size

Biodiversity surveys for conservation potential of Seychelles islands for threatened endemic birds have been conducted for 10 islands (Hill 2002). Initial selection was made on the basis of island size, human population and land ownership. The smallest islands (under 20ha) were rejected because in most cases they could only support small populations of endemic land birds, leaving those populations vulnerable to stochastic risks of extinction. Large islands (over 500ha) were also rejected because whilst they have great conservation potential, they also have a number of disadvantages: most have large human populations with associated introduced animal species, and most have multiple land ownership which complicates management. However, medium sized islands have the advantages of small islands in that most have single ownership, small or non-existent human populations, and the potential to eradicate alien predators and maintain a continued predator free status. A further 5 islands have been included in this current assessment.

In this document, island suitability for flycatchers is assessed using three key criteria important for flycatcher survival, in particular the available area of native broad leaved plateau forest, invertebrate (food) abundance and predator-free status. Six additional factors are also included which further help to define the appropriateness of the islands.

Broad-leaved native forest

On La Digue flycatcher territories comprise predominantly native broad-leaved woodland and range in size from 0.4-2.5 ha (mean 1.04 ha, Currie et al. 2003a). Preferred islands are those that can hold the largest populations i.e. that have the most native broad-leaved plateau forest.

Food availability

Flycatchers feed exclusively on insects, mostly gleaned from the surface of leaves by gleaning or sally gleaning (80% of successful feeding observations, Currie et al. 2003a). Invertebrate counts on leaves are therefore considered to be a good indicator of food availability (Hambler 1992; Currie et al. 2003b). Bi-monthly leaf counts were made on La Digue for 15 months from September 1999 to December 2000. For all assessed islands invertebrate leaf counts were made in both the wetter North-West and drier South –East seasons. These island counts were compared to the La Digue counts made in the same months. Islands with leaf invertebrate counts similar or comparable to La Digue are considered to have sufficient food for flycatchers. Leaf counts can be very variable and are probably affected by local environmental conditions. However, in general, leaf count values for Takamaka and Badamier from most islands appear comparable with La Digue. See table 2 for results of the leaf counts. Hill (2002) concluded that as native trees have higher invertebrate counts than non-native trees; islands with the most native woodland habitat have by far the best potential for insectivorous birds such as the flycatcher.

Table 2: Invertebrate Counts on leaves

Island	Season	Takamaka	Badamier	Natives	Non-natives
Bird	NW	-	-	39.89	19.95
	SE	-	-	-	-
Conception	NW	0.86 (8.83)	-	5.07	1.42
	SE	5.94 (4.93)	-	6.65	6.65
Cousin	NW	-	-	25.88	-
	SE	-	-	49.76	-
Curieuse	NW	2.85 (8.83)	0.49 (5.81)	5.45	3
	SE	9.65 (9.61)	5.55 (4.75)	5.09	57
Denis	NW	10.26 (6.21)	10.50 (5.11)	11.54	9.39
	SE	2.08 (4.95)	3.08 (2.80)	4.68	4.07

Felicite	NW	-	-	-	-
	SE	2.08 (4.95)	-	2.17	-
Grand Soeur	NW	-	-	-	4.57
	SE	13.41 (9.61)	6.64 (4.75)	5.64	-
Marianne	NW	5.52 (6.21)	5.80 (5.11)	8.21	16.56
	SE	4.12 (4.77)	2.79 (3.47)	8.01	6.83
North	NW	11.49 (8.83)	-	10.48	7.18
	SE	5.61 (4.95)	4.83 (2.82)	7.49	4.95
Therese	NW	2.18 (8.83)	1.98 (5.81)	3.05	2.74
	SE	10.13 (4.95)	14.76 (2.80)	10.14	20.74

From Currie et al. 2003

Mean number of invertebrates on foliage (m2) excluding ants and soft bugs (calculated after Hill 2001) on Takamaka *Calophyllum inophyllum* and Badamier *Terminalia catappa* 1999-2000, all native tree species and non-native tree species in the north-west and south-east (SE) monsoons. Data in parentheses are the equivalent values for La Digue sampled in the same months.

Predator Status

Available data show that rats, cats and Seychelles bulbuls are adult and/or nest predators (Currie et al. 2005; JU Bristol pers. comm.) Small establishing populations are more vulnerable to the effects of predation than larger established populations therefore islands without these predators are considered preferable.

Six Additional factors that could influence island suitability

1. Climate

Drought or extremely dry conditions can affect invertebrate availability, and insect counts are almost always lower in the dry South-East monsoon season. Rainfall is therefore a consideration. Rainfall records have not been collected for most of the medium sized islands, but existing information suggests that the smaller, lower islands have generally lower rainfall than the larger higher islands (mean annual rainfall c. 1,500mmm-2,000mm; c.f. La Digue mean annual rainfall 2,026mm-2,128mm.) Most assessed islands will have comparable but slightly lower rainfall than La Digue.

2. Potential for habitat rehabilitation to increase area of habitat suitable for flycatchers

Before any translocation takes place sufficient suitable habitat must exist to support a reasonable-sized population (suggested minimum of 10 pairs). However, habitat rehabilitation potential is also an important factor to consider as it indicates which islands have the best future potential to increase flycatcher numbers. Therefore plateau size (area available for rehabilitation) is an important factor to consider.

3. IUCN translocation guidelines

IUCN translocation guidelines recommend that translocations should be restricted to a species former range. However in the case of Seychelles land birds, little is known of original distribution prior to Newton (1867) by which time the islands and their original habitat had been significantly altered (Rocamora & Skerrett 2001). To date the vast majority of translocations of Seychelles threatened endemic land birds have been to islands outside of known former ranges, and almost all have also been extremely successful. Therefore as long as ecological and ownership requirements are satisfied, Seychelles conservation efforts cannot afford to be restricted by known historical range

4. Island commitment to conservation

In addition to satisfying all biological criteria, it is essential that island ownership or management must be able to demonstrate a history of long term commitment to conservation, and there should be an active island management plan that incorporates conservation objectives in order to receive serious consideration as a recipient for translocated flycatchers (or any other threatened endemic). Management plans are important for forward planning regarding habitat management and other factors such as environmentally friendly pesticide and pollutant policies that will not compromise the well-being of translocated insectivorous birds.

5. Proximity to other islands

Islands in close proximity to neighbouring islands have a greater chance of (re)invasion of unwanted predator species especially rats. Norway rats are known to swim up to at least 1 kilometre and ship rats up to 700m (postgradnews 2004; Towns et al. 2006). Islands closer than 1 km to a neighbour that has predators have a higher risk of alien species invasion.

6. Presence of other species with similar habitat requirements

Many of Seychelles endemic land birds are partially or entirely insectivorous (8 of 12 land birds endemic to the granitic islands). Historical records of species assemblages on islands are poor: however current species assemblages indicate that species with very similar diets can coexist (e.g. Seychelles fodies and Seychelles white-eyes on Frégate; Seychelles fodies and Seychelles warblers on Cousin and Cousine).

Ranking of potential islands

Islands have been ranked using criteria deemed important for flycatchers.

Table 3: Criteria included in Mean Ranks

Rank	Island size	Plateau size	Area of existing broad-leafed native plateau forest	Marsh size	Predator status
Mean Rank 1					
Mean Rank 2					
Mean Rank 3					

Mean Rank 1 ranks islands on their current suitability to support a translocated flycatcher population. Mean rank 1 is ranked on predator status, current area of plateau woodland and marsh size.

Mean rank 2 and 3 give an indication of the future potential of an island to support a flycatcher population. They include island size and plateau size and exclude predator status as predator status can be changed, and plateau and island size give an indication of potential for increasing the area of suitable habitat.

Mean rank 2 is ranked using plateau size, plateau native broad-leafed woodland area, and marsh area. It excludes island size and is irrespective of predator status

Mean rank 3 is ranked on island size, plateau size, marsh size, and area of broadleaved native plateau forest and excludes predator status.

See table 4 for island ranking results. Ranks are in parentheses.

Table 4: Ranking of islands suitability for sustaining a translocated flycatcher population

Island	**Size (ha)**	**Area of plateau (ha)**	**Broadleaf native forest (ha)**	**Marsh/ wetland (ha)**	**Pradator Status Rat/cat/bulbul**	**Mean Rank 1** (area of existing broadleaf native plateau woodland, marsh area, and excluding all islands with predators)	**Mean Rank 2** (plateau size, marsh size and area of plateau woodland)	**Mean Rank 3** (island size, plateau size, marsh size and area of plateau woodland)	**Ownership**	**Historically recorded (Y/N)**	**Current carrying capacity** ##
Aride	68 (12)	5.2 (13)	3 (5)	0.3 (2)	N/N/N	3.5	6.7	8	single	Y	**1.2** [2.9]
Bird	82 (10)	82 (2)	12.6 (3)	0 (4)	N/N/N	3.5	3	4.75	single	N	**5.0** [12.1]
Conception	60.3 (13)	13.1 (10)	0 (11)	0 (4)	Y (R.norvegicus) /N/N		8.3	9.5	single	N	**0** [0]
Cousin	27 (14)	22 (7)	0.7 (9)	0.2 (3)	N/N/N	6	6.3	5.75	single	N	**0** [0]
Cousine	25.7 (15)	6 (12)	>1 (7)	0 (4)	N/N/N	5.5	7.7	9.5	single	N	**0** [1]
Curieuse	286 (3)	74 (3)	27.9 # (2)	0.7 (1)	Y (R.rattus) /N/N		2	2.25	government	N	**11.2** [26.8]
Denis	140 (7)	140 (1)	30 (1)	0.3 (2)	N/N/N	1.5	1.3	2.75	single	N	**12** [28.8]
Felicite	268 (4)	35.4 (5)	0 (11)	0.2 (3)	Y (R.rattus)/Y/N		6.3	5.75	government?	Y	**0** [0]
Frégate	210 (5)	30 (6)	<1 (8)	0 (4)	N/N/N	6	6	8.5	single	N	**0** [0]
Grand Soeur	87 (9)	17.8 (9)	1.8 (6)	0.2 (3)	Y (R.rattus)/Y/N		6	6.75	single	N	**0.7** [1.7]
Marianne	94.7 (8)	17.9 (8)	0.4 (10)	0.2 (3)	Y (R.rattus)/Y/N		7	7.25	single	Y	**0** [0]
North	201 (6)	67.4 (4)	12.2 (4)	0.7 (1)	N/N/N	2.5	3	3.75	single	N	**4.9** [11.7]
Praslin	2756 (1)				Y (R.rattus, R. norvegicus)/Y/Y				multiple ownership	Y	
Silhouette	1995 (2)				Y (R. rattus, R. norvegicus)/Y/Y				single	N	
Therese	73.9 (11)	10.2 (11)	0 (11)	0.3 (2)	Y (R. rattus)/Y/N		8	8.75	single	N	**0** [0]

Much of this plateau forest is Takamaka and has been badly affected by Takamaka wilt disease
Ranks are in parentheses
Current carrying capacity: Figures in **bold** are conservative and calculated from the largest plateau territory sizes on La Digue. The figures in square brackets are calculated from the mean plateau territory size on La Digue

Results of island assessment

Current potential to hold a sustainable flycatcher population

Currently, the most suitable island to hold the largest flycatcher population is Denis Island. This is because;

(i) it is rat, cat and bulbul free.

(ii) it has the largest area of plateau broad leafed woodland of any of the medium sized islands surveyed.

(iii) the woodland is Badamier dominated which means it is not subject to Takamaka wilt disease.

(iv) the owners have a demonstrated commitment to conservation, and have a conservation-minded management plan that includes ongoing rehabilitation to increase the area of habitat suitable to flycatchers.

(v) Denis has a significant area of wetland (which may be important for sustaining flycatcher habitat).

Currently Denis is expected to be able to hold 12-28 pairs. North is the next best scoring island for current translocation, followed by Bird and Aride. However these three islands, for different reasons, are not currently suitable to support a flycatcher population.

Reasons for rejecting North Island:

North has an extensive wetland area and the owners are sympathetic to conservation. Rats were eradicated from the island in 2005 and there is a conservation management plan including plans for extensive habitat rehabilitation. However there is currently only one small area of broad-leafed native plateau woodland and it is extensively damaged by Takamaka wilt disease (Hill 2002; JU Bristol pers. comm.). Significant habitat rehabilitation of native woodland is required before a translocation of flycatchers becomes feasible.

Reasons for rejecting Bird Island:

Bird Island has no wetland. Whilst the owners are sympathetic to conservation, they have expressed a desire not to introduce Critically Endangered birds for the time being as a consequence of the accompanying responsibilities and potential restrictions that accompany such activities. In addition Bird Island has had a serious outbreak of introduced crazy ants; the ants were first recorded on Bird in 1991 and by 1998 had reached very high densities disturbing breeding birds and causing breeding failure, and adversely affecting invertebrate populations (flycatcher food supply) (Hill et al. 2003; Feare 1988, 1999a, 1999b). Pesticides used to control crazy ants may also be harmful to flycatchers.

Reasons for rejecting Aride:

Aride mainly scored highly because of its large area of freshwater wetland. Aride management is sympathetic to conservation and the island is a Nature Reserve. However Aride only has 3 hectares of broad-leafed plateau woodland which is insufficient to hold a self-sustaining flycatcher population.

Future potential of islands to support flycatchers

In terms of future potential to support good sized flycatcher populations, the most important factors are likely to be (i) area of plateau and (ii) island ownership being sympathetic to conservation and willing to implement habitat rehabilitation programmes such as predator eradications and planting of broad-leaved native woodland in plateau areas.

Denis Island scores highly in both these categories because the entire island consists of plateau, and habitat rehabilitation commenced in 2000 and is ongoing.

Curieuse also scores highly, mainly due to proportion of plateau area, presence of marshland and existing area of Takamaka woodland, however with the amount of daily boat traffic to Curieuse and close proximity to Praslin, the likelihood of the island remaining rat free after an eradication attempt (currently not planned) is small. In addition extensive woodland rehabilitation is required as Takamaka wilt has damaged and killed large areas of previously suitable Takamaka woodland and currently only very limited rehabilitation is occurring (S Pillay pers.comm.)

North Island scores 3rd highest as it has a large area of plateau, has already eradicated rats and cats, and has commenced a habitat rehabilitation programme. Currently there is only a small area of native broad-leafed plateau woodland and the presence of Takamaka wilt disease has reduced the quality of existing habitat. Extensive habitat rehabilitation is required before this islands long-term potential for holding flycatchers can be realised.

Historically, Praslin supported a population of flycatchers. The island has extensive areas of plateau and wetland, however it also has a large human population and it would be extremely difficult if not impossible to control predators and to rehabilitate the necessary habitat to support flycatchers. Flycatchers became extinct on Praslin, and unless the factors responsible for that extinction are rectified, the same fate awaits any flycatchers reintroduced there.

Felicite and Marianne were also in the historical range of flycatchers. However, whilst these islands have reasonable areas of plateau, both have next to no plateau broadleaved native woodland and would require significant habitat rehabilitation including predator eradications prior to any translocation and currently there are no plans to undertake habitat rehabilitation. In addition there is evidence of highly toxic and persistent pesticide (Aldrin and Dieldrin) use on Felicite through the 1980's.

Silhouette has some plateau and marshland areas, (information on plateau marshland sizes not available) however it is unlikely that rats and cats will be eradicated given the island's relatively large size and difficult terrain.

Discussion/Conclusion

There are constraints to basing habitat requirement decisions on relict populations; we know populations are sometimes restricted to habitat that is perhaps not typical of historical distributions or representative of all habitat types a species could thrive in. However as Seychelles paradise flycatcher habitat requirements appear to be more specific than other species in the *Terpsiphone* genus (Currie et al. 2001), and given the rarity of the species and the need for the first translocation to succeed, translocation to islands that lack habitat similar to the La Digue plateau would, at this stage, be unwise.

Two major factors underpin the choice of Denis Island as the primary candidate island to be the recipient of the first translocated population of flycatchers. Based on observations of the La Digue population, the primary predictors determining island suitability appear to be (i) the presence of healthy native high canopy broadleaf forest, characteristically found on coastal plateau, and (ii) the predator-free status of the island (Currie et al. 2003). This report identifies strong 'island suitability' as an absence of predators to reduce adult survivorship and/or limit reproductive success, extensive natural or native broad-leaf dominated plateau forest and the presence of freshwater wetland.

Currently, based on the La Digue model, the only island with enough habitat suitable to support a flycatcher population is Denis Island. In its current state Denis Island could hold a conservative minimum of 12 pairs of flycatchers, with additional pairs expected if marginal habitats are utilised or the area of woodland is extended. In addition the Environmental Management Plan for Denis Island sets out plans for considerable increase of the area of native broadleaved woodland on the island.

March 2007.

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Appendix 2 Reintroduction proposal

Proposal for:

A Conservation Introduction of

Seychelles Paradise Flycatcher from La Digue to Denis Island

Prepared under Darwin Initiative Funded project 15/009 "*Investing in island biodiversity; restoring the Seychelles Paradise Flycatcher.*"

By the Project Officer Rachel Bristol and the Project Leader Dr. Jim Groombridge

April 2008

Executive Summary

The objective of the Seychelles Paradise Flycatcher *Terpsiphone corvina* Action Plan is to increase the number of breeding populations to at least 3 by 2006. Currently the species is restricted to a single island population of c.250 individuals on La Digue. Denis is considered the most suitable island to support a second population: Denis Island is free of rats, cats and bulbuls, has several permanent wetland marshes and has approximately 30 hectares (ha) of good quality mature Badamier dominated native broad-leafed woodland very similar to the woodland on the La Digue plateau; prime habitat for flycatchers. In addition further habitat rehabilitation by this project is now underway on Denis to increase the area of this high canopy native broad-leafed woodland. Denis Island management are very supportive of conservation, have a history of threatened bird conservation introductions on Denis, and have zoned a large area of Denis for conservation as detailed in the Denis Island Management Plan.

Approximately 20 Seychelles Paradise Flycatchers (SPF) will be caught on La Digue, transferred by helicopter and released the same day on Denis Island. This operation is scheduled for November 2008, directly prior to the birds' main breeding season.

Intensive monitoring of the flycatchers will be undertaken by Darwin Initiative flycatcher project staff.

1. Introduction

1.1. ***Background***

Originally recorded on at least 5 islands in the granitic Seychelles, the SPF underwent a drastic decline in numbers and range in the late 19th and early 20th century coinciding with the clearance of native broad-leaved plateau forest and draining of wetlands as part of the expansion of the local copra industry and plantation agriculture.

Today the flycatcher is listed as Critically Endangered [B1ab (iii)] by the IUCN due to small population size and very restricted range (IUCN 2007).

2. Objective

The objective of the Species Action Plan is to increase the number of island populations to at least 3 by 2006 (Currie et al. 2001).

3. Rationale

3.1. In order to reduce the threat of extinction there is a recognised need to create additional populations of Seychelles Paradise Flycatcher (Currie et al. 2001, 2003b; Hill et al. 2002a; Watson 1984, 1991; Hambler 1992; Rocamora 1997; Marshall 1997).

3.2. Until recently there have not been suitable islands available to support an introduced flycatcher population; however Denis Island has undergone considerable habitat rehabilitation including the eradication of rats and cats (2002 and 2000 respectively) and the removal of large areas of abandoned coconut plantation and replacement with native broad-leafed woodland favoured by SPF.

3.3. An island assessment of 15 medium sized islands in the granitic Seychelles group, considering all criteria deemed important for flycatchers, identified Denis Island as the only island currently suitable to support a self-sustaining SPF population. Denis is identified as most suitable based on a number of criteria: (i) it is rat, cat and bulbul free; (ii) it has the largest area of plateau broad leafed woodland of any of the medium sized islands surveyed; (iii) the woodland is dominated by Badamier which means it is not subject to Takamaka wilt disease; (iv) the owners have a demonstrated commitment to conservation, and have a conservation-minded management plan that includes ongoing rehabilitation

to increase the area of habitat suitable to flycatchers and (v) Denis has a significant area of wetland (which may be important for sustaining flycatcher habitat).

3.4. Denis Island also scored highest in the island-wide assessment of future potential to support SPF populations. The reason for this is in terms of future potential to support good sized flycatcher populations, the most important factors are likely to be (i) area of plateau and (ii) island ownership being sympathetic to conservation and willing to implement habitat rehabilitation programmes such as predator eradications and planting of broad-leaved native woodland in plateau areas. Denis Island scores highly in both these categories because the entire island consists of plateau, and habitat rehabilitation commenced in 2000 and is ongoing, with work being currently supplemented by restoration activities under this project.

4. Timing of translocation

4.1. The transfer of birds' should be undertaken immediately prior to the breeding season, when the birds are at their peak fitness and when the most food resources should be available, thereby easing the stress of a translocation i.e. in November 2008.

4.2. The Darwin Initiative flycatcher project runs until end 2009 providing project resources, support and experienced personnel ensuring intensive and detailed implementation and monitoring of all aspects of the translocation.

4.3. The Darwin Initiative project has the resources, staffing and capacity to intensively monitor the newly introduced Denis Island population, especially important for this first translocation of SPF.

4.4. The Darwin Initiative project has the resources and staff capacity to intensively monitor the La Digue population concurrently to follow population recovery and to collect comparative data on breeding success between the two island populations.

4.5. The Darwin Initiative project as been working for the past 18 months with the La Digue Development Board (LDDB) and community and they are prepared for the translocation. We need to move ahead now before the momentum and local support fades from memory. Support for the translocation has been provided to the Seychelles government (GOS) (refer to Letter from LDDB to GOS in November 2007).

5. Summary of Suitability

For details of island assessments and suitability of Denis Island please refer to the Island Assessment document submitted to GOS in April 2007 by Bristol & Groombridge.

5.1. ***Predator Status*****:** Denis Island has undergone 2 mammalian predator eradications and is free of rats and cats. Cats were eradicated in 2000 and rats and mice in 2002. Rat exclusion measures are permanently in place and maintained to reduce the risk of reinvasion. Bulbuls, the other major predator of flycatcher eggs and nestlings on La Digue, are also absent from the island.

5.2. ***Habitat*****:** Denis has a land area of c.140ha with approximately 30ha of mature broadleaf native forest. Currently the area of habitat suitable for SPF is sufficient for a c.28 pairs of Flycatchers [based on the average territory size on the La Digue plateau (Currie et al. 2003)]. Further habitat rehabilitation outlined in the Denis Island management plan, and currently underway under the Darwin Initiative flycatcher project will increase the area of broad-leafed native woodland and the carrying capacity of the island.

5.3. ***Food availability*****:** Invertebrate leaf counts and aerial trapping in both the North-west and the South-east seasons have yielded similar invertebrate densities and family assemblages to the La Digue plateau (Hill et al. 2002b; Currie et al. 2003a, 2003b) indicating the island has sufficient invertebrate food for flycatchers.

5.4. ***Anthropogenic factors*****:** Human activities are tightly managed and hence the possibility of accidental death through poisoning, pesticide use etc. is minimal. Denis Island management is aware of the dangers presented by pesticide poisoning to insectivorous birds and other threats to native bird life. Only bird friendly pesticides are used on the island.

5.5. ***Similar species*****:** Seychelles warblers introduced to Denis in 2004 also feed on Invertebrates. They however use different habitat types and feed at different heights in the vegetation. Warblers are more inclined to feed in densely foliated plants and in low vegetation. Historical records indicate flycatchers and warblers co-existed (Marianne and likely other islands) and there is no reason to assume they cannot now. The majority of Seychelles endemic land birds are partially or entirely insectivorous (8 of 12 land birds endemic to the granitic islands). In addition current species assemblages prove that species with very similar diets can coexist (e.g. Seychelles fodies and Seychelles white-eyes on Frégate; Seychelles fodies and Seychelles warblers on Cousin and Cousine).

5.6. ***Disease screening*****:** 182 individuals of common bird species from La Digue (109) and Denis (73) have been health screened for blood and intestinal parasites and diseases. See appendix 1 for disease screening details, results and interpretation. The results can summarised as follows: (i) no haematozoa

(blood parasites) were detected in any of the 182 birds screened (ii) three species of intestinal parasite were detected in the faecal samples *Eimeria* spp, *Isospora* spp and *Capillaria* spp. (iii) all parasites found in the SPF and the other bird species are common to both Denis and La Digue so there is no risk of introducing novel diseases either to the SPF or to Denis as a result of the translocation. Dr Andrew Greenwood (an experienced avian veterinarian advising on this project) interprets the results as follows "I have studied the sample results of the faeces and it seems that there is nothing on Denis which isn't already on La Digue (as we might have expected). The only worrying parasite might have been the *Isospora*, which can become invasive as Atoxoplasma under some circumstances, but it appears to already be in the SPF anyway". Given the outcome of this comprehensive disease survey and conclusions of the avian veterinary partners on this project, there is no impediment to progressing with the translocation of SPF as planned. Indeed, the disease survey has been invaluable in providing baseline data that can be integrated with further avian health screening in the future.

5.7. ***Rainfall*:** Rainfall on Denis is similar to La Digue. In general Denis receives slightly less rainfall than La Digue and the rainfall is more evenly spread through-out the year (Hill et al. 2002b) thus avoiding excessively dry periods which is likely a positive thing for flycatchers. Rainfall records for Denis for the last decade are mostly lacking with the only full year of data recorded in 2006. In 2006 Denis recorded 2023.2 mm of rain with the only month recording no rainfall being February. For the same period (Jan-Dec 2006) La Passe, La Digue recorded 1966.5 mm of rain and Belle-vue, La Digue recorded 1951.0 mm with rainfall recorded in all months (data provided by Seychelles Meteorological Services and Denis Island).

5.8. ***Wetland area:*** Denis Island is unusual for a coralline island in that it has significant areas of freshwater marsh in the interior of the island which may be important for sustaining flycatcher habitat.

5.9. ***IUCN guidelines:*** IUCN guidelines for reintroduction recommend that translocations should be restricted to a species former range (IUCN 1998). Denis is outside the SPF's known former range. However it is very likely that other Seychelles islands, including possibly Denis, held SPFs historically as in the case of Seychelles land birds, little is known of original distribution prior to Newton (1867) by which time the islands and their original habitat had been significantly altered (Rocamora & Skerrett 2001). To date the vast majority of translocations of Seychelles threatened endemic land birds have been to islands outside of known former ranges, and almost all have also been extremely successful. Therefore as long as ecological and ownership requirements are satisfied, Seychelles conservation efforts cannot afford to be restricted by known historical range.

5.10. ***Island ownership:*** Denis Island is owned by the Mason family who have demonstrated a firm commitment to conservation on Denis over the last decade including: (i) they employ a full time Conservation Officer (ii) they have personally funded rat and mouse eradications on Denis (iii) they

have rehabilitated over 30 hectares of native habitat in partnership with Nature Seychelles (iv) they have supported successful translocations of the globally threatened Seychelles fody and Seychelles warbler to Denis, and (v) they have developed a sustainable management plan for Denis which includes the designation of a considerable area (c.40ha) of the island to conservation and the maintenance of tall broadleaved native forest within this area.

6. Availability of release stock

We propose to translocate 20 flycatchers (10 males and 10 females) from La Digue. The current population on La Digue is estimated at c.250 individuals. The removal of 20 birds from this population (c.8% of the population) is acceptable under IUCN guidelines for translocation (IUCN 1998, 1999) and will in no way compromise the La Digue population. Productivity on La Digue indicates that the donor population will recover quickly as productivity is higher than adult mortality; on La Digue habitat rather than productivity is the limiting factor (Currie et al. 2005).

7. Methods

7.1. ***Preparation***

- Agree proposal with La Digue Development Board (agreed)
- Seek relevant permissions from MENR (agreed in principal – details remain to be agreed)
- Recruit translocation team (done)
- Source and order materials (done)
- Health screening of common bird species on Denis and La Digue (done)

7.2. ***Preparation on Denis***

Selection of release site (done)

Agreement of post translocation monitoring logistics between Rachel/Darwin Initiative project and Denis Island Management (agreed: initial intensive monitoring undertaken by dedicated Darwin Initiative Flycatcher project staff, long-term monitoring by Denis Island/Green Island Foundation conservation officer on Denis)

7.3. ***Preparation on La Digue***

Birds selected, captured, ringed and housed in individual transport boxes

Helicopter to Denis and release within 7 hours of capture

7.4. ***Transfer and release***

Helicopter transfer in individual transport boxes

Hard release into previously watered vegetation

Note: Hard release is the chosen method as flycatchers are known to be difficult to manage when held as captive birds, as it is hard to supply the required numbers of live insect foods and the transition to captive insectivore food would likely result in the loss of some individuals (Gary Ward pers. comm.). After discussions with several highly-experienced aviculturalists, we recommend that hard release is the most appropriate and least stressful method for Seychelles paradise flycatchers.

7.5. ***After Care***

Intensive monitoring for 2 years after which the monitoring regime will be reassessed and modified as appropriate

7.6. ***Reporting***

Reporting to GOS and all other project partners quarterly for the first 12 months and annually thereafter

8. **Success indicators**

8.1. Short term 0 - 12 months

Survival of c.75% of transferred individuals.

Breeding to produce juveniles that reach independence

8.2. Medium term 1-5 years

Recruitment of F1 birds to the population

Continued population growth measured by number of individuals, number of breeding territories and nesting attempts

8.3. Long term 5+ years

Maintenance of a self sustaining population

9. **Personnel**

Management	Rachel Bristol (Project Officer)
Capture, transfer and release	Rachel Bristol
	Jim Groombridge (Project Leader)
	Josianna Rose (Conservation Ranger, MENR La Digue)
	Nature Seychelles staff x 1
	Andrew Greenwood (Project Vet)
	Darwin Initiative Project staff member- Denis Island
Denis management/monitoring	Rachel Bristol and dedicated Darwin Initiative Project staff member to be hired
La Digue monitoring	Rachel Bristol and Darwin Initiative project staff.

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Appendix 1. **Disease screening details, results and interpretation**

Table 1. Birds from Denis Island and La Digue examined for haematozoa.

Number of birds examined/number positive

Host	**Denis Island**	**La Digue**
Madagascar fody	25/0	24/0
Madagascar turtle dove	23/0	29/0
Seychelles warbler	3/0	----
Seychelles fody	5/0	----

Seychelles sunbird	----	9/0
Common myna	3/0	4/0
Barred ground dove	14/0	13/0
Seychelles bulbul	----	3/0
Seychelles paradise flycatcher	----	27/0
Totals	**73/0**	**109/0**

Note 2 slides were examined for each individual bird.

Report 1. Examination of blood smears from birds in the Seychelles.

The samples were from two locations - La Digue and Denis Island. The species sampled and the sample sizes are presented in Table 1.

There were two slides for each bird with the exception of Madagascar Fody No 6 from Denis Island for which there was only a single smear.

General comments: The quality of the blood smears and the staining was generally good.

Results: No blood parasites were observed in any of the smears. Given the good quality of the smears it is almost certain that no parasites were missed in screening.

Comments: The negative results from this initial survey were totally unexpected and somewhat surprising. Whilst bird populations on Pacific Islands are frequently free of haematozoa, the converse has hitherto been true for Indian Ocean Islands. Surveys of birds from Madagascar, Mascarenes, Amirantes, Comores and Aldabra have all shown positive results.

It is known that outside of the breeding season, which usually coincides with a lower vector activity, infections with haematozoa frequently enter a latent phase making detection of parasites in peripheral blood circulation scarce. Even so, the odd parasite may still be found even if only a single organism per slide, as has been observed with the Pink Pigeons on Mauritius. Therefore a similar situation may be prevalent in the Seychelles and the samples were simply taken at the wrong time. Alternatively, there may be a genuine absence of infection. But whether this is due to an absence of vectors is currently unknown. Certainly some ornithophilic arthropods are present as micofilariae have been found in White-eyes.

M A Peirce

August 2007

Report on examination of blood smears from birds in the Seychelles. No 2.

As on the first occasion, the samples were from the same two locations – La Digue and Denis Island. The species sampled and the samples sizes are presented in Table 1.

There were two slides for each bird with the exception of Paradise flycatcher No 15 for which there were three smears.

General comments: The overall quality of the blood smears and staining was good, so it is most unlikely that any parasites were missed during screening.

Results: All smears, including duplicates, were screened under low and high power, but not a single parasite was observed.

Comments: As per comments in the previous report, the total absence of any parasites is again unexpected. One would not expect to find leucocyozoids since the simuliid vectors would be absent on sandy/coral atolls. Simuliids will only occur where there are relatively fast flowing rivers or streams which are usually confined to granitic islands such as the central Seychelles (Mahé). Certainly on Mahé there are records of human filariasis and simuliids are known to occur.

Other ornithophilic vectors such as Ceratopogonidae and culincine mosquitos should be present, as too should hippoboscids and acarines. One would have expected to see a similar pattern to that observed on Aldabra. It may be that infections are very seasonal, but one would still expect to find the odd parasite even if most are in a latent phase.

The results from these two islands so far are perhaps giving a false impression that haematozoa are absent from islands in the Seychelles. To date, only microfilariae have been recorded in White-eyes from Conception Island which is in the central Seychelles group around Mahé. Perhaps sampling of sea-birds should be included in the Denis Island and La Digue survey as these birds are usually in more direct contact with acarine vectors in particular. There is also a need to provide samples from the major islands such as Mahé and Praslin to provide a broader perspective of the situation and the distribution of avian haematozoa in general throughout the Seychelles.

M A Peirce

March 2008

Central Science Laboratory Test Results for Rachel Bristol Samples

DATE SAMPLES RECEIVED: 4/03/2008

Package number: PO8-9296

Date examined: 4-6/03/2008

Estimates per gram of faeces were based on counts from one McMaster chamber (volume examined 150µl) and related to an original average wet pellet weight of 0.114g (n=20) homogenised in 1000µl of saturated salt water (i.e. counts multiplied by 59 to get estimate per gram).

No.	Sample ID	Location	Host species	Count	Estimate/ gram	Parasite taxa
1	MTD 2	Denis island	MTD	46	2714	*Eimeria* spp.
2	MTD 9	Denis island	MTD	0	0	-
3	MTD 10	Denis island	MTD	10	590	*Eimeria* spp.
4	MTD 30	Denis island	MTD	81	4779	*Eimeria* spp.
5	MTD 31	Denis island	MTD	1900	112100	*Eimeria* spp.
6	MTD 32	Denis island	MTD	0	0	-
7	MTD 33	Denis island	MTD	45	2655	*Eimeria* spp.
8	MTD 34	Denis island	MTD	40	2360	*Eimeria* spp.
9	MTD16	La Digue	MTD	0	0	-
10	MTD21	La Digue	MTD	0	0	-
11	MTD24	La Digue	MTD	9	531	*Eimeria* spp.
12	MTD30	La Digue	MTD	0	0	-
13	MTD35	La Digue	MTD	0	0	-
14	MTD (1 of 4)	La Digue	MTD	3	177	*Eimeria* spp.
15	MTD (2 of 4)	La Digue	MTD	0	0	-
16	MTD (3 of 4)	La Digue	MTD	10	590	*Eimeria* spp.
17	MTD (4 of 4)	La Digue	MTD	8	472	*Eimeria* spp.
18	MF3	Denis island	MF	37	2183	*Isospora* spp.
19	MF6	Denis island	MF	105	6195	*Isospora* spp.
20	MF7	Denis island	MF	390	23010	*Isospora* spp.
21	MF11	Denis island	MF	2200	129800	*Isospora* spp.
22	MF12 male	Denis island	MF	790	46610	*Isospora* spp.
23	MF13 male	Denis island	MF	2100	123900	*Isospora* spp.
24	MF13admale	La Digue	MF	0	0	-
25	MF14admale	La Digue	MF	0	0	-
26	MF15	La Digue	MF	11	649	*Isospora* spp.
27	MF16	La Digue	MF	0	0	-
28	MF20	La Digue	MF	350	20650	*Isospora* spp.
29	MF21female	La Digue	MF	1000	59000	*Isospora* spp.
30	MF22	La Digue	MF	320	18880	*Isospora* spp.
31	BGD5	Denis island	BGD	0	0	-
32	BGD16	Denis island	BGD	0	0	-
33	BGD17	Denis island	BGD	0	0	-
34	BGD20	Denis island	BGD	0	0	-
35	BGD21	Denis island	BGD	0	0	-
36	BGD22	Denis island	BGD	11	649	*Isospora* spp.
37	BGD25	Denis island	BGD	0	0	-
38	BGD26	Denis island	BGD	0	0	-
39	BGD27	Denis island	BGD	0	0	-
40	BGD35	Denis island	BGD	0	0	-
41	BGD01	La Digue	BGD	0	0	-
42	BGD02	La Digue	BGD	0	0	-
43	BGD03	La Digue	BGD	0	0	-

44	BGD05	La Digue	BGD	0	0	-
45	BGD06	La Digue	BGD	0	0	-
46	BGD07	La Digue	BGD	0	0	-
47	BGD08	La Digue	BGD	0	0	-
48	BGD09	La Digue	BGD	0	0	-
49	BGD10	La Digue	BGD	0	0	-
50	SW29	Denis island	SW	0	0	-
51	SW36	Denis island	SW	0	0	-
52	Indian Myna 02	Denis island	CM	0	0	-
53	Indian Myna 03	Denis island	CM	0	0	-
54	Indian Myna 28	Denis island	CM	0	0	-
55	Myna 1	La Digue	CM	350	20650	*Isospora* spp.
56	Myna 3	La Digue	CM	70 1	4130 59	*Isospora* spp. *Capillaria* spp
57	Myna 4	La Digue	CM	62	3658	*Isospora* spp.
58	TokTok41female	Denis island	SF	29	1711	*Isospora* spp.
59	TokTok42male	Denis island	SF	10	590	*Isospora* spp.
60	2 male	La Digue	SS	8	472	*Isospora* spp.
61	11 adult male	La Digue	SS	100	5900	*Isospora* spp.
62	20 adult male	La Digue	SS	0	0	
63	SPF01	La Digue	SPF	1	59	*Isospora* spp.
64	SPF02	La Digue	SPF	0	0	
65	SPF04 adult male	La Digue	SPF	0	0	
66	SPF05 subadult male	La Digue	SPF	0	0	
67	SPF06 subadult male	La Digue	SPF	0	0	
68	SPF07	La Digue	SPF	0	0	
69	SPF08	La Digue	SPF	0	0	
70	SPF10 adult male	La Digue	SPF	0	0	
71	SPF11 adult male	La Digue	SPF	0	0	
72	SPF12	La Digue	SPF	0	0	
73	SPF14 adult female	La Digue	SPF	0	0	
74	SPF15 adult male	La Digue	SPF	0	0	
75	SPF16 adult male	La Digue	SPF	0	0	
76	SPF17	La Digue	SPF	0	0	
77	SPF18	La Digue	SPF	0	0	
78	SPF19	La Digue	SPF	0	0	
79	SPF21 juv female	La Digue	SPF	0	0	
80	SPF22 adult male	La Digue	SPF	0	0	
81	SPF23 adult female	La Digue	SPF	0	0	
82	SPF grey-grey adult female	La Digue	SPF	0	0	
83	SPF black-black adult male	La Digue	SPF	0	0	
84	SPF black-black adult male	La Digue	SPF	0	0	
85	SPF grey-grey adult male	La Digue	SPF	0	0	
86	SPF grey-grey adult male	La Digue	SPF	0	0	
87	SPF WB-WB adult female	La Digue	SPF	0	0	
88	SPF RW-RW adult male	La Digue	SPF	0	0	
89	Sey-Bulbul 02	La Digue	SB	0	0	
90	Sey Bulbul 10	La Digue	SB	0	0	

Host species key:

MTD=Madagascar Turtle Dove

MF=Madagascar Fody

BGD=Barred Ground Dove

SW=Seychelles warbler

CM=Common myna

SF=Seychelles Fody

SS=Seychelles sunbird

SPF=Seychelles paradise flycatcher

SB=Seychelles Bulbul