Hungarian Academy of Sciences
Doctor of Science (DSc) Thesis

Parrot Conservation and Extinction Risks: Multidisciplinary and Conservation Genetic Approaches

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Introduction

Questions about parrots

To introduce the topic of parrots, it is useful to address a series of fundamental questions about them: who, where, when, why, what, and how? Here I answer these questions briefly from my own experience working on this group for nearly two decades.

Who: Parrot taxonomy

The avian order Psittaciformes WAGLER, 1830, commonly known as the parrots, comprises approximately 400 extant species, unified by distinct morphological features such as their strong, curved bills and zygodactyl feet (Forshaw 2006). This diverse order exhibits a remarkable range in size, from the diminutive, 10-g pygmy parrots (*Micropsitta*) to the majestic, over 1-kg macaws (e.g. *Ara*) and cockatoos (e.g. Palm Cockatoo, *Probosciger aterrimus*). A defining and unique characteristic of parrots is their vibrant colouration. Parrots are the only birds that possess biochrome pigments known as psittacofulvins, which are responsible for their brilliant red and yellow hues (Arbore *et al.* 2024).

For many years, the precise phylogenetic placement of parrots within the avian tree of life remained an enigma. Recent advances in molecular systematics have since provided paradigm-shifting insights (Hackett *et al.* 2008), revealing the surprising sister relationship between parrots and passerines (Passeriformes), with both groups sharing a more ancient common ancestor with falcons (Falconidae). The chapters of this thesis were published across a decade, during which parrot taxonomy evolved significantly. The most complete recent phylogeny for parrots was published in 2024, using phylogenomics with sampling of over 1 million sites across the genomes of nearly all parrot species (Smith *et al.* 2024).

In Hungary, we published the first official Hungarian nomenclature for all extant and now-extinct parrot species (Olah and Bankovics 2022). This standardized naming system is crucial for consistent communication among biologists, taxonomists, bureaucrats, legislators, and wildlife managers, particularly in the context of government regulations concerning species trade and other legal texts. As the taxonomy, conservation status, and legal protection of these species may change in light of new research, so I regularly update an online database to reflect these changes (http://papagajok.info).

Where: Distribution of parrots

Phylogenetic data indicate that parrots originated from the southern supercontinent Gondwana (Tavares *et al.* 2006), while the fossil record has been interpreted to indicate a northern origin (Waterhouse 2006). Parrots are currently native to 124 countries between the latitudes N 35° and S 56°, but are mainly distributed in tropical and subtropical habitats of the southern hemisphere (Chapter 1). At least 70 % of parrots are secondary tree cavity nesters (nesting in preexisting tree cavities), hence primary forest destruction decreases the number of available nest sites and reproductive success (Newton 1994).

In addition to their native ranges, parrots have been transported and traded by humans for at least the last 2000 years, with the majority of recognized species having been involved in

this trade. This has led to a complex new chapter in their distribution. As a result of both accidental escapes and deliberate releases from the pet trade, more than 75 parrot species have established breeding populations in countries where they were not native (Pruett-Jones 2021a). Parrots are now considered among the most widely distributed groups of birds, with naturalized populations becoming common in many parts of the world, particularly in urban areas across Europe and North America. The most widespread and successful of these introduced species are the Rose-ringed Parakeet (*Alexandrinus krameri*) and the Monk Parakeet (*Myiopsitta monachus*), which have established thriving populations in dozens of countries outside their native ranges. While the impact of these naturalized populations on native ecosystems is a subject of ongoing debate, their presence highlights the growing and complex interaction between parrots and human activities worldwide.

When: Evolutionary origin of parrots

Similarly to their area of origin, the time of the origin of parrots is also under debate. A Cretaceous origin, before the Cretaceous-Paleogene extinction event 66 million years ago (Mya), was originally proposed (Wright *et al.* 2008). Other studies also suggested dispersal from Australasia and Antarctica, but later in the Paleogene (66–23 Mya) period (Schweizer *et al.* 2010). The oldest known parrot fossil, a fused jawbone is from the late Cretaceous, which seems to support the mtDNA results (Stidham 1998), while others disagree with this conclusion (Dyke and Mayr 1999). The first undoubtedly parrot fossils date from about 54 Ma from the northern hemisphere (Waterhouse 2006).

Initial vicariance events (i.e. continental breakups) were followed by local radiations and crown group diversification around 58 Mya (Schweizer *et al.* 2011). Current data suggest that parrot crown-group diversification probably happened in the early Oligocene, around 28–34 Mya (Kimball *et al.* 2019). The common ancestor of the *Arini* (Neotropical parrots) and *Psittacini* tribes (African parrots) probably lived in Antarctica (Schweizer *et al.* 2011). It is hypothesized that due to the cooling climate in Antarctica they colonized Africa and South America, and then the two tribes split about 30.8 Ma in the early Oligocene (Schweizer *et al.* 2014). *Arini* colonized the previously parrot-free Neotropics by adaptive radiation with an early concentration of size evolution, sustaining a high diversification rate due to continuously emerging new habitats (mountain orogenesis, new areas in open vegetation, wetland and river dynamics) and continuing speciation even in the Pleistocene period (Schweizer *et al.* 2014). The colonization of Indomalaya occurred several times from Australia about 28 Ma (Schweizer *et al.* 2011).

Why to study parrots

Parrots present fascinating and important subjects for study due to their diverse characteristics and behaviours. They are highly social creatures with complex communication systems, large brains relative to body size, and fission-fusion social groups (Toft and Wright 2015). Their specialized bills, zygodactyl feet, and metabolic abilities allow them to exploit a wide range of plant resources, from toxic but nutritious foods to difficult-to-access seeds. Recent research has challenged the traditional view of parrots as mere seed predators, uncovering their crucial role as ecosystem engineers through interactions like seed and pollen dispersal and the removal of plant parasites (Tella *et al.* 2019).

While parrots are widely recognized for their vibrant colours, intelligence, and sociable nature, they have historically been overlooked in broader studies of conservation, ecology, and evolution. However, this is changing. Researchers are increasingly recognizing parrots as a flagship taxon for biodiversity conservation, given their status as one of the most threatened bird orders. Research on parrots has grown dramatically over the past three decades, with the number of publications increasing from fewer than 50 per year before 1990 to over 250 each year since 2014 (Heinsohn *et al.* 2018). This increase reflects a growing recognition of the valuable role parrots can play in advancing evolutionary biology.

What threatens parrots today

Despite their appeal and intelligence, parrots face pervasive threats from the Anthropocene. They are considered the most threatened bird order, with 28% of extant species classified as threatened under IUCN criteria, and they have the lowest Red List Index of all comparable bird groups (Chapter 1). The primary anthropogenic threats are habitat loss and degradation from agriculture and logging, as well as trapping for the bird trade. This confluence of ecological, socio-economic, and human factors underscores the urgent need for a consolidated international effort to understand and protect this remarkable group of birds. To devise appropriate actions, we first need to better understand the mechanisms behind these threats, which are very complex and depend on many biological and artificial factors, and can differ across taxa. The complex mechanisms underlying these threats are analysed in detail in Part I of this thesis.

How to study parrots

Studying wild parrot populations presents significant challenges, as their often-elusive behaviour and complex social structures frequently render them unsuitable for traditional ecological and statistical approaches such as, capture-mark-recapture studies. These limitations highlight a critical need for innovative methodologies in parrot research and conservation. This thesis addresses this gap by leveraging advanced genetic techniques, which form a cornerstone of my work. My research demonstrates the power of non-invasive genetic tagging, utilizing naturally shed feathers to obtain unique DNA profiles without the need for capturing individuals. Furthermore, the thesis incorporates next generation sequencing technologies, enabling comprehensive analyses ranging from population genetics to the investigation of breeding systems, and the estimation of effective population sizes. This innovative, genomics-driven approach allows for the acquisition of detailed ecological and demographic data that would otherwise be inaccessible, thereby significantly advancing our capacity to study and conserve this highly charismatic avian order.

Research questions

In this thesis, I aim to answer the following key research questions, which are organized around three overarching themes and explored through empirical data and advanced analytical approaches:

I) What biological, socioeconomic, and anthropogenic factors drive the extinction risk of parrots on a global and regional scale?

   a) *What are the global and regional patterns of parrot extinction risk, and what intrinsic biological and socioeconomic traits are associated with higher vulnerability?*
   b) *How do global habitat destruction trends and illegal wildlife trade dynamics contribute to parrot extinction, particularly in biodiversity hotspots?*

II) *How can an interdisciplinary approach, combining field ecology with pioneering genetic methods, provide new insights into the biology and conservation of wild parrot populations?*
   a) *How do ecological factors, such as nest site selection and parasitism, influence the breeding success and survival of wild macaws?*
   b) *How can genetic methods be applied to understand the population structure, movements, and demography of macaws in challenging tropical environments?*

III) *How can advanced genetic and genomic techniques be used to effectively assess the viability of threatened parrot populations and provide data-driven recommendations for conservation management?*
   a) *What is the role of genetic tools in advancing our understanding of the biology of threatened parrot species and conservation?*
   b) *Which is the best method to estimate effective population size based on sibling data, and how do different molecular markers influence population genetic estimates?*

Study sites: Peru, Australia, and Indonesia

While certain chapters of this thesis are founded on comparative meta-analyses conducted at large or even global scales, a significant portion of the work is directly rooted in intensive fieldwork. This fieldwork was carried out either personally, through my collaborations with postdoctoral researchers, or with the involvement of my students.

All fieldwork in Peru for the chapters presented in Part II was conducted by me. The interdisciplinary study of two large macaw species took place in the lowland rainforest of southeastern Peru, specifically within the regions of Madre de Dios and Puno. This tropical moist forest, ranging in elevation between 250 and 800 meters, receives approximately 3,200 mm of rain annually (Tosi 1960; Brightsmith 2004). There are two large, adjacent protected areas in this region: the Tambopata National Reserve (2,747 km^2) and the Bahuaja-Sonene National Park (10,914 km^2). This area is renowned for its exceptional biodiversity, harbouring some of the highest species richness in the entire Amazon Basin, making it one of my most compelling field research sites. The core area of the study was situated in the forests surrounding the Tambopata Research Center, encompassed by four principal forest habitats: terra firme, floodplain, palm swamp, and successional forests.

During my postdoctoral years, I had the privilege of collaborating with and mentoring other postdoctoral researchers studying the Swift Parrot (*Lathamus discolor*) in Tasmania, Australia. This species is unique among parrots as one of only two migratory species, breeding in Tasmania during the Australian spring–summer before migrating north across the Bass Strait to the Australian mainland for its broad wintering range (Saunders and Heinsohn 2008). Its highly specific nesting and food requirements, coupled with the necessity for hollows and flowering to coincide geographically, render the species acutely vulnerable to the ongoing impacts of habitat degradation and loss (Webb *et al.* 2014). Over many years, the long-term

research project at this site accumulated hundreds of genetic samples, which I processed and analysed. The results of these analyses have yielded crucial management information for their conservation, directly applied in the field.

My involvement in supervising students in Indonesia, initiated through my collaboration with the Indonesian Parrot Project NGO (of which I am a board member), providing access to another vital hotspot of parrot diversity and conservation. This collaboration quickly exposed the severe issues of parrot trade within the country, leading to the research presented in Chapters 4 and 5. In Wallacea (comprising Sulawesi, the Lesser Sunda Islands, and the Maluku Islands), nearly half of all parrot species are affected by illegal trapping for the pet trade, establishing this part of Indonesia as a critical region for studying the illegal parrot trade (Eaton *et al.* 2015).

Together, these varied study sites—from the complex conservation challenges of Indonesia's endemic species, through the biodiverse Amazonian rainforests of Peru, to the breeding grounds of Australia's swift parrots—underscore the thoroughly international and global scope of this thesis. My research integrates empirical data from these diverse field locations with broad meta-analyses, demonstrating a comprehensive approach to understanding and addressing parrot conservation challenges across different scales and contexts. This global perspective, combining both intensive field research and macro-level data synthesis, is fundamental to developing robust and universally applicable conservation strategies for this threatened avian order.

Structure of the thesis

This thesis is structured into a three-part narrative that progresses from problem identification and large-scale analysis to the application of specific, innovative solutions. This journey reflects my research focus on conservation genetics and tropical ecology, using next-generation sequencing technology to study birds for their conservation management. The work represents a creative, inter-disciplinary approach to research that connects diverse data types and leverages an international team approach to science.

Part I: Extinction risk in parrots establishes the global context for the research by defining the scope and nature of the threats facing parrots worldwide. Our work confirms that parrots face a higher aggregate extinction risk than other comparable bird groups. Building on this, the thesis first analyses the intrinsic biological, life history, and socio-economic factors associated with this risk (Chapter 1). We then investigate the global trends of habitat destruction, highlighting its profound impact on parrot populations and identifying key conservation hotspots where policy changes are most critical (Chapter 2). This is followed by a more focused comparative study on the parrots of Oceania, where invasive species and poaching are identified as especially severe threats (Chapter 3). Finally, the narrative delves into the specific threat of the illegal trade, with one study applying a criminological model to analyse the factors driving the parrot trade in Indonesia, a country identified as a high-priority area for parrot conservation (Chapter 4). This section concludes with a detailed case study on a biodiverse Indonesian island, assessing the influence of this illegal trade on wild parrot populations (Chapter 5).

Part II: An interdisciplinary approach to macaw research in the Peruvian Amazon transitions from identifying global problems to applying a multidisciplinary approach to solutions through a series of focused, field-based studies. The work begins with an analysis of nest site selection and efficacy of artificial nests for Scarlet Macaws (Chapter 6). This is followed by an investigation into a specific threat, bot fly parasitism in macaw nests, where a new technique for larvae extraction is also described (Chapter 7). The thesis then showcases a key methodological innovation: the development and validation of non-invasive genetic tagging on wild macaws (Chapter 8). This novel technique, which was implemented for the first time on non-invasively sourced bird DNA in a tropical landscape, is then applied to reveal new insights into the population structure and local population size of wild macaw populations (Chapter 9). To further our understanding of dispersal, the final chapter in this section combines population genetic information with remotely sensed data from spatial ecologists, exploring how natural landscape features influence gene flow in the Peruvian Amazon (Chapter 10).

Part III: Conservation genetics of parrots synthesizes the advanced technological and analytical heart of my research program. This part demonstrates the direct application of genetic tools to assess population viability and provide crucial, data-driven advice for conservation management. The section begins with a comprehensive review of genetic methods in parrot conservation, summarizing major advances and highlighting the future potential of applying genomics to study issues like illegal trade (Chapter 11). The narrative then focuses on case studies of the Critically Endangered Swift Parrot, where genetic data is first used to confirm the severe extinction risk of the species (Chapter 12). These tools were then further applied to show how a sex ratio bias and shared paternity negatively impact both individual fitness and long-term population viability for this species (Chapter 13). This part concludes with a direct comparison of techniques for estimating effective population size (Chapter 14) and the influence of different molecular markers on genetic parameter estimates (Chapter 15), providing practical guidance for conservation practitioners.

Finally, this thesis concludes with a comprehensive summary of the collective findings and an outlook on future research directions in parrot conservation.

Publications serving as the basis for the thesis

The thesis consists of the following papers listed in order of the chapters. * indicates shared first authorship.

1. **Olah G.**, Butchart S.H.M., Symes A., Guzmán I.M., Cunningham R., Brightsmith D.J., Heinsohn R. (2016) Ecological and socio-economic factors affecting extinction risk in parrots. ***Biodiversity and Conservation*** 25(2):205–223.
   https://doi.org/10.1007/s10531-015-1036-z (IF_{2016} = 2.265)
   [Cited among the top 1% papers in Environment/Ecology with top 1% Altmetric attention score in the journal Biodiversity and Conservation.]
2. Vergara-Tabares D.L., Cordier J.M., Landi M.A., **Olah G.**, Nori J. (2020) Global trends of habitat destruction and consequences for parrot conservation. ***Global Change Biology*** 26(8):4251–4262.
   https://doi.org/10.1111/gcb.15135 (IF_{2020} = 10.863)

[I had a significant role in this paper conceptualizing the work, supervising the data analyses, interpreting the results and writing the paper.]

3. **Olah G.**, Theuerkauf J., Legault A., Gula R., Stein J., Butchart S.H.M., O'Brien M., Heinsohn R. (2018) Parrots of Oceania: A comparative study of extinction risk. ***Emu – Austral Ornithology*** 118(1):94–112.
https://doi.org/10.1080/01584197.2017.1410066 (IF_{2018} = 1.351)
[Invited paper for the special issue on parrots. The publisher identified it as the most 'talked about' article reflected in its Altmetric attention score and promoted to open access for a while. Video abstract: https://youtu.be/OJn3053dTaM]

4. **Olah G.***, Pires S.F.*, Nandika D., Agustina D., Heinsohn R. (2021) What drives the illegal parrot trade? Applying a criminological model to market and seizure data in Indonesia. ***Biological Conservation*** 257:109098.
https://doi.org/10.1016/j.biocon.2021.109098 (IF_{2021} = 7.499)
[With Dr. Pires we contributed equally to this paper in conceptualising, analysing, and writing it together. Video abstract: https://youtu.be/1GbRyb94qlw]

5. Nandika D.*, Agustina D.*, Heinsohn R., **Olah G.** (2021) Wildlife trade influencing natural parrot populations on a biodiverse Indonesian island. ***Diversity*** 13(10):483.
https://doi.org/10.3390/d13100483 (IF_{2021} = 3.031)

6. **Olah G.**, Vigo G., Heinsohn R., Brightsmith D.J. (2014) Nest site selection and efficacy of artificial nests for breeding success of Scarlet Macaws *Ara macao macao* in lowland Peru. ***Journal for Nature Conservation*** 22(2):176–185.
https://doi.org/10.1016/j.jnc.2013.11.003 (IF_{2014} = 1.646)

7. **Olah G.**, Vigo G., Ortiz L., Rozsa L., Brightsmith D.J. (2013) *Philornis* sp. bot fly larvae in free living scarlet macaw nestlings and a new technique for their extraction. ***Veterinary Parasitology*** 196(1–2):245–249.
https://doi.org/10.1016/j.vetpar.2012.12.052 (IF_{2013} = 2.545)

8. **Olah G.**, Heinsohn R.G., Espinoza J.R., Brightsmith D.J., Peakall R. (2015) An evaluation of primers for microsatellite markers in Scarlet Macaw (*Ara macao*) and their performance in a Peruvian wild population. ***Conservation Genetics Resources*** 7(1):157–159.
https://doi.org/10.1007/s12686-014-0317-2 (IF_{2015} = 0.446)

9. **Olah G.**, Heinsohn R.G., Brightsmith D.J., Espinoza J.R., Peakall R. (2016) Validation of non-invasive genetic tagging in two large macaw species (*Ara macao* and *A. chloropterus*) of the Peruvian Amazon. ***Conservation Genetics Resources*** 8(4):499–509.
https://doi.org/10.1007/s12686-016-0573-4 (IF_{2016} = 0.470)

10. **Olah G.**, Heinsohn R.G., Brightsmith D.J., Peakall R. (2017) The application of non-invasive genetic tagging reveals new insights into the clay lick use by macaws in the Peruvian Amazon. ***Conservation Genetics*** 18(5):1037–1046.
https://doi.org/10.1007/s10592-017-0954-6 (IF_{2017} = 2.025)
[Video abstract: https://youtu.be/Ilr9Llm-d9U]

11. **Olah G.**, Smith A.L., Asner G.P., Brightsmith D.J., Heinsohn R.G., Peakall R. (2017) Exploring dispersal barriers using landscape genetic resistance modelling in scarlet macaws of the Peruvian Amazon. ***Landscape Ecology*** 32(2):445–456. https://doi.org/10.1007/s10980-016-0457-8 (IF_{2017} = 3.833)
[Video abstract: https://youtu.be/uZWFpTQyY_A]

12. **Olah G.**, Smith B.T., Joseph L., Banks S.C., Heinsohn R. (2021) Advancing genetic methods in the study of parrot biology and conservation. ***Diversity*** 13(11):521. https://doi.org/10.3390/d13110521 (IF_{2021} = 3.031)
[Invited to the special issue "Ecology and Conservation of Parrots in Their Native and Non-Native Ranges"]

13. Stojanovic D.*, **Olah G.***, Webb M., Peakall R., Heinsohn R.G. (2018) Genetic evidence confirms severe extinction risk for critically endangered swift parrots: implications for conservation management. ***Animal Conservation*** 21(4):313–323. https://doi.org/10.1111/acv.12394 (IF_{2018} = 3.048)
[With Dr. Stojanovic we contributed equally to this paper in conceptualising, analysing, and writing it together. Video abstract: https://youtu.be/HzACJxhP3Bc]

14. Heinsohn R.G., **Olah G.**, Webb M., Peakall R., Stojanovic D. (2019) Sex ratio bias and shared paternity reduce individual fitness and population viability in a critically endangered parrot. ***Journal of Animal Ecology*** 88(4):502–510. https://doi.org/10.1111/1365-2656.12922 (IF_{2019} = 4.554)
[This paper has been selected for the In Focus and cover image of the April 2019 issue. My roles included conducting the genetic analysis, analysing the molecular data, and major contribution to writing the paper.]

15. **Olah G.***, Stojanovic D.*, Webb M., Waples R., Heinsohn R.G. (2021) Comparison of three techniques for genetic estimation of effective population size in a critically endangered parrot. ***Animal Conservation*** 24:491–498. https://doi.org/10.1111/ACV.12655 (IF_{2021} = 4.377)
[This paper ranked #1 most communicated in *Animal Conservation* based on Altmetric attention score and was promoted to free access for a while by Wiley.
Video abstract: https://youtu.be/4RKhfbTmEhk]

16. **Olah G**, Waples R, Stojanovic D (2024) Influence of molecular marker type on estimating effective population size and other genetic parameters in a critically endangered parrot. ***Ecology and Evolution*** 14(3):e11102. https://doi.org/10.1002/ece3.11102 (IF_{2024} = 2.3)

Part I. Extinction risk in parrots

1. Global extinction risk in parrots

1.1. Introduction

The current worldwide decline in biodiversity requires urgent action (Butchart *et al.* 2010; Pimm *et al.* 2014), but determining appropriate actions is often impeded by poor understanding of the diverse causes of decline and extinction across different taxa and regions of the world (Tittensor *et al.* 2014). The factors that determine a species' extinction risk fall into two main categories: (1) intrinsic biological attributes of species, and (2) exposure to external anthropogenic threats (Fisher *et al.* 2003). Although exposure to threats is the ultimate cause of extinction, a species' biology often determines how sensitive it is to different threat types. Particular biological attributes can allow populations to recover rapidly from depletion and can offer a degree of resilience from external threats (Cardillo *et al.* 2004). Biological attributes that have been shown to decrease the risk of extinction include large geographical distribution, high population density, lower trophic level, and various life history traits including high reproductive rate, small body size, short generation length, and a low degree of habitat specialization (Owens and Bennett 2000; Purvis *et al.* 2000).

The order *Psittaciformes* (parrots) contained 398 extant species in 2014, divided into three families (*Psittacidae* 374, *Cacatuidae* 21, and *Strigopidae* 3 species) of which 111 (28%) were classified as threatened on the IUCN Red List, i.e. in the categories of Critically Endangered, Endangered, and Vulnerable (IUCN 2014). Sixty species were classified as Near Threatened and the rest (227 species) were considered as Least Concern. *Psittaciformes* is the fourth largest bird order after *Passeriformes* (5 913 spp., 10% threatened), *Caprimulgiformes* (593 spp., 9% threatened), and *Piciformes* (484 spp., 7% threatened), but it contains the second highest number of threatened bird species after *Passeriformes* (containing 611 threatened species). Data from BirdLife International underpinning assessments for the IUCN Red List showed in 2014 that 56% of all parrot species were in decline, 35% were stable and only nine per cent had increasing populations (IUCN 2014). These assessments related to the observed, estimated, inferred or suspected direction of population trends, and the underlying basis and evidence for each one were specified under "trend justification" on the Data Zone factsheets (BirdLife International 2016).

Parrots in 2014 were native to 124 countries between the latitudes N 35° and S 56° but are mainly distributed in tropical and subtropical habitats of the southern hemisphere. They have been popular as pets throughout human history, probably due to their colourful appearance, reputedly high intelligence, and remarkable ability to mimic various sounds including the human voice (Benedict *et al.* 2022). This popularity had led many species to be captured from the wild for pets. Although captive breeding has proven possible for many species (Tella and Hiraldo 2014), *Psittaciformes* remains the most common avian order reported in the wildlife trade (Bush *et al.* 2014).

Parrots exhibit many of the traits known to be associated with extinction: for example, many are large-bodied and slow-breeding (e.g. macaws and cockatoos), and 70% (see below) are ecologically specialized (e.g. forest species) (Forshaw 2006). The main anthropogenic

threats to parrots are habitat loss, degradation and fragmentation driven by unsustainable agriculture, logging and commercial and residential development, and hunting and trapping (Beissinger *et al.* 1992; Snyder *et al.* 2000; Toft and Wright 2015). The socio-economic status of a country can determine the severity of anthropogenic threats affecting the species living there. For example, human population density was found to be closely related to the proportion of threatened bird species per country (Kerr and Currie 1995). Human activities such as poaching, hunting, and land clearing can be poverty driven in certain developing areas (Pires 2012a), but the effects of socio-economic drivers on the extinction risk of parrots have never been examined.

Although parrots have long been considered a group of especially high conservation concern (Pasquier 1980), in-depth quantitative analysis of the nature and trends in threats across the order has been lacking. Reviews to date have assessed particular threats (Beissinger and Bucher 1992; Newton 1994; Müller 2000; Pain *et al.* 2006; Pires 2012a), focused on individual species (Snyder *et al.* 2000; Webster *et al.* 2003; Holdsworth and Starks 2006; Baker-Gabb 2011) or assessed general trends in parrot conservation without comparative analysis (Collar and Juniper 1992; Collar 1997; Collar 2000; Martin *et al.* 2014). Jones *et al.* (2006) advocated decision tree analysis for assessing extinction risk and used the IUCN Red List data of parrots from 2000 as an example group, but did not cover extrinsic extinction threats or socio-economic traits, and nor did they control for phylogenetic dependence of data. Our aim is to use comparative analyses to enhance understanding of the extrinsic, intrinsic, global and regional factors associated with endangerment and extinction in this highly threatened taxonomic group.

1.2. Methods

We first examined trends in extinction risk during 1988–2012 for parrots (*Psittaciformes*) and other high profile ecological groups with similar numbers of species using Red List Indices (Butchart *et al.* 2007). These groups included waterbirds, seabirds, and raptors, each of which comprises multiple orders. Other groups included pigeons (*Columbiformes*) and gamebirds (*Galliformes*). IUCN Red List criteria (e.g. absolute population size, range size, rates of decline, etc.) are used to assign species to Red List categories of relative extinction risk, and the Red List Indices are calculated from changes between these categories. Red List Indices for sets of species are based on the number of species in each category, and the number moving between categories owing to genuine improvement or deterioration in status, i.e. increases or decreases in population size, population trends, extent of occurrence, etc. that are of sufficient magnitude to cross the thresholds for lower or higher Red List categories (Butchart *et al.* 2004; Butchart *et al.* 2005; Butchart *et al.* 2007).

1.2.1. Database compilation

We assembled a database of the biological and geographic attributes of all 398 extant parrot species using the 2014 version of BirdLife International and IUCN's database which underpins the IUCN Red List assessments for birds on the BirdLife Data Zone (BirdLife International 2016) and IUCN Red List website (IUCN 2014). Table 1.1 shows all explanatory variables (including sources) used in our statistical models of threat status. An online supplementary provide further details of the derivation of values in the database (Olah *et al.* 2016a).

Table 1.1. Explanatory variables used in the statistical models. Variables marked with an asterisk were loge transformed.

Group	Variable name	Description	N	Median	Data source*
A	Median Latitude	Absolute value of the median latitude of the species distribution (decimal degree)	398	9.5	(1)
	Historical Distribution Size*	Area of the historical distribution size of the species (km^2)	398	177,782	(1)
	Mean Altitude	Mean elevation of the species distribution range (m)	322	322	(2)
	Region	Distribution of the species: (1) Neotropic, (2) Afrotropic, (3) Indomalaya, (4) Australasia/Oceania	398		(3)
	Island Endemism	If endemic to islands smaller than 110,000 km^2 (yes/no) - Large islands were not considered as islands (New Guinea, Borneo, Madagascar, Sumatra, Celebes/Sulawesi, New Zealand, Java)	398		(2)
B	Body Size*	Length of the species (cm)	398	26	(4)
	Number of Habitats	Total number of habitats utilised by species (importance: major, marginal, or suitable)	398	3	(2)
	Migrant Status	(0) Not a Migrant, (1) Nomadic, (2) Altitudinal Migrant, (3) Full Migrant	398		(2)
	Main Diet	(1) Frugivore (fruits, vegetable matters, leaves, fungi, lichens), (2) Granivore (grass seeds), (3) Nectarivore (nectar, pollen, flowers), (4) Tree seeds (hard seeds, acorns, nuts, cone seeds), (5) Specialist	398		(5)
	Social Flocking	Flock size in the non-breeding season: (0) No flocks (alone or pairs), (1) Small flocks (up to 20), (2) Large flocks (more than 20)	397		(4; 6)
	Colony Nesting	yes/no	386		(4)
	Nesting Tree Type	(1) hardwood trees and their branches; (2) palm trees; (3) other nest types (e.g. termite mounds, epiphytes, moss, burrows, grass, etc.)	322		(4; 5)
	Forest Dependency	(0) Non-forest, (1) Low, (2) Medium, (3) High. Scored from published and unpublished information on the ecology of each species.	398		(2)
	Generation Time	Mean generation length in years	398	6	(2)
C	Captive Breeding	yes/no	397		
	Used for Pets	yes/no	398		(2)
	Used for Food	yes/no	398		(2)
	Used for Accessories	yes/no	398		(2)
	Used for Sport	yes/no	398		(2)
D	Single Country Endemic	If endemic to one country (yes/no)	398		(2)
	Per capita GDP	Gross domestic product based on purchasing-power-parity (PPP) per capita in USD	397	10,148	(7; 8)
	Industrial Production Growth Rate	Mean percentage of the industrial production growth rate of countries where species is extant (%)	378	4.1	(7)
	Unemployment Rate	Mean percentage of the labour force that is without jobs of countries where species is extant (%)	395	6.6	(7; 8)

	Human Population Density	Mean population density of countries where species is extant (people/1000Ha)	398	315	(7; 8; 9)
	Urban Population	Mean percentage of population living in urban areas of countries where species is extant (%)	398	68.3	(9)
	Human Population Growth Rate	Mean population growth rate of countries where species is extant (%)	398	1.1	(7)
	Agriculture Area	Mean percentage of agricultural area of countries where species is extant (%)	398	31.3	(9)

* Data source: (1) BirdLife International and NatureServe (2014); (2) BirdLife International (2016); (3) Olson *et al.* (2001); (4) Forshaw (2011); (5) Juniper and Parr (1998); (6) Arndt (2007); (7) CIA (2013); (8) IMF (2013); (9) FAOSTAT (2013).

Because of the large number of explanatory variables, we initially divided the potential explanatory variables into four groups and performed analyses separately for each. We then combined all significant variables from each sub-analysis into a final model (see below). The groups were: (A) geographical and distributional attributes of each species; (B) biological, ecological and life history variables; (C) type of utilization by humans; and (D) socio-economic and demographic attributes of the countries where the species occur.

We used historical distribution size instead of current distribution sizes to test if species with originally small distributions are more prone to be endangered, and in order to avoid possible circularity as current extent of occurrence is a parameter used in the IUCN Red List criteria. Historical distribution is mapped using the same sources as for contemporary distribution, adding areas with historical records prior to 1980 for which the species is now judged to be extirpated owing to lack of recent records despite searches, lack of suitable habitat and informed by expert judgment (BirdLife International and NatureServe 2014). Population size and trend were not used as variables in our models for similar reasons. All the detailed underpinning data for Red List evaluation are available on the BirdLife Datazone (BirdLife International 2016). Because generation time was significantly positively correlated with body size, we calculated and used the residual values from the simple linear regression of generation time versus body size and referred to this variable as 'residual generation time' following Owens and Bennett (2000).

To assess socioeconomic and demographic attributes of the countries in the parrot species' range, we used the World Economic Outlook Database (IMF 2013), the World Factbook (CIA 2013), and the database of the Food and Agriculture Organization of the United Nations (FAOSTAT 2013). We calculated the mean values of each parameter for all countries in which a species occurred (excluding vagrant records). Industrial production growth rate, unemployment rate, human population density, urban population, human population growth rate, and agriculture area were all significantly correlated with per capita gross domestic product (GDP), so we used residual values for these variables after regressing them against GDP. We analysed the traits of 16 extinct species separately comparing trends with values for extant species.

1.2.2. Statistical modelling procedures

We used linear logistic regression models to identify the broad covariates of whether a parrot species is classified as threatened. 'Threatened' was defined as belonging to the IUCN

threat categories 'Vulnerable', Endangered', and 'Critically Endangered', whereas 'Non-threatened' included the categories 'Least Concern' and 'Near Threatened'. We then used ordinal regression models to evaluate in more detail the covariates of the degree of threat faced by the threatened parrot species. Both logistic and ordinal regression models were initially computed separately using each of four categories of variables (Table 1.1), and a final universal model was computed by combining the variables found to be significant in each of the sub-models. We used GenStat 13.7 (Payne 2009) for all modelling.

To control for phylogenetic relatedness between species, we downloaded 1000 possible phylogenetic trees of *Psittaciformes* from birdtree.org. These were randomly selected to account for the branch lengths in addition to nodes separating species (Jetz *et al.* 2012). For each phylogeny, we ran a phylogenetic generalized least squares (PGLS) regression using the 'caper' package of R statistics (Freckleton *et al.* 2002). The explanatory and response variables were those from the best models per category found in the regression models. For each predictor in each model, we report the modified *P*-value accounting for phylogenetic relatedness, and we report λ (lambda transformation that improves the fit of the model to the phylogeny data) for each model. Greater values of λ indicate that the relationship between response and predictor variables is dependent on the phylogeny and trait values are more similar for closely related species. Values of λ closer to 0 indicate that the relationship is unrelated to phylogeny. Since these analyses were repeated for 1000 phylogenetic hypotheses, we report the mean and standard deviation of λ in each case. Additionally, we calculated in R the Blomberg's K for threat status, which is a measure of phylogenetic signal. When values are closer to zero it indicates that the distribution of the trait is not related to phylogenetic relatedness.

We analysed the specific threats associated with high extinction risk in parrots by assessing how many parrot species are affected by each threat type globally and specifically at the regional scale in the Neotropics, Afrotropics, Indomalaya, and Australasia/Oceania. We used threat impact scores estimated from timing, scope and severity for each threat for each species, and we compared the high and medium impact threats worldwide. We also considered the most important conservation actions needed to improve the status of threatened parrots. A formal prioritization analysis has not been carried out for all species, but the most urgent actions are documented by BirdLife International and therefore useful to present a broad level analysis. We draw attention to countries with high risk of parrot conservation using two different methods. (1) We ranked countries by combining their scores for number of parrot species, number of globally threatened species, and number of endemic species, following methods from Croxall *et al.* (2012). We refer to these as 'country priority' scores. (2) We calculated the residual values of the final combined linear logistic regression model accounting for all significant extrinsic and intrinsic variables. We refer to these values as 'unexplained extinction risk' hereafter.

1.3. Results

1.3.1. Red List Index for parrots and geographical differences

The Red List Index for parrots (0.825 in 2012) is lower than that for comparable groups of bird species including waterbirds (0.879), raptors (0.877), pigeons (0.868), gamebirds (0.842),

and seabirds (0.828; Figure 1.1). However, rates of decline are broadly comparable across these different groups: 4.19% for parrots, 4.22% for seabirds, 4.30% for gamebirds, 4.39% for pigeons, and 4.49% for waterbirds and raptors (Figure 1.1).

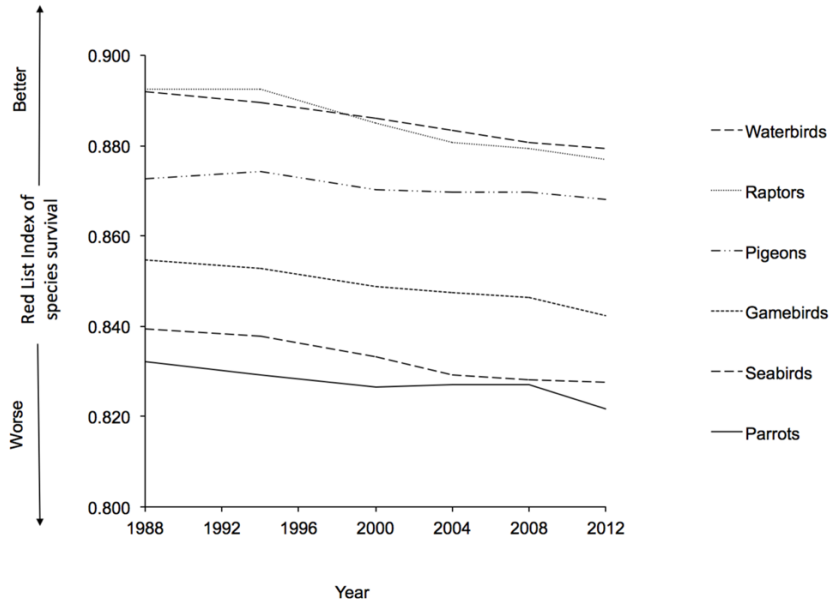

Figure 1.1. Red List Indices of species survival for parrots (N = 398 non Data Deficient extant species; *Psittaciformes*), seabirds (N = 355; *Anseriformes, Podicipediformes, Phaethontiformes, Gaviiformes, Sphenisciformes, Procellariiformes, Pelecaniformes, Suliformes, Charadriiformes*), gamebirds (N = 307; *Galliformes*), pigeons (N = 350; *Columbiformes*), raptors (N = 320; *Accipitriformes, Cathartiformes, Falconiformes*), and waterbirds (N = 852; *Anseriformes, Podicipediformes, Phoenicopteriformes, Gruiformes, Gaviiformes, Ciconiiformes, Pelecaniformes, Suliformes, Charadriiformes*).

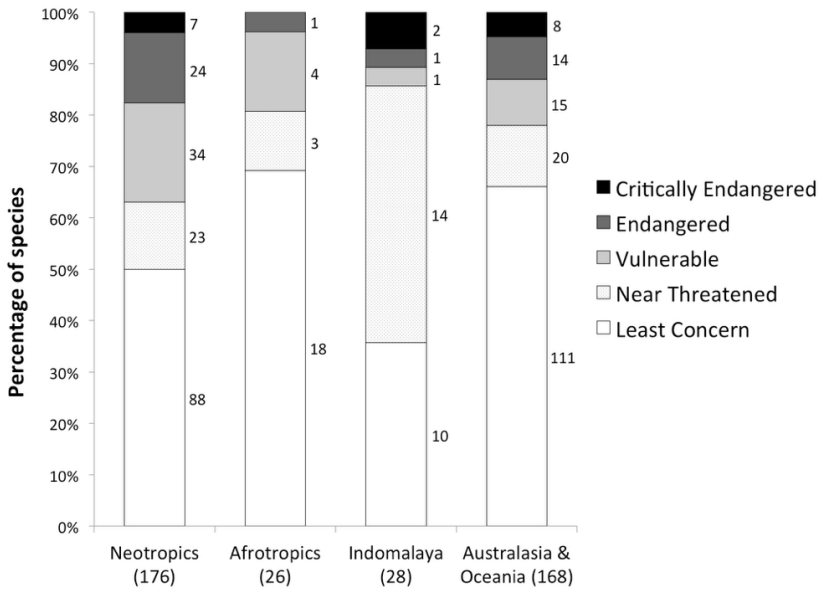

Figure 1.2. Proportion of parrot species in each IUCN Red List category for each region (following Croxall *et al.* 2012). Number of species in each category is shown.

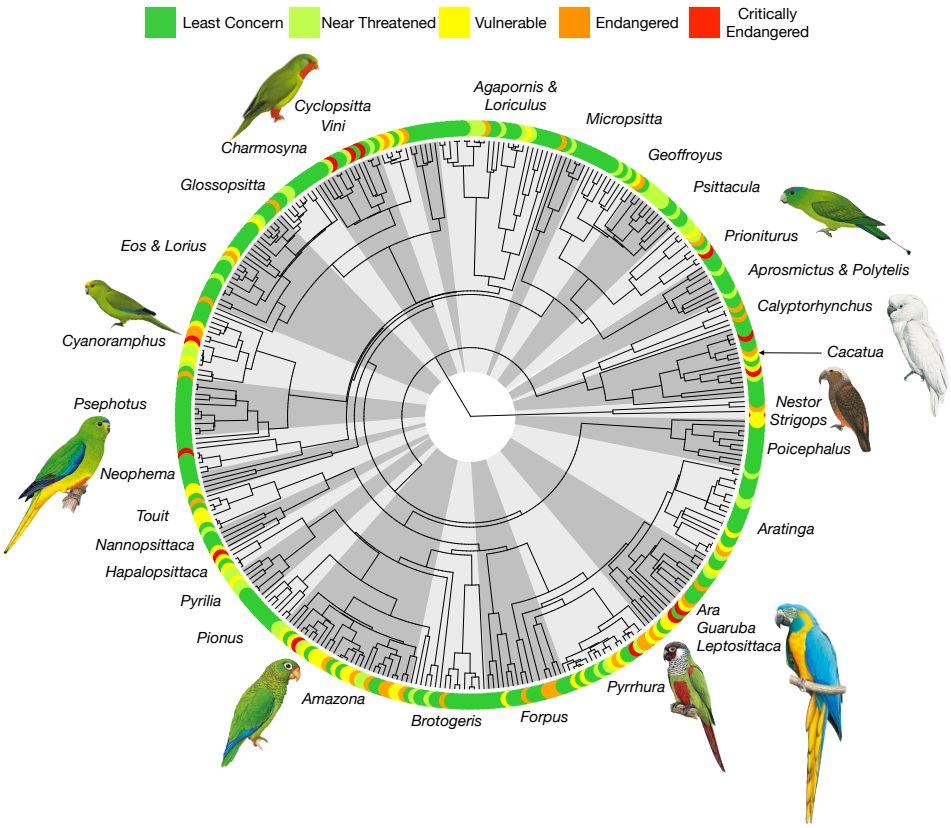

Figure 1.3. Phylogeny of parrots indicating IUCN Red List status of each species. Colours at the tip of the branches represent the IUCN Red List category of each species; grey shading inside the circle represent major genera and groups of related genera as labelled outside the circle. Images are taken with permission from the Handbook of Birds of the World online and represent examples of species that are Critically Endangered. Source of phylogeny: www.birdtree.org

A total of 16 parrot species are considered to be Extinct, 17 are Critically Endangered (one species Possibly Extinct in the Wild, and another species Possibly Extinct), 40 Endangered, 54 Vulnerable, 60 Near Threatened, and 227 are categorized as Least Concern. The Neotropics have the highest proportion of threatened species (37% of the region's parrot species, N = 176), followed by Australasia/Oceania (22%, N = 168 spp.), Afrotropics (19%, N = 26 spp.), and Indomalaya (14%, N = 28 spp.; Figure 1.2). Overall, there was no significant phylogenetic signal in degree of threat (K = 0.05, $P = 0.36$), which means that closely related species are not more likely to share the same Red List category (Figure 1.3).

1.3.2. Linear logistic regression model

The combined linear logistic regression model showed that the probability of a parrot species being threatened decreased with larger historical distribution size ($\chi^2_1 = 64.89$, $P < 0.001$). The probability of being threatened increased significantly with: body size ($\chi^2_1 = 13.18$, $P < 0.001$), residual generation time ($\chi^2_1 = 16.82$, $P < 0.001$), extent of forest dependency ($\chi^2_3 = 29.47$, $P < 0.001$), and the percentage of the human population in the countries of occurrence living in urban areas ($\chi^2_1 = 28.71$, $P < 0.001$). There were no significant interactions among the 27 tested variables (all $P \geq 0.184$). All significant variables in the combined linear logistic regression model were also significant in the PGLS model controlling for phylogeny.

1.3.3. Ordinal regression model

The combined ordinal regression model indicated that the severity of threat among threatened parrot species increased significantly with higher per capita GDP (Table 1.1) of the countries of occurrence ($\chi^2_1 = 8.47$, $P = 0.004$) and when the species is endemic to a single country ($\chi^2_1 = 5.6$, $P = 0.018$). The severity of threat decreased significantly for species that are used as pets ($\chi^2_1 = 4.75$, $P = 0.029$). There were no significant interactions among the 27 tested variables (all $P \geq 0.140$). All significant variables in the combined ordinal regression model were also significant in the PGLS model controlling for phylogeny.

1.3.4. Traits of extinct species

Sixteen parrot species in 12 genera are recorded as extinct. Five of the extinct species lived in the Neotropics (mainly the Caribbean), six on islands near Africa (Mascarene Islands, Mauritius, Seychelles), and five in Australasia/Oceania (4 in Oceania and 1 in Australia). Fourteen out of 16 extinct species inhabited islands only. By comparison one quarter of all extant parrot species (96 out of 398 species) are insular suggesting an over-representation of island species amongst those now extinct ($\chi^2_1 = 28.5$, $P < 0.001$). All but one extinct species were endemic to a single country, but distribution size was highly variable (38 – 557 670 km^2) with the Carolina Parakeet (*Conuropsis carolinensis*) forming an outlier with range size of 3 167 000 km^2 (mean of 240 510 km^2 ± 198 000 SE for all 16 species). The mean of the extinct species' median latitude of occurrence was -6.96 degrees ± 5.00 SE compared with -6.6 degrees ± 0.75 SE for extant species, with no significant difference (ANOVA $F_{1,413} = 0.01$, $P = 0.936$). Extinct species were mainly large bodied with a mean length of 39 cm ± 3.5 SE (ranging between 25–70 cm) compared with a mean of 28.5 cm ± 0.7 SE (ranging between 8–100 cm) for extant parrots (ANOVA $F_{1,410} = 7.62$, $P = 0.006$). Their estimated mean generation time was 8 years ± 0.7 SE compared with 7.3 years ± 0.2 SE for extant species (ANOVA $F_{1,412} = 0.92$, $P = 0.339$). All but one of the extinct species lived in only one habitat type, mainly in forests. Out of the 16 extinct species 38% (6 species) were recorded as used as pets compared to 93% today (372/398 species). A percentage of 69% (11/16 spp.) of the extinct species were used as food compared to 23% today (90/398 spp.).

1.3.5. Threat types, conservation actions, and priority countries

Based on the threat impact scores, the greatest threat to parrots worldwide is from agriculture (impacting 35% of extant species), followed by hunting & trapping, logging, climate change & severe weather, and invasive alien species (Figure 1.4A). The top three threats (agriculture, hunting & trapping, logging) appear roughly similar across regions, however residential development features as a worse threat in the Neotropics and Indomalaya, and invasive alien species is a greater threat in the Afrotropics and Australasia/Oceania (Figure 1.4B). Whether species are threatened depends significantly on five major types of threat and their interactions: invasive alien species, agriculture, hunting & trapping, energy production & mining, and residential & commercial development. Higher categories of threat (i.e. Endangered, Critically Endangered) were especially associated with invasive alien species, even after controlling for phylogeny ($\chi^2_1 = 14.41$, $P < 0.001$).

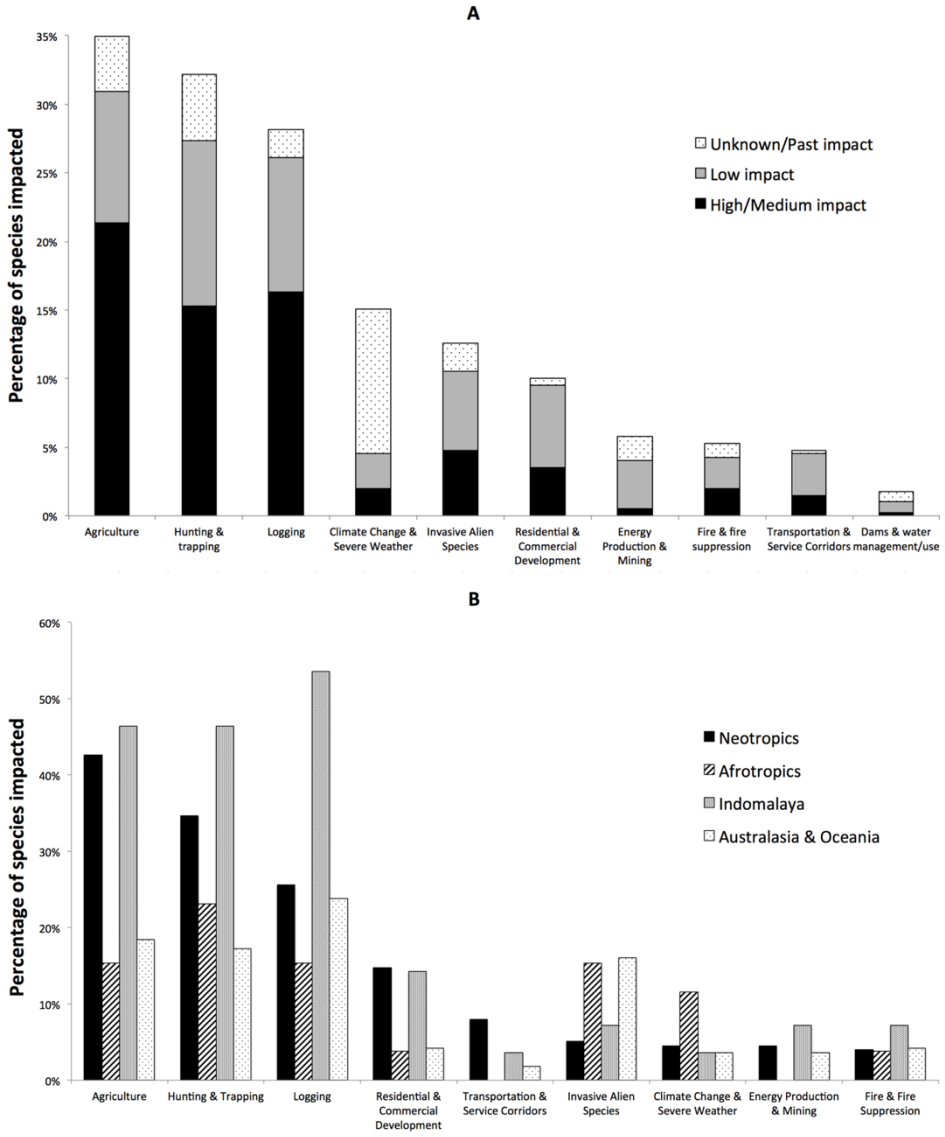

Figure 1.4. Threats: (A) Percentage of parrot species impacted by the 10 major threat types. Impact is calculated from scores for timing, scope and severity (see http://www.birdlife.org/datazone/info/spcthreat); (B) Percentage of species in each region impacted by each threat type. Only threats impacting >15 species were plotted.

The most important conservation actions as determined by BirdLife International (2016) are presented in Figure 1.5. The most common actions needed in the Neotropics are site protection and management, in the Afrotropics they are legislation and ex-situ conservation, and in both Indomalaya and Australasia/Oceania they are awareness & communications, broad-scale habitat protection, and site protection (Figure 1.5).

Our country priority method revealed the following 10 countries with highest priority ranks: Indonesia, Brazil, Australia, Colombia, Bolivia, Ecuador, Peru, Papua New Guinea, Venezuela, and Mexico (more countries in Table 1.2). Examination of the unexplained extinction risk (after all significant intrinsic and extrinsic variables were accounted for in our models) showed that island countries (or territories) accounted for both the lowest and highest values (Figure 1.6).

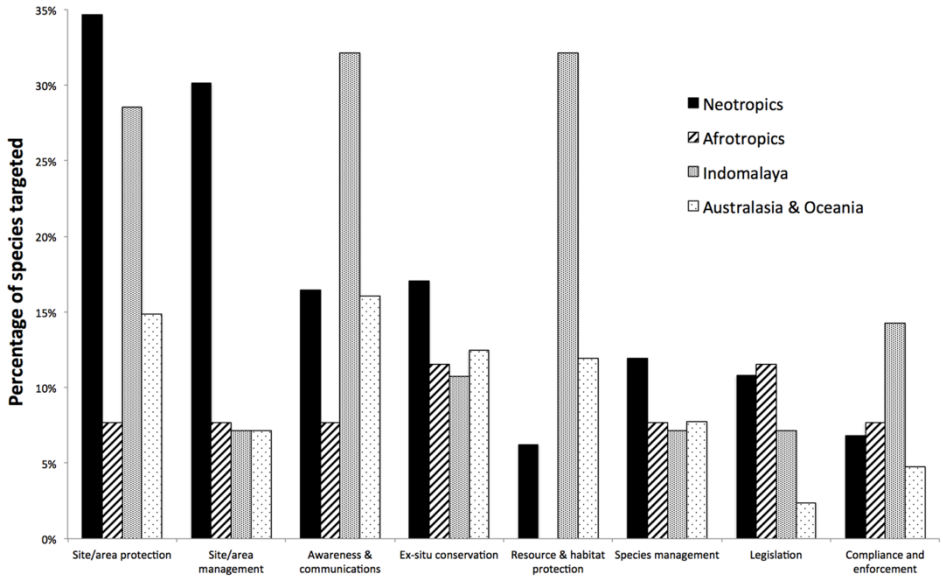

Figure 1.5. Most important conservation actions needed by region. The figure shows for each region the percentage of species for which each conservation action is considered required by BirdLife International (2014). Only conservation actions identified as required for >15 species across all regions were plotted. Order is given by total species showing the globally most important conservation actions first.

Table 1.2. The highest priority countries for parrot conservation, ranked by total numbers of their (a) parrot species, (b) globally threatened species, and (c) endemic species (restricted to one country). Overall rank is derived from the sum of ranks for the three parameters.

Country	Overall Rank	Diversity rank	Threat rank	Endemics rank
Indonesia	1	1	2	2
Brazil	2	2	1	3
Australia	3	4	6	1
Colombia	3	3	2	6
Bolivia	4	5	4	8
Ecuador	5	8	3	9
Peru	5	6	5	9
Papua New Guinea	6	6	11	5
Venezuela	7	7	8	8
Mexico	8	13	6	8
Philippines	9	16	8	4
Argentina	10	10	9	11
Guyana	11	9	11	11
Panama	11	11	10	10
New Zealand	12	20	7	5
Paraguay	12	12	9	11
French Guiana	13	10	13	11
Suriname	13	10	13	11
Costa Rica	14	14	10	11
Guatemala	15	15	11	11

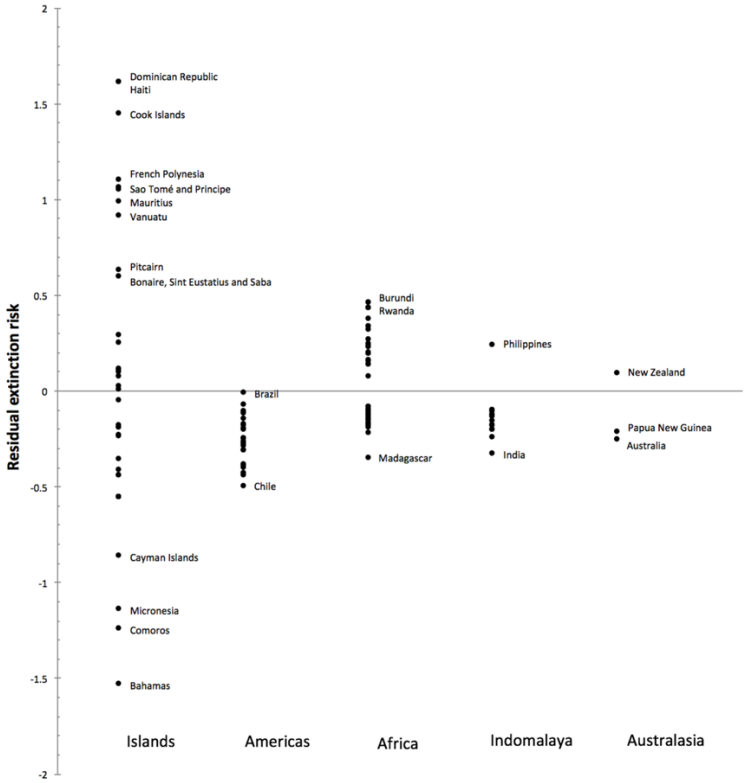

Figure 1.6. Unexplained (residual) extinction risk of parrots by country and region: values shown are each country's mean residuals from the combined linear logistic regression model predicting likelihood of a parrot species being threatened (VU, EN, CR). High positive values indicate high unexplained extinction risk.

1.4. Discussion

Our analysis of Red List Index revealed that parrots are more threatened than other comparable taxonomic groups (Figure 1.1) with consistently negative trends in extinction risk over 25 years. The results show that conservation successes have been outweighed by the number of species being up-listed to higher categories of threat (IUCN 2012). Importantly, our analysis showed no significant effect of phylogeny on threatened status of parrots (Figure 1.3). Extinction risk is usually non-randomly distributed with respect to phylogeny (Fisher and Owens 2004), hence the most illuminating comparative models are those like ours that focus on relatively narrow taxonomic groups (Cardillo *et al.* 2008).

1.4.1. Biological attributes

We found several biological attributes associated with extinction risk in parrots. We found that species with larger historical distributions are less likely to be threatened. This probably reflects the link between historical and present distribution size, and the fact that widely distributed species are often adapted to multiple habitats and less impacted by local threats (Purvis *et al.* 2000; Ewers and Didham 2006).

As for other taxa, large bodied parrots are more prone to extinction risk (Bennett and Owens 1997; Cardillo 2003). Large body size correlates with many known extinction-promoting traits, for example large species tend to have low population densities and slower life histories (Cardillo *et al.* 2005). The significant effect of residual generation time also indicates that

parrots with slower life history (longer generation time) are more likely to be threatened, independently of body size. Bennett and Owens (1997) also found that increasing body size, and residual generation time were associated with increased extinction risk among birds when analysed at the family level. Marsden and Royle (2015) showed that larger bodied parrots are predictably uncommon in the wild.

Our study confirmed that forest dependent parrot species are more likely to be threatened. Most parrots are forest dependent because of their nesting and feeding habits (Snyder *et al.* 2000; White *et al.* 2005a). At least 70% of parrots are secondary tree cavity nesters (nesting in pre-existing tree cavities), hence primary forest destruction decreases the number of available nest sites and reproductive success (Newton 1994). Forest destruction can also lead to the loss of key food resources, as many parrots eat mostly tree seeds and fruits and rely on large areas of suitable habitat to provide year-round sources of these temporally variable food sources (Brightsmith 2005c; Forshaw 2006).

1.4.2. Anthropogenic and socio-economic factors

Our data suggest a subtle effect of the extent of economic development of the countries where the parrots live. Whereas the extent of urbanization (percentage of human populations living in urban areas) seems to relate broadly to whether a parrot species is threatened, the impact of per capita GDP (i.e. developed economies) seems to have most influence by pushing parrot species into higher categories of extinction risk. Urbanization can entail far-reaching transformations in itself but is also linked to broad scale environmental degradation (McKinney 2002; Cohen 2006). As populations and economies of primarily rural countries grow there is often a migration of people to urban areas, and this in turn can be followed by further economic expansion and higher per capita GDP (Moomaw and Shatter 1996; Chang and Brada 2006). Hence urbanization can occur at high levels before GDP is maximized (Brülhart and Sbergami 2009). Our results suggest that urbanization is a good variable accounting for the impact on parrots of the earlier stages of economic development, with the continuing impact of economic development best captured by GDP.

Parrots with distributions limited to one country are also more likely to belong to higher categories of endangerment. Our analysis thus suggests that extinction risk of single country endemics (45% of all parrot species) is often adversely affected by either the singular history or the conservation management practices (or lack thereof) of their single country of occurrence. In the latter case, specific threats may be better controlled in some nations than others (e.g. progressive forestry laws and control of pet trade) with potential advantages to species living in more than one country (Hirakuri 2003; Sunderlin *et al.* 2005; Pires 2012a).

Our finding that threatened species used for pets tend to belong to the lower categories of endangerment may seem counterintuitive but is supported by recent studies which show that the vast majority of species in domestic and international bird trade are non-threatened species (Pires and Clarke 2012; Pires 2012a) and that utilized species in general are less threatened (Butchart 2008). This finding likely speaks much more to how increased threatened status (or decreased abundance) reduces use as pets than it does to how use for pets causes increases in threatened status. Pires and Clarke (2012) report that rare parrot species are only infrequently found in illegal parrot markets, confirming that most parrot poaching is driven by species that are easier to catch rather than by commercially based poachers searching for the rarest species.

1.4.3. Traits of extinct species

Our analysis of the traits of extinct species sheds light on some of our key results concerning extinction risk of extant species. For example, a disproportionate number of extinct parrot species (88% versus 24% for extant species) were insular, yet our models for extant species did not show that island endemism was a significant factor. This suggests that island endemism has been a strong factor in extinction risk but may have been masked in our analyses of extant species because many of the most susceptible species have already become extinct. In keeping with our models, extinct parrot species were larger on average than extant parrots, and most extinct species (94%) were single country endemics (compared to 45% for extant species).

1.4.4. Threat types, conservation actions, and priority countries

Our analysis reveals that the most important threats to parrots are agriculture, hunting and trapping, logging, climate change and severe weather, invasive alien species, and residential and commercial development (Figure 1.4). Invasions by non-native species are often cited as leading causes of species extinctions (Gurevitch and Padilla 2004; Carrete and Tella 2008), but our analysis suggests more complex synergistic effects in combination with other threats, including hunting and trapping, and agriculture. Interestingly, 'invasive alien species' was the sole threat significantly associated with the higher categories of threat status.

We found regional differences in the threats impacting parrots and hence the identified conservation actions required. Agriculture is a particularly important threat worldwide in terms of number of parrot species impacted (Figure 1.4), and especially in the Neotropics where site protection is identified as the major conservation action required (Figure 1.5). In Indomalaya and Australasia/Oceania, the most significant threat to parrots is logging (Figure 1.4), driving extensive deforestation in recent decades (Sodhi *et al.* 2004; Sodhi *et al.* 2010). Reflecting this, the most common conservation actions needed are broad-scale habitat protection, site protection and awareness raising (Figure 1.5). In contrast, the most common threat in the Afrotropics is hunting and trapping (primarily for the cage-bird trade and for use as pets; Figure 1.4), and the most important key actions include enforcing and enhancing legislation (Figure 1.5).

Countries with the highest conservation priority were from all regions except the Afrotropics. Although Indonesia ranked the highest overall and Australia (ranked equal third) had the highest value amongst developed countries, 15 out of the top 20 countries were from the Neotropics (Table 1.2). These countries deserve high conservation interest given the high diversity and endemism of their parrot species, and the high proportion of their parrot species that are threatened. After accounting for all the variables in our model that proved to have significant effects on parrots being threatened (historical distribution size, body size, generation time, forest dependency, and percentage of urban human population in the distribution ranges), the mean residual (or unexplained) extinction risk values of each country suggested some positive and negative trends in country-level performances. Interestingly, island countries and territories have both the highest (on average more threatened species than expected; e.g. Dominican Republic, Haiti, Cook Islands) and lowest trends (less threatened species than expected; e.g. Bahamas, Comoros, Micronesia) in conservation status (Figure 1.6).

Considering only mainland countries, the Afrotropics showed the worst trend compared to the other regions, however each region showed a wide range from low to high residual extinction risk (Figure 1.6). This method may prove important for identifying poorly performing countries (e.g. Dominican Republic, Haiti, Brazil, Burundi, Philippines, New Zealand, etc.) that need both extra conservation attention and further research on the nature of threatening processes.

1.4.5. Conclusion

Our study confirms that parrots are more threatened on average than comparable bird species groups, and that biological factors known to affect extinction risk in other taxa also apply to parrots. Thus, parrot species with small historical distribution size, large body size, long generation time, and forest dependency are most likely to be categorized as threatened (Vulnerable, Endangered, Critically Endangered) under IUCN Red List criteria. Extinct parrot species shared most of these traits but also highlight island endemism as an important factor in the past. The extent of a country's urbanization provides a broad proxy for the major human socio-economic drivers of extinction risk in parrots. Our models also revealed that parrots are more likely to be highly threatened if their distribution falls within a single country's jurisdiction and in countries with higher per capita GDP, presumably because the higher levels of development that these factors are associated with tend to drive the major threats to parrots worldwide including agriculture, hunting and trapping, and logging.

2. Global habitat destruction and its impact on parrots

2.1. Introduction

Human advance on natural habitats is a major cause of biodiversity loss (Ceballos *et al.* 2015). Approximately 39% of Earth's total ice-free surface was converted into agricultural land and settlements, and an additional 37% of global land has become surrounded by anthropogenic biomes (Ellis *et al.* 2010). These processes of transformation represent a profound change in wooded environments, where native communities of flora and fauna are disrupted (Ficetola *et al.* 2017). Many species of birds are highly dependent on forests. The order of Psittaciformes (hereafter parrots) conform one of the most threatened groups of birds (Chapter 1) including a great proportion of forest dependent species. The order encompasses 398 species of parrots divided into 6 families (Provost *et al.* 2018), distributed in four continents, and representing one of the most species-rich groups of birds. Almost a third of the extant parrots (112 species) are threatened by extinction (IUCN 2018): 18 Critically Endangered (CR), 39 Endangered (EN), and 55 vulnerable (VU). Parrots are not only affected by habitat loss but also by other human induced threats. For example, many species are considered as valuable pets, mainly due to their colourful feathers, the capacity to mimic human voice, and their great tolerance to captivity (Forshaw 2006). The human pressure on wild parrot populations has contributed to the decline of many species since the beginning of the 19th century (Forshaw and Knight 2017). Currently, 56% of parrot populations are in decline and the advancing habitat destruction of tropical and subtropical forests represents the main threat to this group globally (Chapter 1).

Although parrots are threatened by several human induced factors, their long history of coexistence with human society is also a consequence of their charismatic image and intelligence, and many of them can be used as flagship species to conserve highly threatened regions (Snyder *et al.* 2000). Indeed, those characteristics of parrots and their high level of threat has recently promoted this group to be moved to centre stage in conservation and evolution as the most threatened bird order (Heinsohn *et al.* 2018). In 2010, the International Ornithologists' Union established the Working Group on Psittaciformes (www.internationalornithology.org/psittaciformes) in order to evaluate and review the concerning situation of parrots worldwide. As a consolidated international effort, this current study follows the line of regional reviews on parrot extinction risk (Martin *et al.* 2014; Olah *et al.* 2016a; Berkunsky *et al.* 2017; Olah *et al.* 2018), this time concerning the global threats of habitat destruction to parrots and evaluating their future in protected areas.

While agriculture, hunting, trapping, and logging have been highlighted as the most important drivers of parrot extinction risk (Wiley *et al.* 2004), their relative importance strongly differ among regions (Chapter 1). While agricultural practices are the main threats to the group, they are especially relevant in the Neotropics, one of the most species rich areas (Berkunsky *et al.* 2017). In other regions like Africa, parrots, such as the Psittacus species, are heavily threatened by poaching, logging, and hunting (Martin *et al.* 2014; Martin 2018). In addition, the introduction of alien species has been pointed out as the main threat for the taxa in the

Pacific Islands, a centre of endemism for the group with almost the half of them threatened (Olah *et al.* 2018). These varied external factors often interact with intrinsic attributes of parrots, such as large body size, narrow range of distribution, or forest dependence (Bennett and Owens 1997). In fact, over 70% of parrot species are forest dependent due to their feeding and breeding habits (i.e. parrots are secondary tree cavity nesters) (Snyder *et al.* 2000; White *et al.* 2005b). Therefore, they are strongly affected by different kinds of human activities in forested regions, and most of them are extremely sensitive to the reduction of nesting sites caused by forest destruction (Newton 1994).

It is not novel that forests are highly threatened by agriculture and urbanization, processes in which original habitats become heavily fragmented or disappear entirely (Laurance *et al.* 2012; Hansen *et al.* 2013). These changes in land use result in serious biodiversity threats (Foley *et al.* 2005; Fischer and Lindenmayer 2007; Jetz *et al.* 2007). Although this process operates at the global scale, its intensity and dynamic are temporally and spatially heterogeneous. Historically, rates of land change during the 20th century and early 21st century were the highest in human history (Goldewijk, 2001; Hansen et al., 2013). Briefly, in North America and Europe, forest conversion processes were rapid in the 19th century but have since declined, whereas in South America, Southeast Asia and western Africa, conversion rates were high during the 20th century, with a continuous increment at present (Hansen *et al.* 2013). The most threatened areas of these continents correspond to tropical and subtropical forests, coinciding with the major reservoirs of global biodiversity, and the distribution hotspots of parrot species (Laurance *et al.* 2012). The future perspectives of forest loss are highly dependent on both biophysical and socioeconomic factors. For example, crop growth depends on local soil and climatic conditions, and human decision-making influences land use in response to global markets (Verburg *et al.* 2011). As almost all the countries harbouring tropical and subtropical forests are emergent or developing countries, their economies are supported by primary production (Sloan and Sayer 2015).

Therefore, the main drivers of forest loss are commercial agriculture and subsistence agriculture (both including forest clearing for cropland), pasture and tree plantations, and permanent subsistence and shifting cultivation (Hosonuma *et al.* 2012). Besides, forest clearing accelerates the human intrusion to adjacent forests and allows the exploitation of additional resources such as timber, bush meat, and animals (Cullen *et al.* 2000; Torres *et al.* 2018). Logging and hunting are commonly associated to the edges of deforested areas and usually coexist in space and time (Brodie *et al.* 2015). While hunting and poaching directly impact parrot populations by the extraction of wild individuals, logging has an indirect but long-lasting negative effect on these populations by reducing their nesting sites. Forest loss (and its inherent human activities, such as logging and hunting) is expected to be continued and increased in the future in America, Africa, and Asia (Hosonuma *et al.* 2012). In this regard, it is unknown how these processes of forest degradation and loss will impact on habitat availability for parrots and their distribution in the future.

In the context of rapid forest habitat loss with associated human impacts (i.e. hunting and logging) and their innate biodiversity, protected areas (PAs hereafter) play a crucial role in maintaining original diversity, hence being the cornerstone of in situ conservation (Pollock *et al.* 2017). Unfortunately, several studies have highlighted that in the past, protected areas have been allocated without proper assessments on species distribution or their ecological needs

(Rodrigues *et al.* 2004; Nori *et al.* 2015; Vergara-Tabares *et al.* 2018; Vieira *et al.* 2019). In general, PAs are designated due to their scenic beauty or the cheap values of lands due to the low potential for extractive and/or primary production (Venter *et al.* 2014; Pressey *et al.* 2015). The dominantly residual nature of PAs, in concordance with their management problems (Watson *et al.* 2014) and their relatively high degree of human disturbances (Jones *et al.* 2018), are the main reasons that PA networks are not capable of properly representing and protecting biodiversity (Rodrigues *et al.* 2004; Venter *et al.* 2014; Nori *et al.* 2015). In that regard, birds are poorly represented in PAs, which largely varies at different spatial scales and in different locations (Vergara-Tabares *et al.* 2018; Prieto-Torres *et al.* 2018). Given these patterns and the perspectives of land changes, it is essential to evaluate the parrot representation under current PA networks, in order to provide better implications for their conservation.

In this study, we use parrots as model group, given their high forest dependency, the several human threats they are facing, and their potential as a surrogate group for conservation planning, to explore how the temporal variation in forest loss (considering past and future land-use scenarios) affects the extinction risk of this group in a global context. Specifically, we aim to (i) identify hotspots for parrot conservation, based on forest dependent species richness, their conservation status, and land-use trends; (ii) analyse potential changes in extinction risk of parrots considering the land-use changes, human pressure (logging and hunting), forest dependency, and decreasing population trends under the thresholds imposed by IUCN (focusing on habitat availability); and (iii) study the efficiency of the current global network of PAs to represent parrot diversity worldwide.

2.2. Methods

2.2.1. Spatial database construction

We downloaded digital range maps (extent of occurrence maps) for 398 parrot species available at BirdLife International and Handbook of the Birds of the World (2018). Using the spatial join tool of ArcGIS 10.3, we associated each range with the RedList conservation status of the species (LC to CR), their population trend (IUCN 2018), and three different habitat needs of the species (hereafter forest dependency). This corresponds with an international and widely accepted classification, where experts classified birds into three categories based on their dependence on forest environments: (a) highly forest-dependent species that only occupy forest environments; (b) medium forest-dependent species; and (c) low or non-forest-dependent species (BirdLife International 2016). We also included the altitudinal range occupied for each species to the attribute table. We calculated the distribution area for each species in km^2.

Global land cover maps with a resolution of 300 m for the year 2000 was downloaded from ESA Climate Change Initiative (2017). This source provides the highest resolution images of forest loss at global scale for this period. Then, they were reclassified in ArcGIS 10.3, in order to generate a new binary raster discriminating those areas occupied by agricultural crops (i.e. pixels with at least 50% of their surface occupied by crops) and urban settlements, from all the other categories. For the current global land cover (i.e. 2018), we downloaded and compiled images provided by the Tree Cover Loss platform (Hansen *et al.* 2013) with resolution of 30 m (https://earthenginepartners.appspot.com/science-2013-global-forest). With these images we assembled a binary raster using the same taxonomical criteria as for the land cover of year

2000 (see above). Given that the Tree Cover Loss platform only provides images of forest loss from 2000 to 2018, in order to obtain an image with the current global forest loss, these images were combined with the global land cover from ESA (showing land covers form previous periods). We also used predicted layers of forest loss and urban areas by the year 2050 from the Harmonized Global Land Use (Chini *et al.* 2014) with a spatial resolution of 0.5 square degrees, which provided the first consistent set of land-use change and emission scenarios for studies of human impacts on the past and future global carbon-climate system. Among the available future scenarios (combinations of different socioeconomic pathways and radiative forcing scenarios), we selected "the middle of the road (SSP2)" as socioeconomic pathway which consider that the world follows a path in which social, economic, and technological trends do not shift markedly from historical patterns. We used the RCP4.5 as radiative forcing scenario that considers medium carbon emission and medium mitigation actions, both coherent with the SSP2 model.

To evaluate the influence of other human threats associated to forest loss, we used a global land cover of wood extraction (Hurtt *et al.* 2011) as the proxy to logging and hunting/poaching. We downloaded a global land cover of wood extraction for 2018 and 2050 that were available from the Harmonized Global Land Use (Chini *et al.* 2014). These global layers are built under our selected socioeconomic pathways (SSP2) and radiative forcing scenarios (RCP4.5). This source provides a temporal series of predicted wood extraction and allows to select the global prediction for any year comprised between 2005 and 2100. These layers provide an estimation of wood extraction per grid cell measured in ton/km^2, so we only considered those grid cells that belong to the third and fourth quartiles (i.e. areas with the highest wood extraction). In addition, we reclassified the grid cells of the third quartile as high wood extraction and those of the fourth quartile as very high wood extraction categories. From the different available environments for which wood harvest biomass can be calculated using Harmonized Global Land Use, we only considered the following: primary forest, primary non-forest (shrubs), secondary mature forest, secondary young forest, and secondary non-forest (degraded shrubs). In this way, we avoided including wood biomass from tree plantations into the analyses, which could generate strong bias.

Finally, we obtained shapefiles of terrestrial protected areas (PAs) around the globe from the World Database on Protected Areas website (UNEP-WCMC and IUCN 2015). We only selected those terrestrial protected areas with the "designated" status (i.e. we did not consider PAs with "inscribed", "non-reported", or "proposed" status), from I to IV management categories defined by the IUCN (i.e. categories which have specific conservation objectives), totalling 85,378 protected areas. The shape file of the selected PAs was rasterized at spatial resolution of 1 km in ArcGis 10.3.

2.2.2. Data analysis

We used the 'raster' (Hijmans *et al.* 2015) and 'maptools' (Bivand *et al.* 2017) packages of R statistics (R Core Team 2013) in order to minimize the inaccuracy of distributional polygons (Ficetola *et al.* 2014). We overlapped each polygon with a raster of altitude at spatial resolution of 1 km, so we were able to extract those pixels of each distribution inside the known altitudinal range occupied by species. This methodology allowed us to generate individual rasterized distribution for each species by refining the information of their polygon with ecological

information of their altitudinal ranges. After that, we multiplied the individual raster of each species by a value corresponding to its conservation status (LC = 1; NT = 2, VU = 3; EN = 4; CR = 5). We used an ordinal scale for the conservation status given that the trends in the resulting index are driven by a relatively large number of species (hence producing a more robust and representative index). This is because a species moving from Least Concern to Near Threatened contributes just as much to the changing score as a Critically Endangered species going Extinct, and the numbers of species in each category (and the number moving in and out of each category) increases disproportionately from Critically Endangered to Least Concern (Butchart *et al.* 2004). Each resulted raster was multiplied by its forest dependence (low and no forest dependence = 0; medium dependence = 1; high dependence = 2). Finally, rasters of overlapping species were merged in order to generate a weighted raster of richness. Because species with low or no forest dependence were multiplied by zero, these species were excluded from the final maps. The weighted map of richness was overlapped with the binary rasters of human-dominated areas (for the three periods 2000, 2018, and 2050), and the portion of the maps of richness overlapping with human-dominated areas were extracted. Then, we superposed the map of richness in human-dominated areas with the original map of richness in order to generate a bivariate global map, showing the value of weighted richness in both human-dominated and wild habitats. In summary, we generated a bivariate map of weighted richness for each period.

We calculated the distribution percentages for each species overlapping with both categories of land cover (human dominated and wild) for each period, by using the 'maptools' (Bivand *et al.* 2017) and 'raster' (Hijmans *et al.* 2015) packages of R statistics (R Core Team 2013) with the refined shapefiles of the species distributions and the binary land cover raster of each period. In the same way, we overlapped the individual raster of each species with the map of PAs and calculated the degree of representation for each species in the global network of PAs (mean ± standard deviation). We also calculated the mean value of wood extraction per km^2 across each parrot distribution for 2018 and 2050. Additionally, we identified those forest dependent species that currently had a decreasing tendency in population size and cross some of the thresholds imposed by the criterion B1 of IUCN. The criterion B is based on the geographic range of the species (B1 = extent of occurrence and/or B2 = area of occupancy) and imposed several thresholds that define a conservation category. To establish a conservation status of a species based on criterion B, it is also necessary to demonstrate at least two of the following conditions: (a) severely fragmented or known to exist at only a certain number of locations; (b) continuing population decline; and (c) extreme fluctuations in occupancy, subpopulations or mature individuals (IUCN 2012). Although, our approach only considered the variation of each parrot species' geographic range and their current population tendencies, it has been largely evidenced that habitat reduction induces fragmentation and increased extinction risk of subpopulations (Brooke *et al.* 2008; Hanski *et al.* 2013).

2.3. Results

From the 398 parrot species, 76 had low or no forest dependency and hence were excluded from subsequent analysis. Out of the included species, 68.9% (222 species) were categorized as non-threatened (i.e., LC or NT). From the 100 remaining species, 48 were categorized as

VU, 35 as EN, and 17 as CR. From the 76 non-forest-dependent species, only 14.7% were categorized as threatened, including seven VU, four EN, and one CR species.

Both weighted and non-weighted maps of richness (Figure 2.1) indicated that the most important conservation hotspots for the group are located at the northeast of the Amazon forest, in the southern Amazon Basin, with a maximum index value of 44 in the weighted map of richness and 31 species in the non-weighted map of richness. There are other areas with high index values ranging from 22 to 33. These are located in South America (including the northern Amazon Basin, portions of the Tropical Andes, small areas confined within the Atlantic Forest, and the Cerrado; Figure 2.2A), and in Australasia (including the temperate forest of southern Australia and the New Guinea highlands; Figure 2.2B). The geographic pattern showed in the maps of weighted richness (Figure 2.2) did not differ from the non-weighted maps using all parrot species (Figure 2.1).

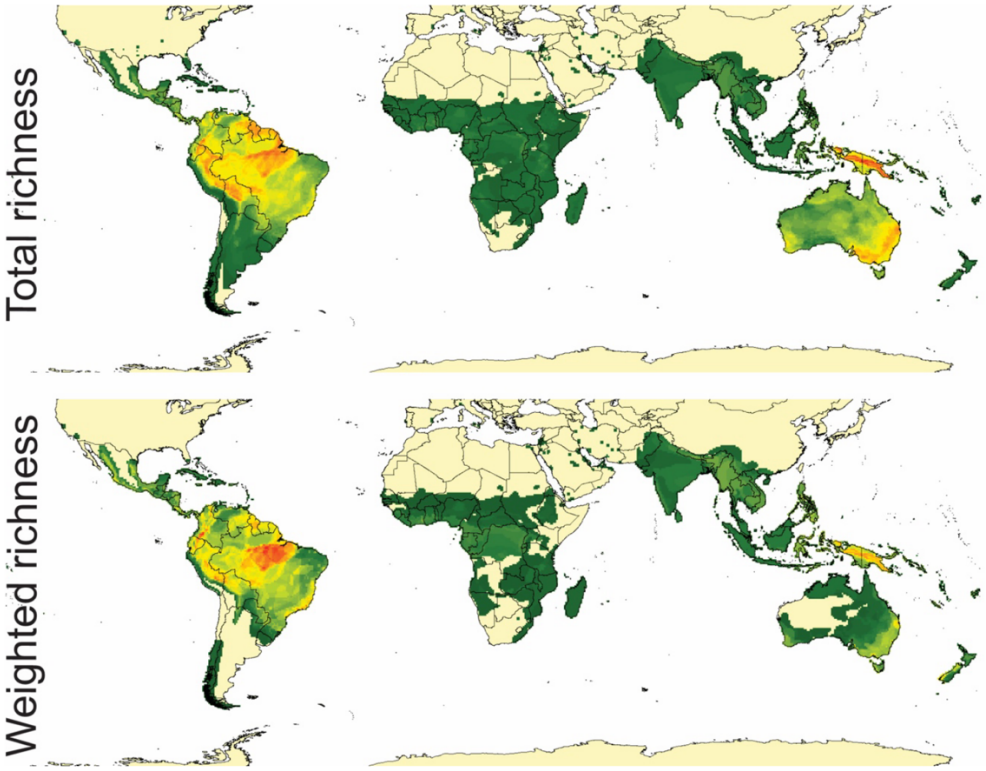

Figure 2.1. Comparison of weighted and total richness maps of parrot species. Orange areas indicate greater richness and green areas lower richness.

We found that human-modified environments are quickly encroaching over the most important areas for parrots, when overlaps between weighted richness and land-use changes in different time-periods were analysed. The most parrot species-rich areas in South America (located in the eastern Amazon Basin) were practically intact in 2000, but human-modified environments increased in 2018, and our forecast for 2050 indicated a stabilization of intensive human activities in the region. Trends also indicated dramatic increases in human-dominated lands in other important Neotropical areas including the Brazilian Atlantic Forest, the Andes, intermontane valleys in Colombia, and Central America toward 2050 (Figure 2.2A). On the other hand, trends in New Guinea and the central and western portions of the Amazon Basin

and the tropical Andes indicated that parrot hotspots will not be heavily threatened by intensive human activities in the near future (Figure 2.2). The temperate forest of Australia and the large islands of Indonesia were already heavily degraded by human-modified landscapes in 2000, and the trends toward 2050 indicate that additional changes due to human activities will affect these regions in the near future (Figure 2.2B). Finally, in Africa (with lowest parrot richness relative to the global parrot distribution), the land-use change will advance mainly in the west-central areas and in the mountains of the east-central part of the continent in the future (Figure 2.2C).

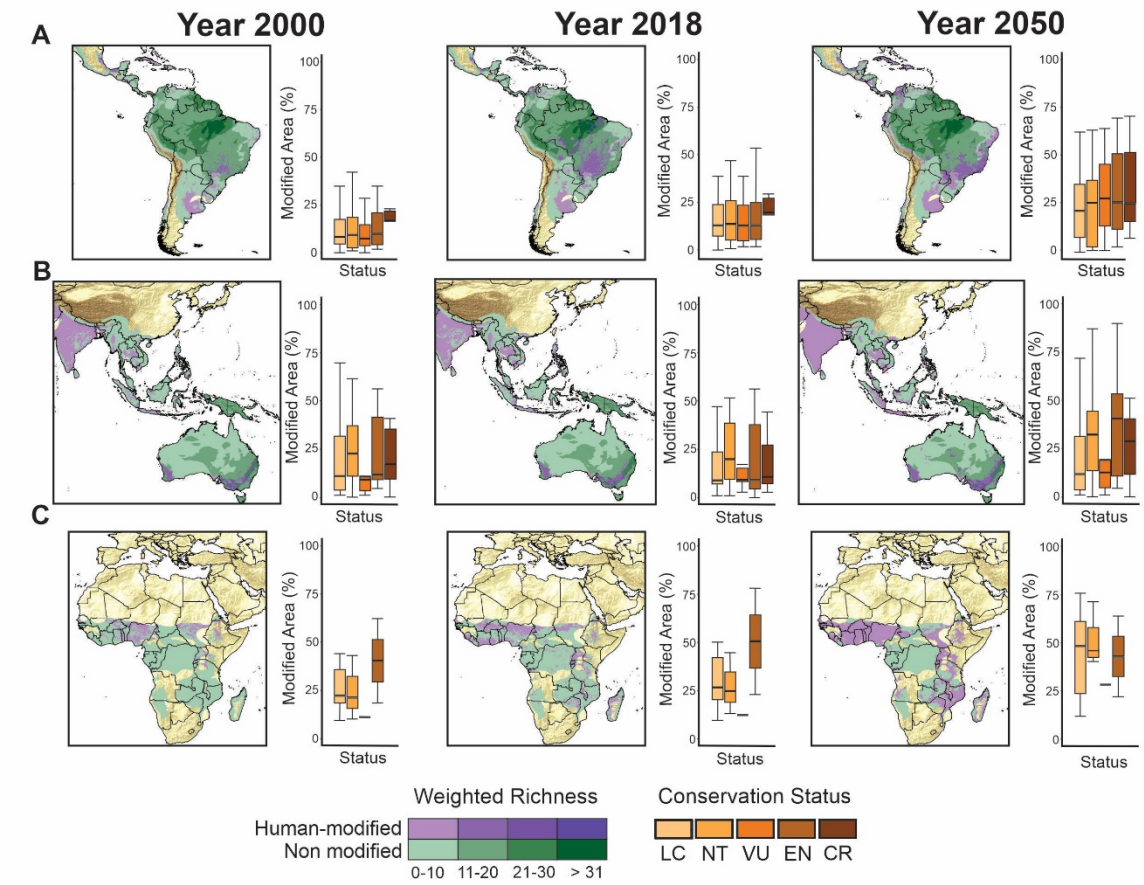

Figure 2.2. Maps of conservation hotspots for parrot conservation in relation to land use. Colour gradients indicate the overlap of maximum existing/expected weighted richness for parrot species with different land use: green gradient represents non modified areas; purple gradient represents human-modified areas (intensive agriculture and urban areas). Darker areas indicate greater weighted richness for both colour gradients. Global maps cover (A) Central- and South America; (B) Australasia and Oceania; and (C) Africa. In vertical directions are represented the three different temporal scenarios analysed. Box plots indicate the loss of area per conservation status including all parrot species distributed on the associated map. Lines in boxes are medians, boxes indicate 25th and 75th percentiles, whiskers indicate the data range, and points are outliers.

We found that the most important hotspots for parrot diversity (northwestern Amazon and western New Guinea) will be suffering very high rates of wood extraction (Figure 2.3A and Figure 2.3C) by 2050. According to our forecasts, this picture will be more extensive in area

but less intense in the Amazon basin (Figure 2.3A), and it will be greatly reduced in New Guinea by 2050 (Figure 2.3C). Contrary, in central Africa there is currently an intense wood extraction, which would be exacerbated toward 2050 (Figure 2.3B). In addition, some important regions without intensive wood extraction at the moment (e.g. central Bolivia and northeastern Argentina), are expected to suffer drastic increases by habitat destruction in the near future (Figure 2.3A). Finally, several scattered areas worldwide will keep on with very high levels of wood extraction until 2050, including central-western Africa, eastern India, and many islands of Indonesia (Figure 2.3B and Figure 2.3C).

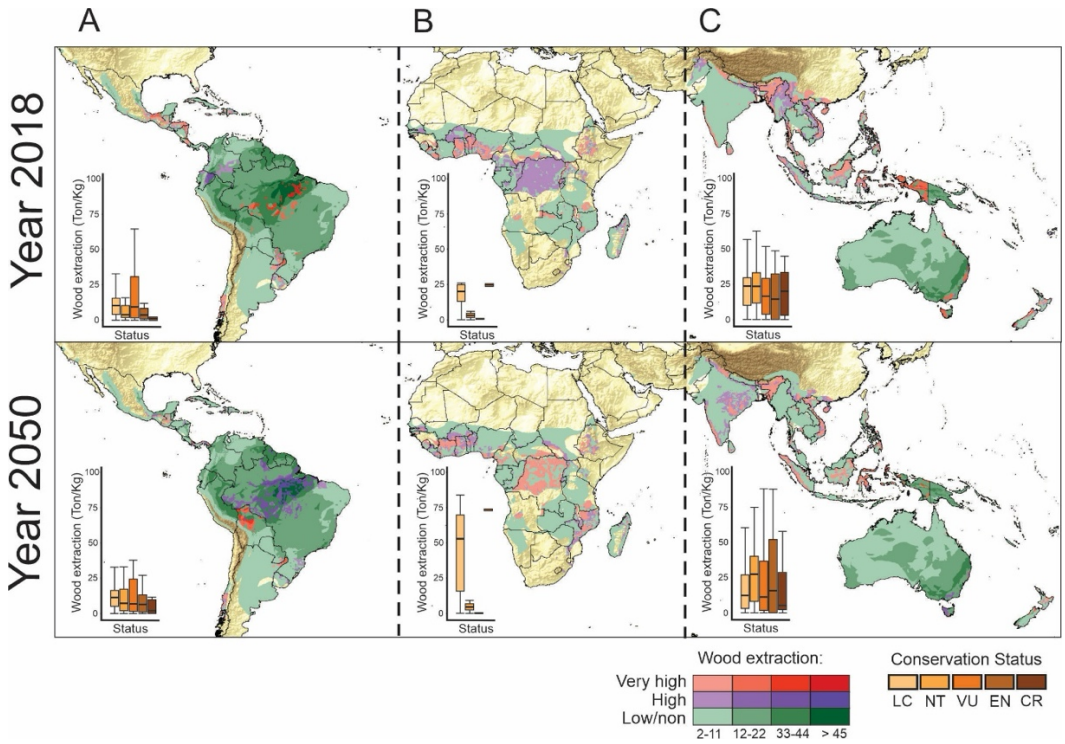

Figure 2.3. Maps of conservation hotspots for parrot conservation in relation to timber extraction. Colour gradients indicate the overlap of maximum existing/expected weighted richness for parrot species with different levels of wood extraction: green gradient represents areas with low or null wood extraction (proxy of logging and hunting/poaching); purple gradient represents areas with high wood extraction (defined by the limits of the third quartile of wood extraction distribution); and red gradient represents areas with very high wood extraction (defined by the fourth quartile of wood extraction). Darker areas indicate greater weighted richness for all colour gradients. Global maps cover (A) Central- and South America; (B) Australasia and Oceania; and (C) Africa. Box plots indicate the wood extraction (ton/km^2) per conservation status including all parrot species distributed on the associated map. Lines in boxes are medians, boxes indicate 25th and 75th percentiles, whiskers indicate the data range, and points are outliers.

On average, only 10.2% (±13.4 SD) of the geographic range of all parrot species overlap with protected areas. The results are similar when analysed regionally, with the average overlap varying from 8% in America to 23% in Africa. Finally, when we considered the conservation status of the species in the analysis, the least represented species in PAs were those classified as CR (with 4.46% in America and 21.1% in Australasia). The best represented groups of

parrots in PAs were those categorized as EN with average overlap of 15% in Australasia, and those categorized as VU with 11.1% in America and 14.5% in Africa (Figure 2.4).

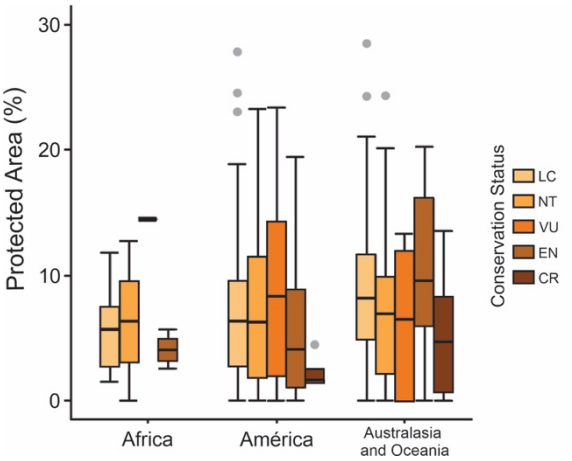

Figure 2.4. Average overlap of the geographic range of parrots with protected areas, partitioned by global regions and the conservation status of the species. The values were calculated using the land cover map of 2018.

Regarding forest dependent parrot species with decreasing population trends, our results show that some of these inhabiting America, Southeast Asia, and Oceania could change their conservation status by 2050, given the current land-use trends. The species for which we expect a greater overlap with human-dominated environments will most probably cross at least one of the thresholds imposed by the B1 criterion of IUCN. We identified 26 species crossing these imposed thresholds: 10 in America and 16 in Australasia and Oceania (Figure 2.5).

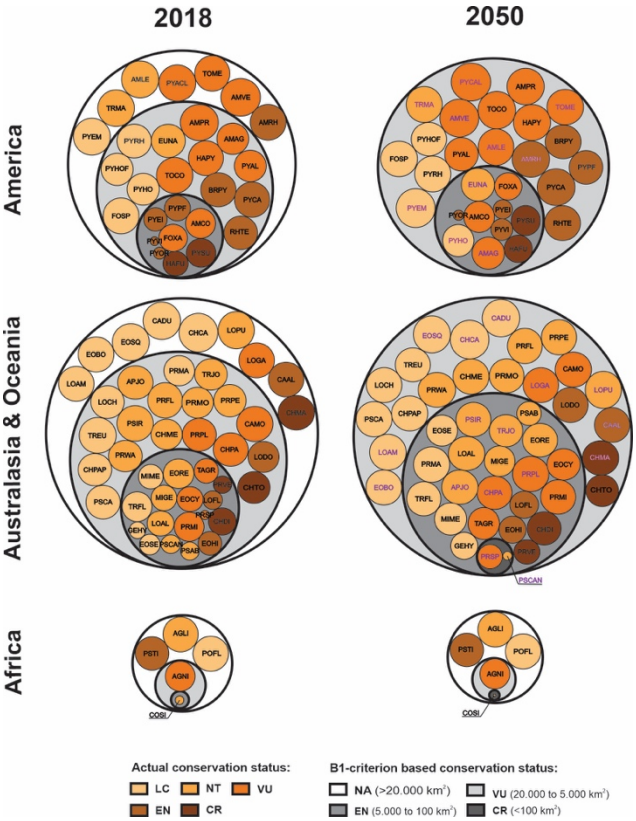

Figure 2.5. Current and predicted conservation status of forest dependent parrot species. Internal circles represent each forest dependent parrot species that has less than 20,000 km^2 of range distribution occupied by non-human dominated habitat. The surface of each circle indicates the log of the surface in km^2. External circles (in bold) represent the thresholds from the criterion B1 for conservation status. The parrot circles were partitioned by geographic region and period (present and future). Species acronyms for 2050 in purple letters indicate that the species will decrease habitat surface overstepping the threshold for conservation status (B1 criterion). Acronyms in blue letters indicate an expected increase in habitat surface. Different colours of parrot circles indicate the current conservation status of IUCN. Acronyms represent species.

2.4. Discussion

In this work, we provide a global picture on the effect of human habitat transformation (for past, present, and future periods) for the conservation of forest dependent parrots, identifying particular areas where human encroachment would threat important hotspots for their conservation. Worryingly, some of these hotspots are heavily threatened or show an uncertain forecast, compromising the conservation of parrots as the most threatened bird group, and warning policy-makers to generate suitable conservation policies. Combining parrot richness with their conservation status and forest dependence, we detected four parrot conservation hotspots worldwide. These areas are in concordance with previous studies (see Chapter 1): two in the Neotropics (eastern Amazon Basin and the northeastern Andes) and two in Australasia/Oceania (New Guinea and southeastern Australia). In regard of the two land-use trends analysed in this study, the current picture for parrot conservation clearly differs among these hotspots. In addition, it is expected that these differences will increase towards the future. The areas of New Guinea and the Amazon Basin have currently a low overlap with human-modified landscapes, with low changes expected in the near future. In contrast, the area located in southeastern Australia shows a great overlap with human-modified landscapes, with a substantial increase expected in the future. While the current overlap with human-modified environments is low in the northeastern Andes, one of the most important parrot conservation hotspots, a great increase is expected by 2050. Most importantly, all parrot conservation hotspots are affected by intensive wood extraction, which also affect parrot populations by reducing availability of nesting sites and/or increasing the accessibility to hunting and poaching activities (Brodie *et al.* 2015). Additionally, the size of the affected areas by these threats is expected to increase in the near future, while their intensity would show a great variation among regions. All these results suggest that the future of parrots is subject to policy-making in specific countries, and so decision making is especially important in countries sharing the eastern Amazon Basin, as it was shown for many other key groups of species (Soares-Filho *et al.* 2006; Miranda *et al.* 2019).

We highlighted the northeastern Andes and southeastern Australia as two of the most important hotspots for parrot conservation, however the current situation of these areas is not reassuring. They had high deforestation rates during the last decades (Hansen *et al.* 2013) and have a worrying future under problematic conservation policies (Clerici *et al.* 2019). According to our predictions, the change in land-use could strongly compromise most of the distributions of threatened parrot species sensitive to these changes. This could have a drastic effect on parrots by 2050 if we consider that many species will overlap with the increased land-use, which in turn will increase their conservation status by crossing thresholds imposed by the B1 criterion of IUCN. Considering the already Endangered and Critically Endangered parrot species, this poses an extremely elevated extinction risk to them in the near future. The extinction of parrot species may entail the deterioration or loss of some ecological functions, such as long-distance dispersal of large trees (Blanco *et al.* 2016; Tella *et al.* 2019). The conservation of ecosystem function and the great diversity in these two regions may be planned using parrots as surrogate (or umbrella) species given several characteristics that the group represent in the area (i.e. high richness and endemism, their conservation problems, and the empathy that they generate in humans).

Importantly, in the phylogenetic context of parrots, Australasia and Oceania (especially New Guinea and southeast Australia) also become very relevant for their conservation (Provost *et al.* 2018), hosting three endemic parrot families. The rough terrain of New Guinea indirectly protects many parrot species from land-use change, mainly confined to its mountainous regions, but the western side of the island belonging to Indonesia is experiencing very high wood extraction that may represent an unevaluated threat (Figure 2.2B). This further supports the assumption of Chapter 1 that the surprisingly low number of threatened parrot species in New Guinea is mainly given by the lack of information about those species and the threats they are facing, and that further evaluation might lead to revisions of their conservation status. The situation of the Australian parrot conservation hotspot is much more uncertain. Currently, this hotspot is mostly overlapped with human-modified landscapes (Figure 2.2B) and land-use increment is expected in this area (Verburg *et al.* 2011). Given the current situation of shortage of forest remnants, the future of the group will depend on the rate of forest loss and other stochastic factors as well as natural disturbances (e.g. frequency of fires which are especially high in the region) or biological invasions (Heinsohn *et al.* 2015).

Considering the predictions for agricultural expansions for the near future, it is expected that land-use changes will have a great general impact on the conservation status of parrots worldwide. Most of the forest dependent parrot species that are expected to increase their conservation status considering the criterion B1 of IUCN by 2050 are distributed in the Neotropics and southeastern Australia (Figure 2.5). Among these neotropical species, four are currently listed under Least Concern and Near Threatened categories (*Pyrrhura emma*, *P. hoematotis*, *Triclaria malachitacea,* and *Eupsittula nana*) and may be seriously affected by increasing urbanization and agriculture in the future. In addition, many parrot species are mainly distributed in the West Indies, the northeastern Andes, and the Atlantic Forest regions where a high percentage of habitat destruction is expected due to agriculture by 2050. In addition, those regions harbour a high proportion of endemic species that, according to our results, could be threatened seriously in the near future (e.g. *Eupsitytula nana* and three *Amazona* spp. in the West Indies; *Triclaria malachitacea, Touit melanonotus*, and *Amazona rhodocorytha* in the Atlantic Forest; and three range restricted species from the northeastern Andes and Venezuela).

We predicted that the most changes in the conservation status of parrots will happen in the region of Australasia and Oceania in the near future, mainly because there are more species expected to experience great habitat reductions and the majority of them are endemic to islands. From the 16 species that are expected to cross a threshold of IUCN in these regions, six are categorized as Least Concerned and four as Near Threatened, most of them distributed in archipelagos of the Moluccas, Timor, and the Solomon Islands. In addition, six species with higher categories of threat are also distributed in the Solomon Islands, Moluccas, the Philippines, and Fiji. Many of these species will not only experience habitat reduction but also be threatened by invasive species that increase their extinction risk (Olah *et al.* 2018). Finally, regarding African parrots, there are only a few species inhabiting small areas (close to 20,000 km^2 in total), but a large proportion of them (3–6 species) will experience some habitat destructions, most of which will predictably happen in central Africa.

Timber extraction is another important threat to parrots, which can adversely affect their natural populations in the long term, given their strong dependence on nest hollows for

reproduction. Traditionally, this threat was mainly evaluated locally in a case-to-case basis (e.g. Heinsohn *et al.* 2003; Olah *et al.* 2014) but here we analysed it in a global scale. The current trend is worrying, as very high rate of wood extraction occurs over a large area of the Amazon Basin, the most important hotspot for parrot conservation. Additionally, this fact is directly associated with poaching, one of the main threats to neotropical parrot species (Berkunsky *et al.* 2017). Moreover, our predictions showed that timber extraction will affect an even larger area of the Amazon Basin by 2050, elevating this already important parrot conservation hotspot to a higher priority. Other important hotspots for parrot conservation are also predicted to suffer similar fate due to wood extraction, including New Guinea, central Africa, and southeastern Australia. In summary, our results warn that wood extraction can be seen as one of the most important threats for parrots, and is occurring in key areas for their conservation. Considering that many wood extraction activities are illegal, and when legal are frequently associated with other illegal activities like poaching, there must be strict governmental regulations of wood extraction activities with efficient law enforcement, especially in the mentioned key areas.

The global system of PAs should also play an essential role in maintaining populations of threatened and key species free from human-modified encroachments. However, this is not the case for parrots. We highlighted that, in fact, the most threatened group of parrots (CR) is the less represented in PAs globally and also locally in each region (Figure 2.4). This is an additional proof of the inefficiency of PAs at the global scale, i.e. not protecting efficiently nearly 85% of the threatened terrestrial vertebrates (Rodrigues *et al.* 2004; Venter *et al.* 2014; Jones *et al.* 2018), and specifically the diversity of birds (Vergara-Tabares *et al.* 2018). Historically, the conservation of biodiversity has not been the primal criteria for establishing PAs (Rodrigues *et al.* 2004; Pressey *et al.* 2015; Nori *et al.* 2018), and today most of them are still located in residual places where the potential for extractive uses is low (Devillers *et al.* 2015). The predominantly residual nature of PAs (mainly in developing and highly diverse countries) is one reason why species continue to go extinct, even when most countries have increased the number and extent of established PAs over the past 10 years (Watson *et al.* 2014).

Analysing parrot distributions, their diversity, habitat dependence, and conservation status, here we present a global overview of conservation hotspots and conservation trends of this highly diverse, charismatic, and most threatened group of birds. Unfortunately, the degree of deterioration of many parrot conservation hotspots are (and will be) high. In addition, it is expected that the current global changes may generate further increases in the conservation status of most parrot species, pushing many of them to the edge of extinction. Protected areas seem not to play a significant role in reverting, stopping, or at least slowing down this process. These negative trends that we obtained may entail new challenges in a good moment, when the concern for nature conservation is high and protected areas are increasing worldwide (Jones *et al.* 2018). The future of wild living parrots is strongly subject to the highlighted parrot conservation hotspots, especially in southern Australia and the Amazon Basin. In these hotspots, decision-makers should use the flagship image of parrots as a tool for guiding conservation policies.

3. Status of parrots in Oceania

3.1. Introduction

Parrots (Psittaciformes) originated on the Gondwanan supercontinent, likely during the Cretaceous period (Stidham 1998; Wright *et al.* 2008), and experienced a major radiation between 60 and 65 million years ago in Australasia (Cracraft 2001; Schweizer *et al.* 2010). There were 398 parrot species worldwide in 2016, and 112 (28%) were categorised as threatened according to the 2016 IUCN Red List (BirdLife International 2016; IUCN 2016). Among these, 55 are considered Vulnerable (VU), 39 are Endangered (EN), and 18 are Critically Endangered (CR). Of the remaining species, 60 are classified as Near Threatened (NT), and 226 are of Least Concern (LC). Parrots carry a higher risk of extinction than most other birds (Butchart *et al.* 2004), which makes them an important group for identifying the factors underlying a wide and complex range of threatening processes.

Comparative analyses are useful tools in conservation biology because they help to prioritise conservation actions while taking phylogenetic factors into consideration (Fisher and Owens 2004). They have been used, for example, to predict which groups of species warrant additional conservation attention and to identify the underlying biological and anthropogenic factors associated with conservation issues (Bennett and Owens 1997). Chapter 1 used comparative methods to assess the factors associated with higher extinction risk in parrots worldwide, and found that parrots were more likely to be threatened if they were endemic to a single country, or if they occurred in a country with a large urban population, or a high GDP. Parrots also faced a high risk of extinction if they had limited historical distributions, high dependency on forest, large bodies, or long life spans (Chapter 1). Each of the studied regions had a distinct pattern of threats, suggesting that conservation solutions would differ on a regional basis. Recently, other authors have reviewed the specific issues affecting parrots in the Afrotropics (Martin *et al.* 2014), and in the Neotropics (Berkunsky *et al.* 2017). Forshaw and Knight (2017) also published a thorough overview of the world's extinct and threatened parrots.

Here, we focus on the factors affecting extinction risk in Oceania (i.e. Australia, New Zealand, New Guinea, Wallacea, and the Pacific Islands). This area contains 167 extant parrot species (42% of the world total; Figure 3.1) from 48 genera (55% of 88 parrot genera), many of which are endemic to the region (Forshaw 2011; Forshaw and Knight 2017).

Oceania includes the large, developed countries of Australia and New Zealand, as well as a mixture of other developed and developing territories. New Guinea is one of the largest islands in the world, and is governed by the developing nations of Indonesia and Papua New Guinea. It is of high priority for parrot conservation, particularly due to the level of endemism, and the diversity of species found there. Indonesia also governs most of the islands in Wallacea (excluding Timor-Leste), which are rich in endemic species. For the purposes of this study, we treat the Pacific Islands as three discrete archipelagos: Micronesia, Melanesia (aside from New Guinea), and Polynesia (aside from New Zealand).

In this chapter, we reviewed the parrot species of Oceania both qualitatively, by describing the different threats affecting them, and quantitatively, by using comparative models to identify the factors affecting extinction risk. We placed particular emphasis on Australia and the Pacific Islands, two subregions with sufficient parrot species and ecological data to investigate threatening processes in detail. We were also interested in assessing the impact that island endemism has on predisposing species to extinction, particularly considering that Oceania contains the highest rate of threatened endemic parrots in the world, and most extinct parrots occupied small islands (Chapter 1). As many islands in the region are plagued by invasive species (Theuerkauf *et al.* 2010; Heinsohn *et al.* 2015), we predicted that this threat will have a large influence on the conservation status of parrots in Oceania. We highlight the effects of data deficiency of parrots in the region, and suggest directions for focused conservation research by highlighting the species and locations most in need of attention.

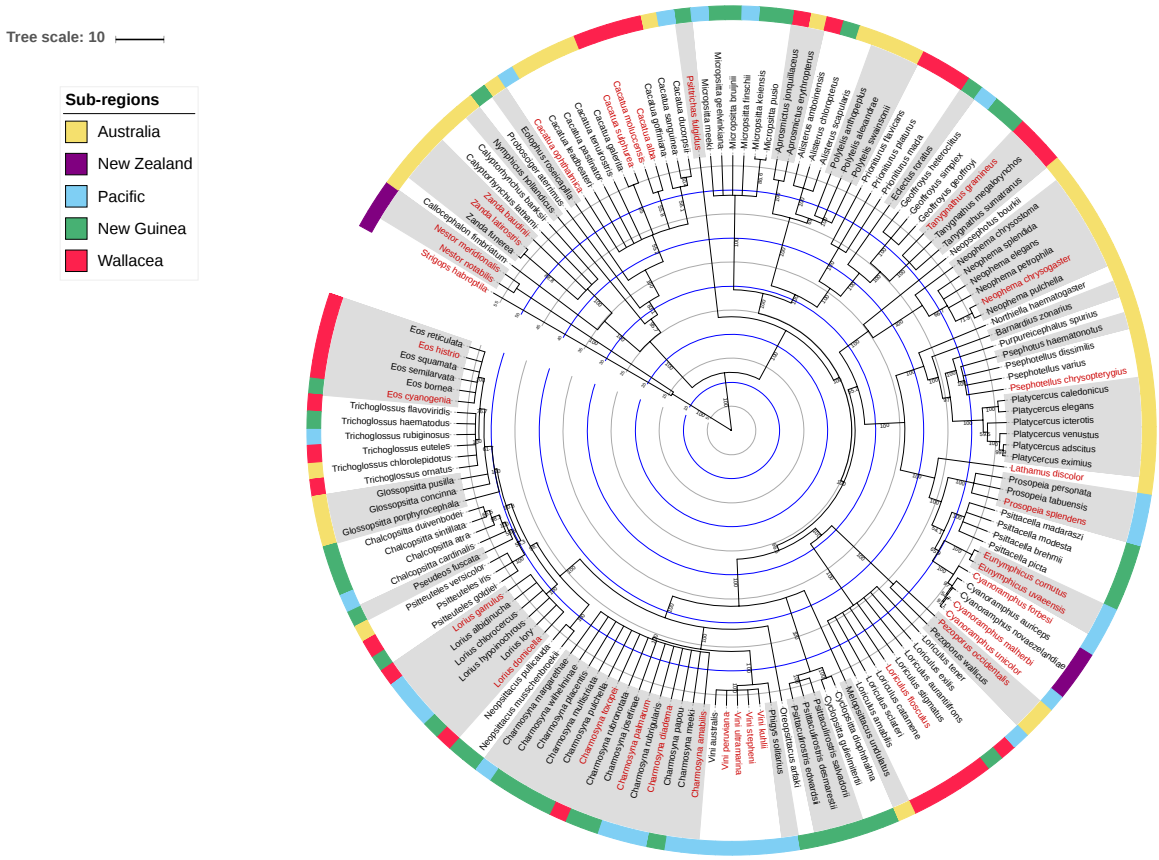

Figure 3.1. Consensus tree with 50% majority-rule of parrot species in Oceania. Coloured strips on the circumference represent the main subregions associated with each species: Australia (yellow), New Zealand (purple), the Pacific (blue), New Guinea (green), and Wallacea (red). Grey shading is used to distinguish genera. Threatened species (Vulnerable, Endangered, and Critically Endangered) are labelled in red, and non-threatened species (Least Concern and Near Threatened) are labelled in black. Values on the nodes indicate the consensus support (%) based on 1000 trees from birdtree.org. The phylogenetic tree is available in colour at https://itol.embl.de/shared/olahgy

3.2. Methods

3.2.1. Database construction

We followed the taxonomy used by BirdLife International (2016) for non-passerines. Using BirdLife's data on species' distributions, we grouped the 167 parrot species from Oceania into five subregions (Figure 3.1): Australia (50 species), New Zealand (6), New Guinea (46), Wallacea (34), and the Pacific Islands (31). For the response variable of conservation status, we used the 2016 IUCN Red List (IUCN 2016) to assign a value of '1' to threatened species (VU/EN/CR), and a value of '0' to non-threatened species (LC/NT). We divided the large number of explanatory variables into four groups: (A) geographical and distributional attributes of each species, (B) biological, ecological and life history variables, (C) type of utilization by humans, and (D) socio-economic and demographic attributes of the countries where the species occur. For detailed descriptions, source of data, and values of each variable see Table 3.1. We used ArcGIS 10.2 to calculate the extent, and average latitude, of the distribution of each species. We calculated the size of each species' historical range from species range maps (BirdLife International and HBW 2016). We mapped historical distributions using the same sources as for contemporary distributions but added in areas where the species was recorded before 1980, yet either had no recent survey records, lacked suitable habitat, or were deemed inappropriate based on expert opinion (BirdLife International and HBW 2016). Historical range size and body size were normalized using natural logarithms.

Table 3.1. Explanatory variables used in the statistical models. The four groups of variables represent (A) geographical and distributional attributes of each species, (B) biological, ecological and life history variables, (C) type of utilization by humans, and (D) socio-economic and demographic attributes of the countries where the species occur.

Group	Variable name	Description	Values	Data source*
A	Average Latitude	Absolute value of the average of northern and southern limits of a species range	decimal degree of latitude	(1)
	Historical Distribution Size [e]	Previous extent of the species' range based on modern and historical records	km^2	(1)
	Mean Altitude	Mean elevation of the species range	m	(2)
	Subregion	Distribution of the species (categorical variable)	(1) Australia, (2) New Zealand, (3) Pacific islands, (4) New Guinea, (5) Wallacea	(3)
	Island Endemism	If the species is endemic to islands smaller than 110,000 km^2 (this excludes large islands, such as New Guinea, Borneo, Sumatra, Celebes/Sulawesi, New Zealand, Java)	yes/no	(2)
B	Body Size [e]	Length of the species	cm	(4)
	Migratory Status	The stage of the migratory status of the species (categorical variable)	(0) Not a Migrant, (1) Nomadic, (2) Altitudinal Migrant, (3) Full Migrant	(2)

	Main Diet	The main food items consumed by the species (categorical variable)	(1) Frugivore (fruits, vegetable matters, leaves, fungi, lichens), (2) Granivore (grass seeds), (3) Nectarivore (nectar, pollen, flowers), (4) Tree seeds (hard seeds, acorns, nuts, cone seeds), (5) Specialist	(5)
	Social Flocking	Flock size in the non-breeding season (categorical variable)	(0) No flocks (alone or pairs), (1) Small flocks (up to 20), (2) Large flocks (more than 20)	(4; 6)
	Colony Nesting	If the species nests in colonies	yes/no	(4)
	Nesting Tree Type	The main nest type used by the species (categorical variable)	(1) hardwood trees and their branches; (2) palm trees; (3) other nest types (e.g. termite mounds, epiphytes, moss, burrows, grass, etc.)	(4; 5)
	Forest Dependency	Scored from published and unpublished information on the ecology of each species (categorical variable)	(0) Non-forest, (1) Low, (2) Medium, (3) High.	(2)
	Generation Time	Mean generation length	year	(2)
C	Captive Breeding	If the species breeds regularly in captivity	yes/no	(6)
	Used for Pets	Pets are defined as those species recorded as being kept in captivity, either as personal pets, or for display in zoos, collections etc.	yes/no	(2)
	Used for Food	If the species is used for food	yes/no	(2)
	Used for Accessories	If the species is used for making accessories	yes/no	(2)
	Used for Sport	If the species is used for sport purposes	yes/no	(2)
D	Single Country Endemic	If the species is endemic to one country	yes/no	(2)
	Per capita GDP	Gross domestic product based on purchasing-power-parity (PPP) per capita	amount in USD	(7; 8)
	Industrial Production Growth Rate	Industrial production growth rate of countries where species is extant	mean percentage	(7)
	Unemployment Rate	Unemployment rate of countries where species is extant	mean percentage	(7; 8)
	Human Population Density	Mean population density of countries where species is extant	number of people/1000Ha	(7; 8; 9)
	Urban Population	Human population living in urban areas of countries where species is extant	mean percentage	(9)
	Human Population Growth Rate	Human population growth rate of countries where species is extant	mean percentage	(7)
	Agriculture Area	Agricultural area of countries where species is extant	mean percentage	(9)

***** Data source: (1) BirdLife International and Handbook of the Birds of the World (2016); (2) BirdLife International (2016); (3) Olson *et al.* (2001); (4) Forshaw (2011); (5) Juniper and Parr (1998); (6) Arndt (2007); (7) CIA (2013); (8) IMF (2013); FAOSTAT (2013). [e] loge transformed variables.

3.2.2. Statistical modelling

We fitted a logistic regression model (correlating explanatory variables to the binary response variable) using the 'GLM' function in R statistics (R Core Team 2013). We carried out analyses separately for each group (A–D) of explanatory variables. First, we performed a likelihood ratio test (LRT) on each group, including all possible variables. We used this to select the best model with only the statistically significant variables. Then, we ran the model with the chosen variables to calculate *P*-values for each of the included variables. We generated one model for all of Oceania (167 species), and two additional models for Australia and the Pacific Islands, as these regions contain sufficient species and information to assess local threats in more detail. For the Australian analysis, we used the Environment Protection and Biodiversity Conservation Act (EPBC 2016) to determine conservation status. We conducted the analysis on 54 native Australian parrots, including four species whose main range is located elsewhere: Double-eyed Fig-parrot (*Cyclopsitta diophthalma*), Eclectus Parrot (*Eclectus roratus*), Red-cheeked Parrot (*Geoffroyus geoffroyi*), and Palm Cockatoo (*Probosciger aterrimus*). We carried out a separate analysis on the 31 parrot species native to the Pacific Islands.

3.2.3. Phylogenetic control and tree

To control for the effect of possible phylogenetic relatedness among species in the statistical models, we used 1000 possible phylogenetic trees of parrots, by accounting for the branch lengths and nodes separating species (Jetz *et al.* 2014; Provost *et al.* 2018). For each phylogeny, we ran a phylogenetic generalized least squares (PGLS) regression using the 'caper' package in R (Freckleton *et al.* 2002). The explanatory and response variables were the same as those used in the regression models. For each explanatory variable, we report the modified *P*-value accounting for phylogenetic relatedness. For each model, we report a variable (lambda transformation) that improves the fit of the phylogenetic data. Greater lambda values indicate that the relationship between response and explanatory variables correlates with the phylogeny, and that the values of the explanatory variables are more similar for closely related species. We also include the mean and standard deviation of lambda, calculated from 1000 phylogenetic hypotheses for each model.

We created a 50% majority-rule consensus tree with Geneious R6 (Kearse *et al.* 2012), using 1000 trees downloaded from birdtree.org. For each node, we report the clade credibility value (consensus support of the given node, as a percentage). Only 151 species were included in the tree, as 16 species were missing from the birdtree.org dataset. We generated Figure 3.1 using iTOL v3 (Letunic and Bork 2016).

3.2.4. Heat maps and analysis of threats

We estimated the area of each species' range using digital distribution maps (BirdLife International and HBW 2016). These ranges were derived from a variety of sources (for detailed description see Buchanan *et al.* 2011). We used the union function in ArcGIS 10.4.1 to generate heat maps of species richness by overlaying the range of each parrot species in Oceania. The number of species in each overlapping polygon were then summed, excluding Extinct presence and non-native species. We also generated a heat map showing where the most threatened species occur, following the 2016 IUCN Red List categories (VU/EN/CR).

We analysed the threats affecting parrots using the same procedures as in Chapter 1. First, we estimated impact scores based on the timing, scope and severity of threats listed by BirdLife International (2016), then we pooled the medium and high impact scores. For all of Oceania, we determined how many species were impacted by each threat, and to what extent. We also estimated how many parrot species were impacted by the threats in each subregion. As few of the extinct parrots in Oceania were studied in detail, we pooled data from both Extinct and Critically Endangered species, then compared their collective traits with those of extant species using means and 95% confidence intervals in R (R Core Team 2013).

3.3. Results and Discussion

Oceania possesses 42% of the world's parrot species, including half (9 out of 18) of those classified as Critically Endangered. It was a focal point for the early diversification of parrots and includes many early branching lineages of the psittaciform phylogeny (Wright *et al.* 2008; Schweizer *et al.* 2010; Schweizer *et al.* 2011). The region accounts for much of the phylogenetic diversity of this charismatic order of birds (Figure 3.1). However, 37 of the 167 extant parrot species in Oceania are currently threatened with extinction, and the specific conservation requirements of many species remain poorly understood. We have attempted to address some of these knowledge gaps by extracting information from large, international datasets (BirdLife International 2016; IUCN 2016). While we acknowledge that there are shortcomings and inaccuracies in such databases (see below), they are invaluable for broad-scale analyses of extinction risk, and useful for identifying data deficiencies. Our analysis identifies the major threats impacting parrots in Oceania, and the species and areas most urgently in need of conservation.

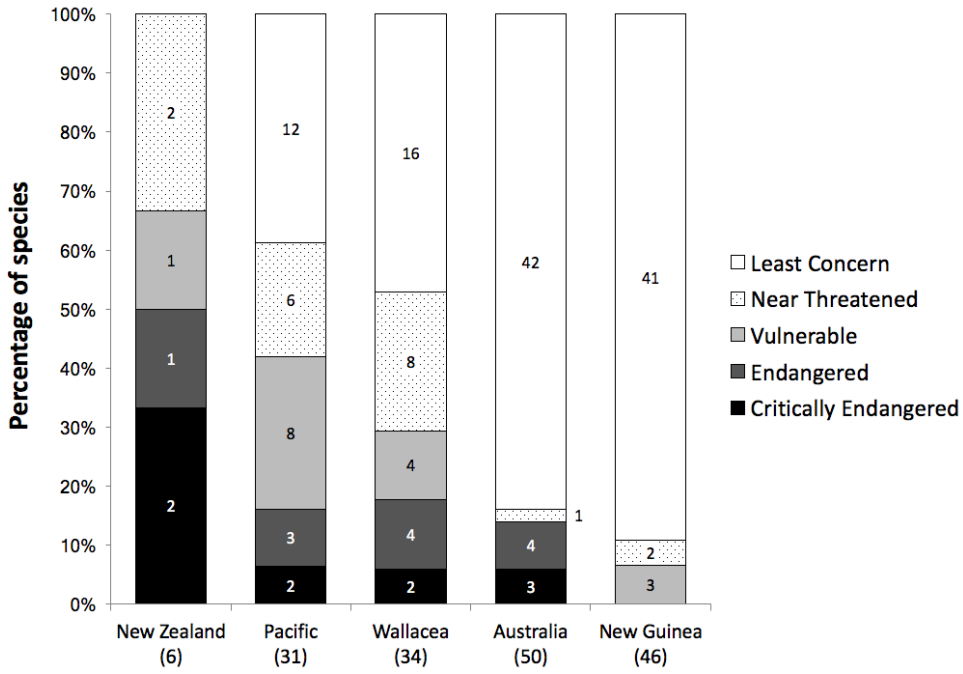

Figure 3.2. Extinction risk of parrot species in Oceania, derived from BirdLife International's assessments for the 2016 IUCN Red List. Columns are broken into the percentage of species per category in each subregion. Digits represent the number of species.

In Oceania, five parrot species are considered Extinct, nine are Critically Endangered, 12 are Endangered, 16 are Vulnerable, 19 are Near Threatened, and 111 are categorised as Least Concern. New Zealand has the highest percentage of threatened parrot species (67%), followed by the Pacific (42%), Wallacea (29%), Australia (14%), and New Guinea (7%; Figure 3.2 and Figure 3.3).

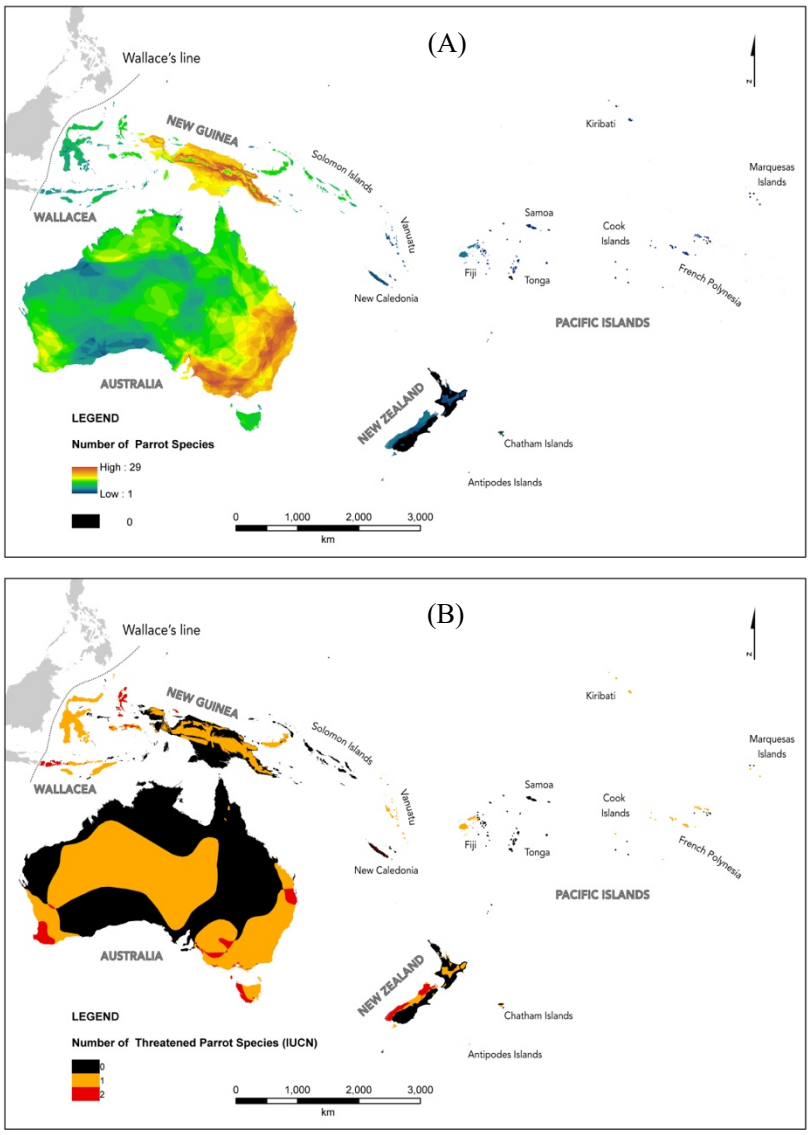

Figure 3.3. Heat maps of parrot species diversity and extinction risk in Oceania. (A) Species richness map of parrots in Oceania, generated by overlaying the distribution maps of parrot species in the region. (B) Richness of threatened (Vulnerable, Endangered, and Critically Endangered) parrot species in Oceania, generated by overlaying the current distribution maps of threatened parrot species in the region. Colour codes indicate the number of parrot species.

Historical range size and single-country endemism are important predictors of extinction risk for the parrots of Oceania (Table 3.2). Species with naturally small ranges appear to be more prone to threats than others, possibly as a result of limited habitat availability and lower dispersal capacity. Although island endemism was not a predictor of conservation status, its importance may have been overshadowed by the inclusion of other variables, such as historical range size and endemism to a single country.

Table 3.2. The statistical significance of four groups of variables as predictors of the likelihood of parrot species in Oceania being threatened (VU/EN/CR). Results of the binomial logistic regression are provided with the likelihood ratio test (LRT), degree of freedom (d.f.), and chi-square *P*-values (P_{chi}). Results from the phylogenetic generalized least squares model (PGLS) are provided as the means of the estimate with their standard deviation (SD) in brackets, *P*-values (P_{PGLS}), and lambda (λ) values for each PGLS model with SD in brackets.

Group*	Variable	LRT	d.f.	P_{chi}	PGLS estimate (SD)	P_{PGLS}	λ (SD)
A	Average Latitude	9.19	1	0.004	-0.009 (0.001)	<0.001	0.039 (0.103)
	Historical Range Size ($\log_e$)	10.15	1	<0.001	-0.089 (0.001)	<0.001	
	Mean Altitude	4.47	1	0.101			
B	Body Size ($\log_e$)	4.77	1	0.018	0.192 (0.008)	0.037	0.363 (0.167)
	Forest Dependency	5.44	4	0.002	0.136 (0.002)	<0.001	
C	Used for Pets	7.02	1	0.026	-0.287 (0.009)	0.009	0.534 (0.063)
	Used for Sport	5.56	1	0.987			
D	Single Country Endemic	12.36	1	0.004	0.212 (0.006)	0.002	0.412 (0.109)
	Unemployment rate	3.90	1	0.037	0.028 (0.001)	0.008	

* A: geography and distribution, B: biology, C: type of utilization by humans, D: socio-economy and demography.

In Oceania, the probability of extinction was higher for larger parrot species, and those with stronger dependence on forest (Table 3.2). Larger body size is correlated with lower reproductive outputs, which may hinder population recovery after disturbance (Cardillo *et al.* 2005). Like other parts of the world, parrots in Oceania rely on trees for nest cavities and food (Snyder *et al.* 2000), and it is this dependency that makes them so vulnerable to forest loss and degradation.

Throughout Oceania, parrots were more likely to be threatened in less-developed countries with high unemployment rates (Table 3.2). These countries often have higher rates of poaching, causing a high degree of pressure on parrot populations, particularly where there is a shortage of paid work (Wright *et al.* 2001; Barré *et al.* 2010). There is little published information about the extent of trapping in Oceania, but the parrot trade is generally more active in less-developed countries (Pain *et al.* 2006). Parrot species that are kept as pets tend to be less threatened in Oceania (Table 3.2), and elsewhere in the world (Chapter 1). However, this might be because common species are more likely to be kept as pets (Butchart 2008; Pires 2012a; Vall-llosera and Cassey 2017). Although latitude is not a significant factor in explaining extinction risk among parrots globally (Chapter 1), it appears to be a significant factor in Oceania, with higher-latitude species more likely to be threatened (Table 3.2). This is possibly driven by the relatively high proportion of threatened parrots in Tasmania and New Zealand (Figure 3.3B), which have suffered from the introduction of non-native predators (Dilks *et al.* 2003; Heinsohn *et al.* 2015).

Parrots in Oceania were mainly threatened by logging (23% of species), agriculture (18%), hunting and trapping (17%), invasive species (16%), fire and fire-suppression, outside of natural range or frequency (4.2%), residential and commercial development (4%), energy production and mining (4%), and climate change and severe weather (4%; Figure 3.4). On a

global scale, agriculture has the largest impact on the extinction risk of parrots (Chapter 1), but in Oceania parrots were mostly threatened by logging (i.e. harvesting trees for timber, fibre, or fuel), and for many species this impact is considerable (Figure 3.4A). The conversion of native forest to tree plantations (e.g. oil palm plantations in Indonesia) is known to have an adverse effect on native biodiversity (Koh and Wilcove 2008), and also reduces habitat for most forest-dwelling parrots in the region. Invasive species threaten a greater proportion of parrots in Oceania than elsewhere in the world (Chapter 1). Of the other threats facing parrots in Oceania, only logging, agriculture, and poaching have negative impacts of comparable magnitude (Figure 3.4A).

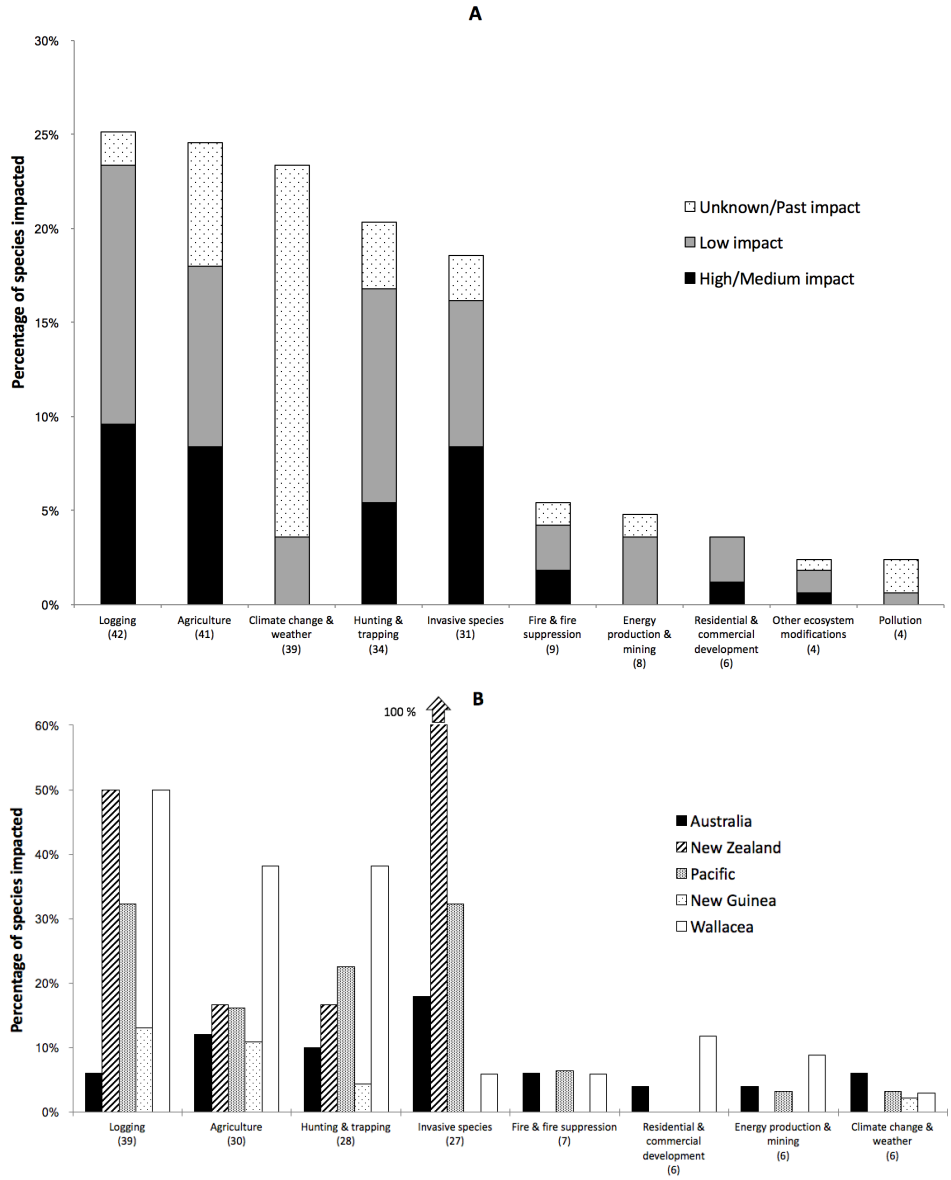

Figure 3.4. Percentage of parrot species impacted by different threats in (A) Oceania, and (B) each subregion of Oceania. Threat impact is extracted from BirdLife International (2016), calculated from scores for timing, scope and severity of threats. Only threats impacting more than five species were plotted. The number of species impacted by each threat is given in brackets.

3.3.1. Parrots of Australia

Australia, including its offshore islands, has 54 species of parrots, of which 15 are currently listed as threatened (EPBC 2016). In our analysis of this subregion, we found that Australian parrots were more likely to be threatened if they had small historical ranges (LRT = 2.82, df = 1, P_{PGLS} = 0.001). On average, the historical range of threatened parrot species was 721,000 km^2 (SD = 931,000), compared to 1,673,000 km^2 (SD = 1,851,000) for non-threatened species. Coxen's Fig-parrots (*Cyclopsitta coxeni*) and Palm Cockatoos (*Probosciger aterrimus macgillivrayi*) provide examples of threatened rainforest taxa with relatively small ranges that are severely affected by deforestation and habitat degradation (Coxen's Fig-Parrot Recovery Team 2001; Murphy *et al.* 2003; Heinsohn *et al.* 2009).

We also found that larger Australian parrots were more likely to be threatened by extinction than smaller species (LRT = 4.95, df = 1, P_{PGLS} = 0.005). Threatened species had a mean body size of 40 cm (SD = 16), compared to 29 cm (SD = 10) for non-threatened species. Palm Cockatoos, along with four other threatened black-cockatoo species (*Calyptorhynchus* spp. and *Zanda* spp.), exemplify the conservation status of large-bodied parrots in Australia. The south-western subspecies of the Red-tailed Black-cockatoo (*Calyptorhynchus banksii naso*) is threatened by the loss of nest hollows from mining (Chapman 2007) and the transformation of forest into agricultural land (Abbott 1998). The south-eastern subspecies (*C. b. graptogyne*) is threatened due to major clearing of its woodland habitat (Maron 2005). Long-billed Black-cockatoos (*Zanda baudinii*) and Carnaby's Black-cockatoos (*Zanda latirostris*) have suffered from habitat loss as well. The Kangaroo Island subspecies of Glossy Black-cockatoo (*Calyptorhynchus lathami halmaturinus*) has also suffered major population declines, mainly due to increased predation by Common Brushtail Possums (*Trichosurus vulpecula*) and forest clearing (Garnett *et al.* 1999; Lee *et al.* 2013).

In contrast with the trends observed in Oceania as a whole, Australian parrots were most likely to be threatened by invasive species (18%), followed by agriculture (12%), and hunting and trapping (10%; Figure 3.4B). For instance, the migratory Swift Parrot (*Lathamus discolor*) has suffered a severe decline in Tasmania due to habitat loss caused by residential and industrial development, and because of predation by introduced Sugar Gliders (*Petaurus breviceps*) (Heinsohn *et al.* 2015). Another migratory species from Tasmania, the Orange-bellied Parrot (*Neophema chrysogaster*), is currently on the brink of extinction in the wild due to degradation of wintering habitat by grazing, agriculture, urban development, and predation by introduced foxes (*Vulpes vulpes*) and feral cats (*Felis catus*) in their breeding range (Department of Environment Land Water and Planning 2016). Reasons for the decline of the recently rediscovered, cryptic Night Parrot (*Pezoporus occidentalis*) are unknown, but are likely to include predation by feral cats and foxes, loss of mature spinifex habitat by altered fire regimes, and habitat degradation by introduced rabbits (*Oryctolagus cuniculus*) and camels (*Camelus dromedarius*) (Whitlock 1923; Blyth 1997; Murphy *et al.* 2018).

Many Australian parrot species (e.g. black-cockatoos) are threatened by the transformation of their preferred habitat into agriculture. This threat was the second highest in Australia (Figure 3.4B), having impacts on 12% of parrot species. The third highest threat was trapping, impacting 10% of the Australian parrot species. Although trapping for the international trade is kept to a minimum through strict biosecurity frameworks (Vall-llosera and Cassey 2017),

this threat class also includes persecution for pest control, a more common issue affecting Australian parrots.

3.3.2. Parrots of New Zealand

As part of the first major landmass to split from Gondwana, New Zealand is home to an ancient parrot family (Strigopidae), which includes the Kakapo (*Strigops habroptila*), Kea (*Nestor notabilis*) and Kaka (*Nestor meridionalis*; Figure 3.1), and is a sister clade to all other parrots (Schweizer *et al.* 2011). The remainder of New Zealand's parrots are *Cyanoramphus* species, including the Yellow-crowned Parakeet (*Cyanoramphus auriceps*), Red-crowned Parakeet (*Cyanoramphus novaezelandiae*), and Orange-fronted Parakeet (*Cyanoramphus malherbi*). The Chatham Islands and Antipodes Islands also possess several *Cyanoramphus* parakeets, but these have been treated here as Pacific Islands, on account of their isolation.

While there are too few parrot species in the region for statistical analysis, some generalisations can be made. Of the six species assessed, four were threatened with extinction, and all were impacted by invasive species (Figure 3.4B). Half of the species were impacted by logging (Figure 3.4B), but this was mostly in the past, or localised.

It is worth noting that several of the mammalian predators introduced to New Zealand are absent from other islands in the Pacific, which may explain why New Zealand has suffered such a disproportionate loss of native species. Stoats (*Mustela erminea*) and Common Brushtail Possums have been implicated in the decline of a range of endemic birds, including the Yellow-crowned Parakeet, Red-crowned Parakeet, and Orange-fronted Parakeet, as well as the larger parrots, such as Kea, Kaka, and Kakapo. Rats also pose a serious threat to New Zealand's parrots. For example, less than four years after the establishment of Black Rats (*Rattus rattus*) on Big South Cape Island, populations of Red-crowned Parakeets and Yellow-crowned Parakeets were experiencing major declines (Atkinson 1985; Ortiz-Catedral and Brunton 2010). Rodents and rabbits are also capable of sustaining abnormally high densities of predators, especially cats, which have the potential to decimate populations of native species.

In New Zealand, the impact of predators is exacerbated by a phenomenon known as 'masting'. The southern beech forests (*Fuscospora* spp.) provide extensive habitat for parrots, and in 'mast' years, they produce copious quantities of seed (Fitzgerald *et al.* 2004). This presents an opportunity to breed, not only for parakeets, but also for mice, rats, and subsequently, stoats and weasels (*Mustela nivalis*). When the seed runs out, rodent numbers crash, and mustelids target native birds instead (Fitzgerald *et al.* 2004).

The Orange-fronted Parakeet is currently facing extinction in the wild, largely as a result of these predator plagues (Kearvell *et al.* 2002). The decline of Yellow-crowned Parakeets has not been as dramatic, perhaps because they forage less frequently in the understorey (Elliott *et al.* 1996; Kearvell *et al.* 2002). In contrast, Red-crowned Parakeets have been extirpated from most parts of the mainland, presumably due to their tendency to forage and nest on the ground (Greene 1998). The decline of the Red-crowned Parakeet was probably accelerated by an increase in browsers, especially deer, which generate a more open environment for cats, and other predators, to hunt in (Kearvell *et al.* 2002).

Several other suggestions have been proposed to account for the decline of Orange-fronted Parakeets, including competition with Yellow-crowned Parakeets for food and nesting hollows, competition with introduced finches (*Carduelis* spp.) for seed, and competition with

invasive wasps (*Vespula* spp.) for invertebrates (Kearvell *et al.* 2002). Given that a considerable proportion of parakeet nestlings die of starvation in the wild (Greene 2003; Ortiz-Catedral *et al.* 2013), it is possible that competition could influence reproductive output, but this is only likely to be an issue if competitors are sufficiently abundant to restrict food availability.

While Kaka are subject to competition from wasps and possums, their main limiting factor appears to be predation, primarily from stoats and possums, which prey upon eggs, chicks, and nesting females (Wilson *et al.* 1998).

Kea populations have also declined as a result of nest predation, most likely by stoats and possums (Elliott and Kemp 2004). Although Kea nest in cavities on the ground, their tendency to select very steep nest sites near the bush-line probably reduces their accessibility to predators (Elliott and Kemp 2004).

Kakapo are particularly vulnerable to introduced mammals, especially stoats and cats (Powlesland 2006). This large, flightless parrot would likely be extinct if the last known individuals had not been translocated to offshore islands that are free of mammalian predators (Clout 2006). The last natural population was discovered on Stewart Island, one of the few sites in New Zealand that has not been invaded by stoats (Powlesland 2006).

3.3.3. Parrots of the Pacific Islands

There are 31 extant parrot species in the Pacific Islands (Figure 3.1), but nearly half (47%) are threatened with extinction (Figure 3.2). Recent population estimates suggest that the New Caledonian Parakeet is also threatened with extinction (Legault *et al.* 2013). However, it was included here as a subspecies of the Near Threatened Red-crowned Parakeet (*C. novaezelandiae*), following del Hoyo and Collar (2014). Nevertheless, genetic evidence (Boon *et al.* 2000), biogeographical discrepancies, and differences in calls and behaviour (Theuerkauf *et al.* 2009) suggest that the taxon should be treated as a full species.

In our analysis of the parrots from this subregion, we found that species were more likely to be threatened if they occurred at higher latitudes (i.e. further south from the equator; LRT = 4.37, df = 1, $P_{PGLS} = 0.001$). On average, threatened species in the Pacific Islands had ranges with a mean latitude of 21°S (SD = 13), while non-threatened species had ranges with a mean latitude of 8°S (SD = 6). This is largely attributed to the inclusion of the Chatham Parakeet (*Cyanoramphus forbesi*) from the Chatham Islands (44.0°S), and the Antipodes Parakeet (*Cyanoramphus unicolor*) from the Antipodes Islands (49.7°S), both of which are classified as Vulnerable. Although these two southern archipelagos are territories of New Zealand, we treated them as Pacific Islands for this study.

We also found that parrot species in more urbanised countries within this subregion were more likely to be threatened (LRT = 8.96, df = 1, $P_{PGLS} = 0.002$). The loss of habitat associated with urbanisation is often more permanent and destructive than other types of land use (McKinney 2002), and it tends to have a disproportionate impact on small islands. Urbanisation is also linked to other forms of economic development and activity, which can pose additional risks to parrots (Chapter 1).

Invasive species were the most frequently reported threats (32%) to parrots in the Pacific Islands (Figure 3.4B). In this subregion, feral cats and Black Rats are the most detrimental introduced predators. These species are known to prey occasionally upon parrots in New

Caledonia, and they have probably influenced the distribution of parrots in some forested areas (Legault *et al.* 2013). Interestingly, rainforests on oligotrophic soils appear to provide some refuge for New Caledonian Parakeets and Horned Parakeets (*Eunymphicus cornutus*) (Legault *et al.* 2011), possibly because rat density is lower there. Small parrots, particularly those belonging to the genus *Charmosyna* or *Vini*, appear to be particularly vulnerable to Black Rats (Rinke *et al.* 1992; Ziembicki 2003). Even populations of relatively widespread species, such as the Blue-crowned Lorikeet (*Vini australis*), may not be secure. While many island nations have biosecurity measures in place to prevent the establishment of rats, and other alien pests, introductions still occur (Theuerkauf *et al.* 2010). As exemplified with the Ultramarine Lorikeet (*Vini ultramarina*), declines associated with such introductions can be swift (Ziembicki 2003). Rats can also have an indirect effect on parrots by acting as a food source for introduced predators such as cats (Atkinson 1985). Predators take advantage of the increased availability of prey, and as their populations expand, the pressure on native species escalates. For example, parakeets coexisted with cats for over 60 years on Macquarie Island, yet the release of rabbits in 1879 provided such an abundant food source that, in little more than a decade, cats had multiplied and spread across the island, and the endemic Macquarie Island Parakeet (*Cyanoramphus novaezelandiae erythrotis*) had been driven to extinction (Taylor 1979).

The clearing of land for agriculture has had major impacts in the past, and continues to affect 16% of parrot species on Pacific Islands (Figure 3.4B). For example, much of the fertile, lowland terrain has been burnt or cleared in New Caledonia, and elsewhere in the Pacific. Before European settlement, Melanesians and Polynesians used fire to establish and maintain an open landscape, and to improve the productivity of crops. In the 19th century, European colonisers subsequently removed vast tracts of native vegetation for agriculture, livestock and timber (Bouchet *et al.* 1995; Stevenson 2004; McWethy *et al.* 2010). Today, logging for timber poses a serious threat to parrots in the Pacific Islands, with 32% of species being impacted (Figure 3.4B). In New Caledonia, it is likely that the absence of parakeets in lowland areas is largely due to forest loss, rather than altitudinal preferences (Legault *et al.* 2011; Legault *et al.* 2013). Deforestation in lowland areas has undoubtedly displaced many species, and is likely to have led to the extinction of others. It is possible, for example, that the New Caledonian Lorikeet (*Charmosyna diadema*) was seasonally dependent upon the sclerophyll forests along the west coast of New Caledonia (Ekstrom *et al.* 2002), which have all but disappeared (Bouchet *et al.* 1995; Veillon *et al.* 1999).

Trapping threatens 23% of the parrot species native to the Pacific Islands (Figure 3.4B). The extent of the parrot trade on most Pacific Islands is largely unknown, and needs further investigation. However, in the Solomon Islands, falsified claims about the origin of wild-caught parrots has led to an alarming number of parrots being exported (Shepherd *et al.* 2012). Although invasive species and habitat loss remain the main threats to parrots in the Pacific Islands, disease is of growing concern, particularly with the rise of travel and tourism, which facilitate the spread of infectious agents around the world. Climate change also has the potential to place parrot species at risk by accelerating the loss and fragmentation of suitable habitat (Legault *et al.* 2013). Insular species are particularly vulnerable, not only from shifting climate envelopes, but also from climate-induced changes in sea level (Weeks *et al.* 2016).

Among the parrots of the Pacific Islands, the New Caledonian species have been most intensively studied (Theuerkauf *et al.* 2009; Legault *et al.* 2013). For French Polynesia, there are a number of publications on the conservation of parrots (Graves 1992; Kuehler *et al.* 1997; Lieberman *et al.* 1997; Ziembicki 2003; Ziembicki and Raust 2004), but few concerning their biology (Gerischer and Walther 2003). There is some information available on the parrots of Tonga (Rinke 1989; Rinke *et al.* 1992), Solomon Islands (Webb 1992; Kratter *et al.* 2009; Danielsen *et al.* 2010; Read 2013), and Fiji (Jackson and Jit 2007; Masibalavu and Mucklow 2008; Franklin and Steadman 2010; Morley and Winder 2013). However, there is limited data for the parrots on most other islands, such as Kiribati (Watling 1995), Niue (Powlesland *et al.* 2006), Vanuatu (Kratter *et al.* 2006), and Cook Islands (Steadman 1991; Wilson 1993).

3.3.4. Parrots of Wallacea and New Guinea

The islands of Wallacea are inhabited by 34 extant parrot species (Figure 3.1), of which 29% are threatened (mostly in the Moluccas and the Lesser Sundas; see Figure 3.2, Figure 3.3B). Parrots in this subregion are impacted by the same major threats affecting Oceania as a whole: logging (50%), agriculture (38%), and hunting and trapping (38%; Figure 3.4B). Lowland forests on the islands of Wallacea, which provide the most important habitat for threatened parrots, face continued threat from deforestation (Marsden and Fielding 1999). For example, the Blue-fronted Lorikeet (*Charmosyna toxopei*) persists in low numbers on a single island with rapidly shrinking habitat (BirdLife International 2016).

In other regions, such as the Neotropics, parrot trapping is mostly an opportunistic activity undertaken by the inhabitants of poor villages (Pires and Clarke 2011). However, in Indonesia, it can be a profession, and certain villages specialise in bird trapping (Jepson *et al.* 2001; Butchart *et al.* 2010). The Yellow-crested Cockatoo (*Cacatua sulphurea*) is now Critically Endangered as a result of unsustainable trapping for the pet trade (Collar and Marsden 2014; BirdLife International 2016), and only a few individuals of the subspecies *C. s. abbotti* have survived on one island belonging to the Masalembu archipelago (Metz *et al.* 2009; Nandika and Agustina 2012).

New Guinea is similarly rich in parrot diversity, with 46 native species (Figure 3.1), though only 7% are considered threatened (Figure 3.2). Most of the threatened species inhabit the satellite islands of New Guinea, with the exception of Pesquet's Parrot (*Psittrichas fulgidus*), which lives on the mainland (Figure 3.3B). Although there are surprisingly few threatened parrot species in New Guinea, many are very poorly known (Marsden and Symes 2006), and further information may lead to revisions of their Red List status.

3.3.5. Extinct and Critically Endangered parrots in Oceania

A total of five parrot species are known to have become extinct in Oceania since the 16th century (IUCN 2016). One of these extinct species was native to Australia, and the other four species occurred in the Pacific Islands (Table 3.3). Of the extant species, nine are classified as Critically Endangered, including three in Australia, two in New Zealand, two in the Pacific Islands, and two in Wallacea (Table 3.3). After pooling the Extinct and Critically Endangered parrots, we noted that all but one species (93%) were endemic to a single country. In contrast, only 60% of the species in lower risk categories were endemic to a single country. The mean historical range of Extinct and Critically Endangered species was smaller (129,000 km^2

±128,000 CI 95%, N = 14) than that of less-threatened species (564,000 km^2 ±190,000 CI 95%, N = 158). On average, Extinct and Critically Endangered species were distributed at higher latitudes (23.8°S ±6.4 CI 95%, N = 14) than less-threatened species (14°S ±2 CI 95%, N = 158).

Table 3.3. List of Extinct (EX) and Critically Endangered (CR) parrot species in Oceania, including scientific and common names, 2016 IUCN Red List category, subregion of occurrence, distribution, average latitude of range (degree), current size of their mapped range (km^2), and estimated population size (number of mature individuals).

Scientific name	Common name	Red List category	Subregion	Distribution	Latitude (°)	Range (km^2)	Population size
Psephotellus pulcherrimus	Paradise Parrot	EX	Australia	Eastern Australia	-26.5	NA	0
Cyanoramphus ulietanus	Raiatea Parakeet	EX	Pacific	Raiatea, French Polynesia	-16.8	NA	0
Cyanoramphus zealandicus	Black-fronted Parakeet	EX	Pacific	Tahiti, French Polynesia	-17.7	NA	0
Eclectus infectus	Oceanic Parrot	EX	Pacific	Tonga	-18.7	NA	0
Nestor productus	Norfolk Kaka	EX	Pacific	Norfolk Island, Australia	-29.1	NA	0
Cyclopsitta coxeni	Coxen's Fig-parrot	CR	Australia	Eastern Australia	-26.2	93,600	<250
Lathamus discolor	Swift Parrot	CR	Australia	Eastern Australia	-31.8	21,500	1,000–2,500
Neophema chrysogaster	Orange-bellied Parrot	CR	Australia	Eastern Australia	-35.5	12,800	20-25
Cyanoramphus malherbi	Orange-fronted Parakeet	CR	New Zealand	New Zealand	-41.9	118,000	<250
Strigops habroptila	Kakapo	CR	New Zealand	New Zealand	-41.7	95,100	108
Charmosyna amabilis	Red-throated Lorikeet	CR	Pacific	Fiji	-17.2	37,100	<50
Charmosyna diadema	New Caledonian Lorikeet	CR	Pacific	New Caledonia	-21.4	1	<50
Cacatua sulphurea	Yellow-crested Cockatoo	CR	Wallacea	East Timor & Indonesia	-4.6	1,360,000	1,500–7,000
Charmosyna toxopei	Blue-fronted Lorikeet	CR	Wallacea	Buru, Indonesia	-3.4	9,100	<250

Large-bodied and island-inhabiting parrot species were found to be over-represented among extinct species at a global scale (Chapter 1). Steadman (2006) reviewed the biogeography and extinction of parrots in Oceania, using archaeological evidence to record the prehistorical ranges of species, and reported at least six species that are now extinct in the tropical Pacific. Along with two *Cyanoramphus* and one *Eclectus* species, from French Polynesia and Tonga, respectively (Table 3.3), archaeological evidence suggests that one *Cacatua* species from New Caledonia and two *Vini* species from the Cook Islands also existed in the past (Steadman 2006). All four extinct *Cyanoramphus* and *Vini* species were larger than the closely related extant species, lending support to the finding that large-bodied parrot species are more prone to extinction (Chapter 1). Biodiverse island ecosystems, such as those in Oceania, can accumulate many vulnerable and endemic species over time, which ultimately raises the extinction risk in this region (Weeks *et al.* 2016).

3.3.6. Priorities for research and conservation

The status of parrots is reassessed by BirdLife International every four years (this study was based on the 2016 evaluation) using published information (e.g. Danielsen *et al.* 2010; Freeman *et al.* 2013; Freeman and Freeman 2014; Sam and Koane 2014), unpublished reports, theses (e.g. Heptonstall 2010), and information provided directly by scientists, conservation practitioners and birdwatchers. However, assessing the status of poorly known species remains challenging. For example, the Red-throated Lorikeet (*Charmosyna amabilis*) may have been extirpated on some islands, but survey data remains insufficient to confirm its status (Watling 2013).

In Table 3.3, we listed the threatened parrot species in Oceania that are particularly in need of conservation research and attention. Most of these species are now restricted to very small areas, mainly individual islands, which makes them highly vulnerable to invasive species and stochastic factors, such as extreme weather events. For certain species, 'insurance' populations in safe breeding facilities may be required as part of a wider recovery plan (Holdsworth and Starks 2006; Collar and Butchart 2014). This pertains not only to Endangered and Critically Endangered species, but also to those species that lack sufficient data to be adequately assessed. Even species that are categorised as Near Threatened may prove to be of significant conservation concern once further information is obtained on their distribution, population trends, and threats.

We identified hotspots for parrot diversity and conservation in Figure 3.3. These maps indicate that threatened parrots in the Pacific are scattered over many islands, yet New Caledonia stands out as a priority for conservation. Other important locations for parrot conservation are the South Island of New Zealand, the Australian island of Tasmania, and the Moluccas and Biak Island in Indonesia. Indonesia is undoubtedly a global priority for parrot conservation (Chapter 1), and our results reinforce the need for conservation research across its vast archipelago. Although focused research and conservation initiatives are in place in New Caledonia, New Zealand, and Australia, similar programmes are lacking in Indonesia, and may be difficult to instigate due to restricted funding and access to certain locations. Nevertheless, we hope that the information provided here will draw attention to the threats facing parrots throughout Oceania, and foster support for the species and ecosystems most in need of protection.

4. The illegal parrot trade in Indonesia

4.1. Introduction

The biological diversity of our planet is rapidly depleting due to the direct and indirect consequences of human activities. Direct global effects are habitat destruction, expanding urban and agricultural areas (Vergara-Tabares *et al.* 2020), and wildlife crime that includes the illegal killing, taking, possessing, or trading of plants and animals (Kurland *et al.* 2017). Wildlife trade and utilisation, whether legal or illegal, are also responsible for the potential emergence and spread of many zoonotic diseases (e.g. SARS, COVID-19, Avian flu) that can cause novel human diseases (Allen *et al.* 2017), which stresses the importance of regulating such activities. CITES has been regulating the international trade of threatened species since 1975 (www.cites.org), and parrots (order Psittaciformes) have become the most traded animal taxon according to their database (trade.cites.org). Parrots may thus provide the best source of data for investigating the causes and consequences of animal trade. As a result of global and domestic demand for parrots as pets, illegal trapping and removal of parrots from the wild has largely contributed to their decline (Clarke and de By 2013). A global evaluation revealed that one-third of the nearly 400 parrot species are threatened by extinction, with Psittaciformes having higher aggregate extinction risk (IUCN Red List Index) than any other comparable bird group (Chapter 1).

Southeast Asia is both a major hotspot for biodiversity and an epicentre for illegal wildlife trade world-wide (Nijman 2010). Indonesia was identified as the highest priority country for parrot conservation because it has the greatest diversity of species (89 parrot species) and the highest proportion of threatened and endemic species of any nation (Chapter 1). Before 2018, only 12 parrot species were listed as threatened in Indonesia and were, thus, regulated using catch-quotas (Republic of Indonesia 1999). However, such quotas were rarely enforced and most parrot species were illicitly removed from the wild (Setiyani and Ahmadi 2020) and trafficked to other provinces and countries (Aloysius *et al.* 2019). Although illicit removal from the wild is only the first step in the illegal wildlife trade, it is the primary activity that harms wild populations, and further understanding of its drivers is essential for understanding the broader issue of illegal trade of wildlife.

While the illegal parrot trade remains an active problem throughout Indonesia, it cannot be assumed that every species faces the same threat from illicit removal from the wild. Recent studies in the Neotropics have demonstrated that there is considerable variation among parrot species traded and sold in illicit markets (Gastañaga *et al.* 2011). Using a criminological framework to understand variation in what is sold—and not sold—in these markets, Pires and Clarke (2011; 2012) have suggested that the illegal parrot trade is mostly an opportunistic crime. That is, species that are easier to remove from the wild are traded more frequently in illicit markets. The CRAAVED model (standing for *concealable, removable, accessible, abundant, valuable, enjoyable,* and *disposable*) was originally developed by Clarke (1999) for understanding theft variation of commonly stolen products and has been used to analyse variation of parrot species involved in the live pet trade (Pires and Clarke 2011). CRAAVED

analyses suggest that "hot products", or those products that are stolen often and re-sold in illicit markets, tend to be highly attractive to thieves and consumers alike, but are often also the easiest to steal (Clarke 1999). Univariate CRAAVED analysis of Neotropical parrots showed that species that were more common in the wild (i.e. *abundant*), had larger distributions especially in closer proximity to illicit markets (i.e. *accessible*), and were easier to remove from nests (i.e. *removable*), were traded significantly more often than other species (Pires and Clarke 2012; Pires 2014). Conversely, species that were more *enjoyable* (i.e. attractive) or *valuable* (i.e. rare), otherwise known as demand-side components, were traded significantly less often. Unfortunately, these studies suffered from data limitations and could not control for competing explanations using multivariate models.

Using a multivariate statistical model on CRAAVED variables, a study found that the most *attractive* and *valuable* species were actually captured more often when controlling for relative abundance and accessibility (Tella and Hiraldo 2014). This study concluded that demand-side components were the main driving force behind illicit trade. In addition, a more recent study of parrot trade in Colombia also found wildlife crime to be driven by selective removal of *attractive* species from the wild, not opportunity (Romero-Vidal *et al.* 2020). These studies were limited, however, because they did not operationalize each component of the CRAAVED model in order to control for all competing explanations, nor did they account for phylogenetic relationships in their statistical models. Controlling for phylogenetic dependence among the studied species is important in these multivariate analyses in order to truly understand the driving factors behind trade without inflating the sample size due to multiple representation of similar species in the dataset (Chapter 1).

Divergent results in studies to date suggest that it is not yet clear whether opportunity, demand, or both drive the illegal parrot trade. It is often assumed that offenders disproportionately target highly valuable and charismatic species, which is thought to be driving the anthropogenic Allee effect that hastens the risk of extinction (Courchamp *et al.* 2006). This theory predicts that under a critical population size (Allee threshold), the elevated value of rare species can provide financial incentives for targeted poaching and eventually lead to accelerated extinction (Holden and McDonald-Madden 2017). At the same time, the finding that the illegal parrot trade is an opportunistic activity is consistent with a number of recent wildlife crime studies that can explain why particular species of flora and fauna are poached (Pires *et al.* 2016; Kurland *et al.* 2017; Kurland *et al.* 2018). For example, Maingi *et al.* (2012) found bodies of water, roads, and abundance of elephants were spatially predictive of where elephant poaching occurred in south-eastern Kenya. This line of research suggests opportunity structures both in the built (e.g. roads) and natural environment (e.g. bodies of water) facilitate the illicit removal of species from the wild.

The present study has the broad objective of furthering our understanding of the forces driving the illegal parrot trade in Indonesia by quantifying the relative importance of opportunity and demand-based factors. We use sophisticated multivariate models, controlling for phylogeny, to assess all CRAAVED factors that could potentially drive the illegal domestic and international parrot trade originating from Indonesia, a task that has not been accomplished to date.

4.2. Methods

4.2.1. Trade data

This study used a combination of seizure data and previously published market surveys of parrots from Indonesia to gather information for species at risk of being removed from the wild. Six data sources were used in total to gather a complete picture of which species are more commonly traded within Indonesia. These data were obtained from six different provinces spanning the archipelago (Figure 4.1). Species traded were shown to be consistent across data sources (ICC = 0.804, 95% confidence interval 0.732–0.861; $F(89,356) = 5.104$, $P < 0.001$) giving us confidence these data are reliable and representative (Figure 4.2).

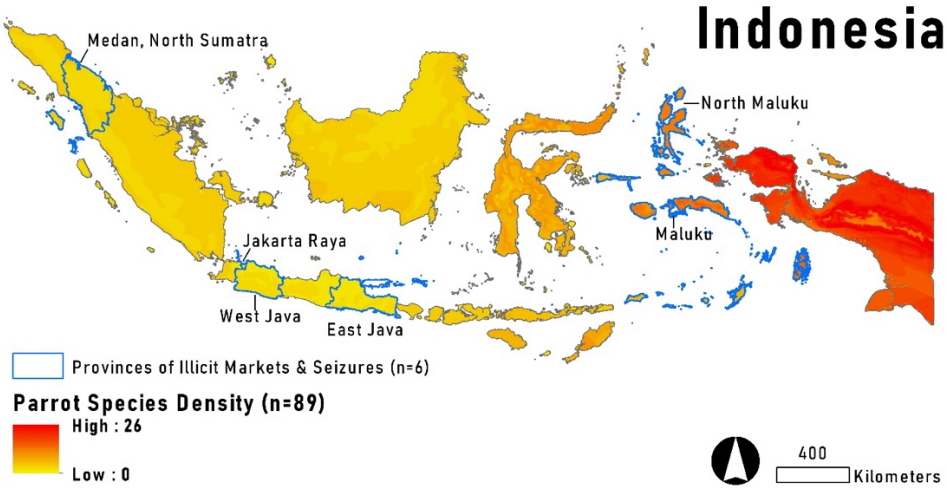

Figure 4.1. Locations of illicit markets and seizures used in this study along with the distribution of parrot species density in Indonesia.

Seizure data were obtained from the Natural Resource Conservation Center of the Ministry of Forestry (Balai Konservasi Sumber Daya Alam, BKSDA hereafter) (Setiyani and Ahmadi 2020) and the Regional Police of East Java (Reskrimsus Polda Jatim). BKSDA confiscated illicitly obtained parrots between 2016 and 2018 in the provinces of North Maluku and Maluku, and the Regional Police in East Java in 2018 (District Court of Jember, Indonesia 2019). Market survey data were obtained from four sources. The first was based on Shepherd's (2006) survey of three markets in Medan, North Sumatra, where monthly market surveys were conducted between 1997 and 2001, and again in 2005, finding a total of 27 parrot species for sale. The second was based on extended field surveys on Obi Island in North Maluku in 2012, where they found eight species for sale (Cottee-Jones *et al.* 2014). The third was a published report by TRAFFIC (Chng *et al.* 2015) that documented the number of birds per sale in three of the largest markets in Jakarta in 2014, registering 14 parrot species for sale. In the fourth study, Chng *et al.* (2016) surveyed the Sukahaji wildlife market in Bandung, West Java in 2016 and found nine parrot species for sale.

Given the wide time-scale of the source data (1997–2018), we divided the sources into two periods for the analyses: (1) a 'past' dataset with Shepherd's (2006) extensive survey between 1997 and 2005 containing 27 traded species, and (2) a 'recent' dataset with all the other sources

recorded between 2012 and 2018 containing 23 parrot species in the trade (Figure 4.2). Trade patterns may have changed over time in Indonesia, and we wanted to isolate these two time periods as well as combine them to get a fuller picture.

First, we analysed the data separately in the two time-periods and then altogether as an 'overall' dataset. We measured CRAAVED components following the methods used by recent studies of illegal parrot trade (Pires 2014), summarized in Table 4.1, and details are given in the appendix of Pires *et al.* (2021). We conducted a Pearson correlation test among the components to explore potential multi-collinearity issues. The complete dataset is available in FigShare (https://doi.org/10.6084/m9.figshare.13681393).

Table 4.1. CRAAVED components used in the study to evaluate the likelihood of Indonesian parrot species being traded in past (1997–2005), recent (2012–2018), and overall (1997–2018) time-periods.

Variable	Definition	Measure	Data source*
Concealable	Species that could not be legally trapped and are therefore harder to conceal from law enforcement. *Opportunity-based*.	Nationally protected (1) or unprotected (0) species in Indonesia.	(1)
Removable	How easy is it to remove species from the wild? *Opportunity-based*.	Easy (1) e.g. from nests near to the ground; medium (2) e.g. using glue or mist netting; difficult (3) e.g. high nests, noose techniques.	(2)
Accessible	Species found in areas where there is a greater number of people may be at an increased risk of trade. *Opportunity-based*.	Human population size within the species range in the year 2000 (past), 2015 (recent), and the average of their sum (overall time-scale).	(3; 4)
Abundance	How common species are. *Opportunity-based*.	To reduce variability between global and Indonesian estimates, average population sizes were converted to (1) 1–9,999, (2) 10,000–49,999, (3) 50,000–99,999, and (4) over 100,000 individuals.	(3; 5)
Valuable	IUCN RedList categorization as a widely used proxy for value. *Demand-based*.	(1) Least Concern, (2) Near Threatened, (3) Vulnerable, (4) Endangered, and (5) Critically Endangered.	(3)
Enjoyable	Species attractiveness to potential customers. *Demand-based*.	Composite values (4–8) from the sum scores of low (1) or high (2) of the colourfulness, percentage of body brightly coloured, body length, and ability to mimic sounds.	(5; 6)
Disposable	The ease with which species can be sold in illicit or licit markets. *Demand-based*.	Number of exported parrot specimens between 1997–2005 (past), 2006–2018 (recent), and their sum (overall time-scale).	(7)

* Data source: (1) Republic of Indonesia (1999); (2) Dudi Nandika, Dr. La Eddy; (3) IUCN (2019); (4) Schiavina *et al.* (2019); (5) Juniper and Parr (1998); (6) Tella and Hiraldo (2014); (7) CITES trade database (trade.cites.org).

4.2.2. Statistical analysis

Utilizing studies and data with different methodologies meant that the exact number of birds observed in markets or confiscated was not always comparable. For example, some market surveys were monthly, while others were conducted over a 3-day or 1-day period at wildlife markets. Hence, we treated our response variable as a binary measure to simply reflect whether the species was traded ('1') or not traded ('0'). In addition, data were assembled according to which species were traded within the past, recent, and overall time-periods. First, we fitted a binary logistic regression model using the 'GLM' function in R statistics (R Core Team 2013) to calculate *P*-values for the seven explanatory variables. Then, we compared the

Akaike information criterion (AIC) of all combinations of the seven variables without interactions and selected the best model with the lowest AIC (i.e. including features that maximize the predictive ability of the model) using the 'MuMIn' package in R (Bartoń 2023). We repeated these steps for each of the time-periods.

For phylogenetic relatedness control, we downloaded 1000 possible phylogenetic trees with the Ericson taxonomic backbone of parrot species distributed in Indonesia from birdtree.org (Jetz *et al.* 2014) to account for the branch lengths in addition to nodes separating species (available with our FigShare dataset). For each tree we ran a phylogenetic generalized least squares (PGLS) regression using the 'caper' package in R (Orme *et al.* 2018).

The explanatory and response variables were the same as those used in the logistic regression model. We ran the PGLS model with all seven variables in the three time-periods, and then with the variables represented in the best models from the previous step. For each explanatory variable, we report the modified *P*-value accounting for phylogenetic relatedness, the estimates, and for each model we also report the λ transformation that improves the fit of phylogenetic data. Greater λ-values indicate that the relationship between response and explanatory variables correlates with the phylogeny, and that the values of the explanatory variables are more similar for closely related species.

We used the random forest machine learning (RFML) algorithm (Breiman 2001) in the 'randomForest' package of R, to model the relationship between the traded parrots and CRAAVED variables. RFML is a computational machine learning approach that fits multiple decision trees to our trade dataset using a random subset of the CRAAVED input variables for each tree constructed for the parrot species. For each model, we present the percentage of variance explained in traded species, and for each CRAAVED component the mean decrease in Gini (i.e. higher decrease meaning that a particular predictor variable plays a greater role in partitioning the data into traded/not traded) and their relative importance in each time-period.

4.3. Results

Indonesia hosts a total of 89 parrot species, of which two are Critically Endangered, four are Endangered, seven are Vulnerable, 16 are Near Threatened, and 60 are of Least Concern (IUCN 2018). Based on the six data sources reviewed by this study, 31 parrot species (34% of total in Indonesia) were reported as traded species (one CR, two EN, two VU, eight NT, and 18 LC). Four species are listed in CITES Appendix I: *Cacatua goffiniana*, *C. moluccensis*, *C. sulphurea*, and *Probosciger aterrimus* (Figure 4.2).

The best linear regression models explaining the likelihood of a parrot species being traded in Indonesia in the past (1997–2005) contained the CRAAVED components *disposable*, *enjoyable*, and *accessible*, while in a later time period (2006–2018), *concealable* was also highlighted alongside these other variables (Table 4.2). Species were significantly more prone to trading if they were more *disposable* in all of the time-periods analysed (PGLS $P_{past} = 0.003$, $P_{recent} = 0.001$, and $P_{overall} = 0.009$; Figure 4.3A). More *enjoyable* species were also traded significantly more in both the past and recent periods (PGLS $P_{past} = 0.026$, $P_{recent} = 0.014$; Figure 4.3B) but this component was not significant when we pooled the two periods (PGLS $P_{overall} = 0.065$). Species that were more *concealable* showed up as significant only in the linear

regression model in the recent time-period (GLM $P_{recent} = 0.04$) but this could not be confirmed when we controlled for phylogenetic relationships (PGLS $P_{recent} = 0.927$).

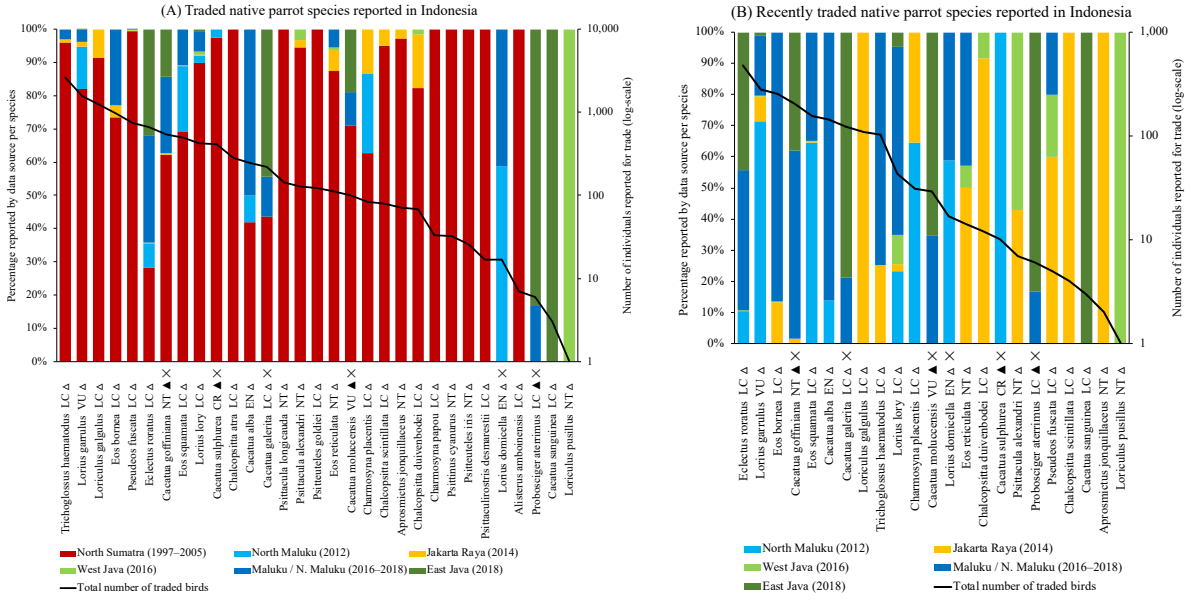

Figure 4.2. Traded native parrot species reported by six sources in Indonesia for (A) an overall period of two decades (1997–2018), and (B) a recent time-scale between 2012 and 2018. Each column represents a species and its relative appearance in market/confiscation data sources (different colors indicate the percentage of total individuals reported by species; left axis). Species are sorted by the total number of individuals reported for trade shown by the continuous line and the right axis. After the species name are their IUCN RedList (2018) status (CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Near Threatened, and LC = Least Concern), CITES listing (▲ = Appendix I and △ = Appendix II), and protection in the national level in Indonesia pre-2019 (×).

Of the total variance between traded and non-traded species data, 21% was explained by the seven CRAAVED components in the overall analysis, while 19% in the past and 23% in the recent time-periods. Of the total effects (100%) of the components, *accessible* showed the highest relative importance in all time-periods (between 23% and 24%) followed by *disposable*, *removable, enjoyable*, *abundance, valuable*, and *concealable* (Figure 4.4). When only the components from the best models were kept, 19% of the variance was explained by four variables retained in the overall analysis, while 18% in the past and 19% in the recent time-periods including three and four variables respectively. In these cases, the highest relative importance was again associated with the *accessible* component (38–41%; Table 4.2, Figure 4.4). Some CRAAVED components were correlated, the highest correlation was between *abundance* and *valuable*.

Table 4.2. Best models explaining the likelihood of Indonesian parrot species being traded in different time-periods: past (1997–2005), recent (2012–2018), and overall (1997–2018). Presented are results from the best models (selected based on AIC) evaluating the importance of each CRAAVED variable: the Wald statistic and chi-square *P*-values (P_{GLM}) from logistic regression (GLM) models; *P*-values (P_{PGLS}), estimates, and lambda (λ) values from phylogenetic generalized least squares (PGLS) regression models with corresponding standard deviation values (SD); and mean decrease in Gini and relative importance of each variable from random forest machine learning (RFML) models. *P*-values lower than 5% are presented in bold.

Time-period	Variable	Wald test	P_{GLM}	P_{PGLS} (SD)	PGLS Estimate (SD)	λ (SD)	Gini	Relative Importance
Past	Accessible	1.71	0.088	0.213 (0.045)	0.002 (0)	0.127 (0.058)	10.4	38%
	Enjoyable	2.24	**0.025**	**0.026** (0.005)	0.147 (0.002)		4.9	25.7%
	Disposable	3.67	**<0.001**	**0.002** (0)	0.009 (0)		14.9	36.3%
Recent	Concealable	2.05	**0.04**	0.927 (0.061)	0.003 (0.015)	0.360 (0.041)	2.8	9.5%
	Accessible	1.88	0.06	0.218 (0.026)	0.002 (0)		11.2	40.7%
	Enjoyable	2.24	**0.025**	**0.013** (0.002)	0.152 (0.004)		4.6	19.7%
	Disposable	3.44	**0.001**	**0.001** (0)	0.016 (0)		13	30.1%
Overall	Concealable	1.83	0.067	0.752 (0.112)	-0.04 (0.021)	0.414 (0.083)	1.3	6.5%
	Accessible	1.86	0.062	0.292 (0.037)	0.002 (0)		13.1	39.9%
	Enjoyable	1.76	0.078	0.065 (0.012)	0.133 (0.005)		4.6	17.1%
	Disposable	3.75	**<0.001**	**0.009** (0.004)	0.008 (0)		19.1	36.6%

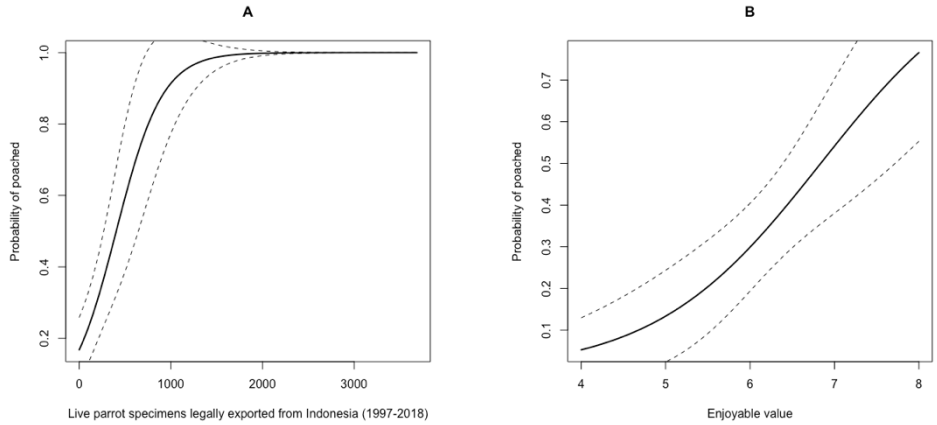

Figure 4.3. Predicted effects of (A) *disposable* and (B) *enjoyable* components of the CRAAVED model on the probabilities of Indonesian parrot species being traded in the overall analysis. Continuous lines are predicted values, and dashed lines represent 95% CI upper/lower bounds.

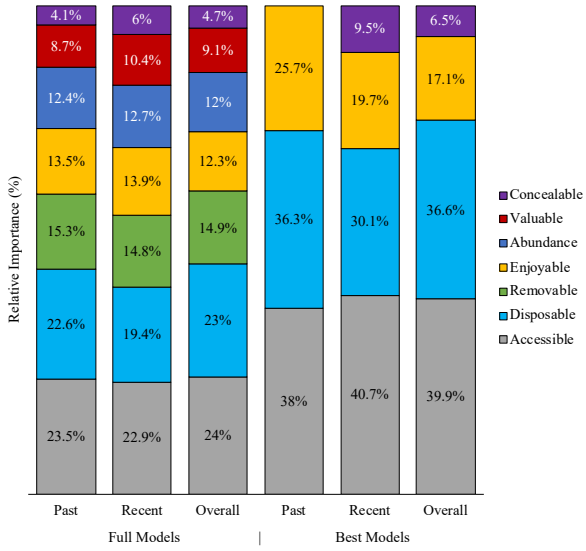

Figure 4.4. Relative importance of CRAAVED components in explaining the variance of parrot species traded in Indonesia by the random forest machine learning (RFML) models in past (1997–2005), recent (2012–2018), and overall (1997–2018) time-periods. Results from full and best models (based on AIC values) are presented.

4.4. Discussion

The objective of this study was to determine whether illegal parrot trade patterns in Indonesia can best be explained by demand, opportunity, or both factors using the criminological model CRAAVED (Clarke 1999). The drivers of the illegal wildlife trade have become a common theme in the literature and this chapter addresses this with specificity to the live pet trade. It is often assumed that offenders target the most valuable and attractive wildlife species for the live pet trade and two recent parrot poaching studies have supported this view (Tella and Hiraldo 2014; Romero-Vidal *et al.* 2020). Some coveted species for the pet trade may have large distributions, are in close proximity to urban populations and illicit markets, and may be easier to remove. Whether this finding holds true for wildlife products, is the basis of this study. Modelling all relevant factors that could influence the decision-making process of offenders is critical to understanding how the wildlife trade operates, and to inform preventive policies.

The contributions of this chapter are twofold. First, this is the only wildlife crime study that operationalizes all CRAAVED components while utilizing sophisticated multivariate models and controlling for phylogeny. Previous conservation studies have either operationalized all CRAAVED components, but were limited to univariate analyses (Pires and Clarke 2011; Pires and Clarke 2012) or ran multivariate models without operationalizing all components (Tella and Hiraldo 2014). Second, Indonesia is home to the largest number of parrot species—roughly a quarter of all Psittaciformes—and the largest number of genera of any nation (Chapter 3). As a result, findings from this context are likely to be more generalizable to other Psittaciformes found elsewhere, especially considering findings from this study have been partially corroborated previously in Mexico, Peru, and Bolivia. Altogether, accumulating knowledge based on a collection of nation-based studies is a suitable method to generalize factors that drive the illegal parrot trade more globally.

As a result of operationalizing all components of CRAAVED and using multivariate models, it is not surprising that our study draws different conclusions to those of previous studies. Our results reveal that both demand-side and opportunity-based factors can explain variation in trade in Indonesian parrots with approximately 20 percent of the variance explained

in all models. While this variance statistic may seem low, it is consistent with explanatory power results published in criminology outlets (Weisburd and Piquero 2008). More specifically, our regression models found the most *disposable* and *enjoyable* species were significantly more likely to be traded while controlling for all other potential explanations and the phylogenetic relationships of the birds (Table 4.2, Figure 4.3). In addition, using a random forest model, the results suggest the relative importance of *accessibility*—an opportunity-based factor (Table 4.1)—is greater in explaining trading variation than the other components in all models (Table 4.2, Figure 4.4), with *disposability* also highly supported by this model.

4.4.1. Demand-side factors

The finding that demand-side factors—*enjoyability* and *disposability*—were found to be significant suggests that people are targeting attractive species that are easier to sell in licit markets, partially supporting Tella and Hiraldo (2014). The most attractive species in Indonesia, according to our *enjoyable* metrics, were the Chattering Lory *Lorius garrulus*, Eclectus Parrot *Eclectus roratus*, and Salmon-crested Cockatoo *Cacatua moluccensis*. All three were traded in multiple provinces indicating their popularity in the trade over time. Conversely, only four of 20 species scoring the lowest on our *enjoyable* metric were traded. Related, the most *disposable* species (exported from Indonesia) were significantly more likely to be traded in Indonesia in all three models. This suggests that there is a cross-cultural preference for particular parrot species, especially ones that have been historically overexploited (Tella and Hiraldo 2014). Separately, our analysis of live psittacines exported from Indonesia between 1997 and 2018 showed a substantial decline since 2006 (mean = 1,640/y) as compared to pre-2006 (mean = 6,271/y), which may be due to the 2007 EU ban on imports of wild-caught birds (Reino *et al.* 2017).

At the international level, only four Indonesian parrot species are listed on CITES Appendix I and the remaining species are on Appendix II (Figure 4.2). These four species only represent 13% of all parrot species reported from trade in this study. Many CITES Appendix II parrot species regularly appear in recent confiscation datasets and often in large quantities (Figure 4.2B). For example, the Chattering Lory is one of the most traded parrots in Indonesia (Figure 4.2) and it is listed as Vulnerable by IUCN with the justification that "this species is undergoing a rapid population decline that is projected to continue as a direct result of habitat loss and human exploitation for the cagebird trade" (BirdLife International 2017). However, the species is listed under CITES Appendix II only, even though it falls into the criterion C of CITES Appendix I with "a marked decline in the population size in the wild, which has been observed as ongoing or as having occurred in the past" (www.cites.org). Indeed, the Chattering Lory was reportedly overharvested in rural areas (Tamalene *et al.* 2019), sold in the close locality, and was registered as the most kept pet species in North Maluku. An urgent re-evaluation is required of the 27 parrot species traded in Indonesia that are currently listed under Appendix II of CITES.

Incidentally, the significant overlap between the domestic and international trade of certain Indonesian parrot species also suggests the possibility that a high number of wild-caught birds in Indonesia are purposefully mislabelled as 'captive-bred' in order to be legally exported. Recently, many parrots of Indonesian origin (including Sulphur-crested Cockatoo *Cacatua galerita*, Palm Cockatoo *Probosciger aterrimus*, Eclectus Parrot, Pesquet's Parrot *Psittrichas*

fulgidus, Red-and-blue Lory *Eos histrio*, Rainbow Lorikeet *Trichoglossus haematodus*, Black-capped Lory *Lorius lory*) were confiscated in the Philippines and repatriated in Indonesia, including the Maluku and Papua regions (D.N. and D.A. unpublished data, 2020). Two studies have also shown a substantial number of wild-caught reptiles from Indonesia are 'laundered' via breeding facilities for the legal international trade (Nijman and Shepherd 2009; Lyons and Natusch 2011). In total, 420 parrots confiscated by the Regional Police of East Java—data first used in this study—were for the purpose of wild bird laundering for international trade. In this case, the owner of these parrots was unable to prove that the traded birds were captive bred, nor could they show pedigree data for the breeding facility either (District Court of Jember, Indonesia 2019). Auditing of captive bred parrot facilities in Indonesia should follow to prevent laundering of wild-caught birds.

4.4.2. Opportunity-based factors

With respect to the opportunity-based components of the CRAAVED model (Table 4.1), only *accessibility* appears likely to be an important predictor based on the consistent results of the random forest models (Figure 4.4). Prior studies in Mexico, Bolivia (Pires and Clarke 2011; Pires and Clarke 2012), and Peru (Pires 2014) found that *accessibility* was one of the leading factors explaining trade variation. Species that are disproportionately targeted are often the ones closer to more humans and open-air illicit markets. This consistent finding across nations and regions suggests that illicit removal of parrots from the wild is partly an opportunistic crime. This idea that crime is an opportunistic activity is long-standing in the discipline of criminology. Both routine activity and rational choice theories posit that crime is a function of suitable opportunity structures (Cohen and Felson 1979). That is, crimes that are easier and less risky to commit will be committed more often, especially in certain places and times. As it relates to the parrot trade, these birds nest in predictable areas and often in specific trees, generally during the same time of year, every year. Predictable behaviour and proximity to offenders make foraging for parrots and their nests relatively easy (Pires and Clarke 2011).

Nevertheless, many opportunistic factors that were previously significantly related to trade variation were found to be unrelated in the present study. *Abundance*, or the number of estimated wild parrots, was not significantly related to the probability of being traded in Indonesia. This result could be due to the nature of our binary outcome measure or because abundance was correlated with other components. Our study also showed no significant effect of *removability* from the wild, which is supported by three previous studies (Pires and Clarke 2011; Pires 2014; Tella and Hiraldo 2014). This may be because there was little variability in how parrots nested in Indonesia as in many cases they are captured on roosting trees using glue (Jepson *et al.* 2001).

The extent that species were *concealable* (i.e. non-protected) was also found to be unrelated to the probability of being traded and consistently showed up as the least informative component in the random forest models (Figure 4.4). This indicates that Indonesian market sellers are not fearful of law enforcement encounters and their outcomes if protected species are no less likely to appear in wildlife markets compared to non-protected species. Finally, *value* was not statistically related to being traded. This may be a result of using a proxy for monetary value (IUCN RedList status) because a limitation in the data was the absence of market prices for 83 of the 89 Indonesian parrot species.

The finding that both demand and opportunity-based factors can explain variation in parrot trade is supported by studies of other taxa. With respect to illegal, unreported, and unregulated (IUU) fishing, CRAAVED research has shown both demand and opportunity-based factors influence target choices. Petrossian and Clarke's (2014) analysis of fish caught illegally for commercial purposes fish found that all CRAAVED components were significantly predictive of illegal fishing. In addition, Petrossian *et al.* (2015) found illegally caught crabs and lobsters were significantly associated with *abundant*, *valuable*, and *enjoyable* measures. Altogether, these findings indicate that the decision-making process of wildlife offenders is influenced by the ease of capturing targeted species as well as their perceived value and desirability.

4.4.3. Conservation and policy implications

During the period of this study, 19% of the parrot species appearing in our trade database were protected by national law, hence their trade was considered to be illegal. Although many parrot species were not protected during this period (1997–2018), our results hold important implications for their conservation especially now, that new national legislation protects all 89 native parrot species (Republic of Indonesia 2018). In addition to protecting a greater number of species, two main strategies should be implemented to reduce the illegal trade of parrots in Indonesia.

First, interventions should focus on the species that are commonly targeted for the trade, including non-threatened species that score high on *enjoyability*, *disposability*, and *accessibility*. While such species are typically not prioritized for protection, they are at potentially greater risk of becoming threatened given their existing numbers in the trade and propensity to be targeted. For example, the Eclectus Parrot is of Least Concern (IUCN 2019), but scored highest on *enjoyability*, is frequently exported to other countries, has a range that overlaps a population of over 7.1 million people, and was found traded in 5 of the 6 provinces in our dataset. Strategies to reduce the trade of such species could take the form of nest protection, educational awareness campaigns targeting children and consumers, and using the power of media (e.g. films) in science communication (Fernández-Bellon and Kane 2020). Such strategies should be evidence-based and micro-target known hot spots and potentially even 'hot times' when nest poaching is likely to occur for particular species (Pires *et al.* 2016).

Second, place-specific illicit markets have been found to facilitate theft, and thus increase crime, by allowing illicitly obtained products to be quickly and easily exchanged for cash (Eck 1995). Eliminating open-air illicit wildlife markets can theoretically reduce illicit removal of wildlife as offenders would be unable to dispose of parrots as easily. Some displacement to online or non-public markets could be expected if open-air markets were shut down, but a net reduction in crime should follow (Pires and Clarke 2011).

4.4.4. Conclusion

The wider implications of this study suggest the criminological model CRAAVED, is suitable for analysis of other taxa commonly found in the illicit pet trade. More specifically, a CRAAVED analysis could be applied to lizards, snakes, songbirds, cacti, orchids, primates, tortoises, and turtles, which all exhibit large variation in illicit removal and trafficking among species (Kurland and Pires 2017). In doing so, the factors that are driving the illicit trade in pets and plants along with the *modus operandi* of offenders could be better understood across

taxonomic groups. For such analyses to be conducted, more data on poaching and trafficking at the local, regional, and national levels needs to be published (e.g. by relevant NGOs), and informed by a variety of data collection methods (i.e. market surveys, offender interviews, seizure data, field observations). Our study provides a robust framework to analyse such wildlife trade data using various methods and timeframes.

5. The impact of wildlife trade on parrot populations on a biodiverse island

5.1. Introduction

Parrots (order Psittaciformes) are the most threatened taxon of birds, with one-third of the nearly 400 species classified as threatened under IUCN criteria (IUCN 2021). In a global analysis, Indonesia was identified as the highest priority country for parrot conservation based on the number of species, endemics, and threats (Chapter 1). Further, among CITES-listed species, parrots are by far the most traded and they are declining faster than any other comparable groups of birds (Chapter 1). In Indonesia, all parrot species (except *Psittinus abbotti*) have been protected by law since 2018 (Republic of Indonesia 2018), but this legislation is poorly enforced (Pires *et al.* 2021). A higher proportion (almost half) of the parrot species in the Wallacean region of Indonesia are affected by trapping than in neighbouring regions or compared to the world average (Chapter 3).

Seram Island is in central Wallacea, between New Guinea and Sulawesi. It hosts 11 parrot species of which three are endemic to the Maluku archipelago: the Salmon-crested Cockatoo *Cacatua moluccensis*, Purple-naped Lory *Lorius domicella*, and the Blue-eared Lory *Eos semilarvata* (Table 5.1). The island is also home to five subspecies of Wallacean endemic parrots: the Moluccan King-parrot *Alisterus amboinensis amboinensis*, Eclectus Parrot *Eclectus roratus roratus*, Great-billed Parrot *Tanygnathus megalorynchos affinis*, Red-cheeked Parrot *Geoffroyus geoffroyi rhodops*, and the Chattering Lory *Eos bornea rothschildi* (Table 5.1). Finally, there are also three non-endemic parrot species: the Red-breasted Pygmy-parrot *Micropsitta bruijnii*, Red-flanked Lorikeet *Charmosyna placentis*, and the Coconut Lorikeet *Trichoglossus haematodus* (Table 5.1).

Parrot trade in Indonesia is driven by both demand and opportunity-based factors, and serves both national and international markets (Pires *et al.* 2021). In the illegal wildlife market, there is a high demand for at least two parrot species endemic to the Moluccas, *C. moluccensis* and the *L. domicella*. Local authorities have processed cases of illegal parrot trade originating from the outskirts of Manusela National Park in Seram (Setiyani and Ahmadi 2020). Although legislation regulating the wildlife trade in Indonesia is in place, law enforcement, monitoring, and awareness are often lacking. Knowledge of trading routes and source populations is sorely needed to help governments shut down the illegal wildlife trade and to aid the rewilding of the increasing number of confiscated parrots.

The high demand for parrots in the illegal trade threatens parrot populations in the wild, so it is crucial to understand the current population status of parrots in their natural habitat and how the trade affects them directly. We conducted a two-month population census of parrots in 2020 at three locations throughout Seram. The aims of this study are to (1) record current demographic measures of wild parrot populations, including density, species richness, diversity, and evenness, and (2) investigate the effects of poaching on the wild populations. Our results will serve as an important reference for the local authorities in making decisions and management programs regarding law enforcement in the local parrot trade and future rewilding work.

Table 5.1. Endemism and conservation status of the parrot fauna in Seram, Indonesia. Presented are endemism, scientific and English names, national protection, CITES status (Appendix I or II), and IUCN Red List conservation status (LC = Least Concern, NT = Near Threatened, VU = Vulnerable, EN = Endangered). * The current study includes recommendations to change these statuses (see details in text).

Endemism	Scientific name	English name	National protection	CITES	IUCN RedList
Species endemic to Seram	*Cacatua moluccensis*	Salmon-crested Cockatoo	protected	I	VU*
	Lorius domicella	Purple-naped Lory	protected	II*	EN*
	Eos semilarvata	Blue-eared Lory	protected	II*	NT*
Subspecies endemic to the Maluku archipelago	*Alisterus amboinensis amboinensis*	Moluccan King-parrot	protected	II	LC
	Eclectus roratus roratus	Eclectus Parrot	protected	II	LC
	Tanygnathus megalorynchos affinis	Great-billed Parrot	protected	II	LC
	Geoffroyus geoffroyi rhodops	Red-cheeked Parrot	protected	II	LC
	Eos bornea rothschildi	Chattering Lory	protected	II	LC
Non endemic	*Micropsitta bruijnii*	Red-breasted Pygmy-parrot	protected	II	LC
	Charmosyna placentis	Red-flanked Lorikeet	protected	II	LC
	Trichoglossus haematodus	Coconut Lorikeet	protected	II	LC

5.2. Methods

5.2.1. Study area

The study was conducted in the Manusela National Park, on Seram Island, Central Maluku District, Maluku Province, Indonesia (129◦ 9’ 3” – 129◦ 46’ 14" E and 2◦ 48’ 24" – 3◦ 18’ 24" S). Manusela NP was established in 2014, covering an area of 174,545.59 ha in the central region of the island (Figure 5.1; map was constructed with QGIS 3.20). The topography of Manusela NP consists of lowland (0–500 m), highland (500–1,500 m), mountain (1,500–2,500 m), and sub-alpine (up to 3,000 m) habitats (Taman Nasional Manusela 2014). The extraordinary biodiversity of the Manusela NP includes endemic and unique flora and fauna, including a high diversity of orchids, pteridophytes, ferns, marsupials, bats, reptiles, and insects (FAO 1981; Edgar and Lilley 1993; Isherwood 1998; Taman Nasional Manusela 2014; GPS Wisata Indonesia 2017). Bowler & Taylor (1993) recorded 197 species of birds in the national park, including 124 resident species and 73 migrant bird species.

The census was conducted from late March to early May 2020 at three locations including seven observation tracks in Masihulan Camp (1) covering 1.25 km^2; seven observation tracks in Sasarata Camp (2) of 1.25 km^2; and the buffer zone of the Manusela NP (3) including three observation tracks in Negeri Masihulan of 0.54 km^2 and one observation track in Negeri Pasahari of 0.18 km^2 (Figure 5.1). The survey locations also represented different habitat types, including mountain forests (in Masihulan- and Illie Camp tracks) (Bowler and Taylor 1989), habitats over 1,000 m dominated by mosses and ferns, lowland habitats (alluvial land, mangrove, and savanna in Sasarata Camp and Pasahari), secondary forest, seashore, and

cultivated land (in the buffer zone with plants including clove *Syzygium aromaticum* nutmeg plant *Myristica fragrans*).

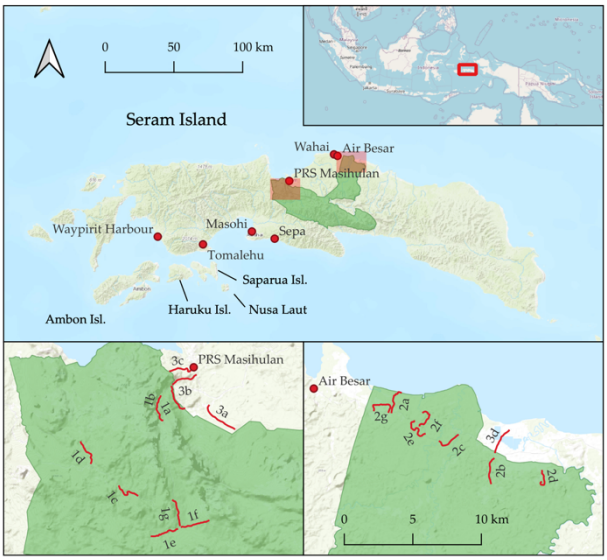

Figure 5.1. Map of the study area in Seram Island, Indonesia with the Manusela National Park (green). Census transects represent (1) Masihulan Camp sites: (1a) Masihulan camp track, (1b) Pos pantau, (1c) Km 20, (1d) Hatusaka track, (1e) Illie Camp track 2, (1f) -track 3, (1g) -track 1; (2) Sasarata Camp sites: (2a) Mangrove track, (2b) Wae Masin, (2c) Wae Mual, (2d) Pasahari fire track, (2e) Wae Patan, (2f) Wae Faung, (2g) Wae Masinatu; and (3) Manusela NP buffer zone (BZ) sites: (3a) Negeri Masihulan (NM) Galian C, (3b) NM shelter track, (3c) NM cultivation, (3d) Negeri Pasahari Wae Masin.

5.2.2. Survey methods

The surveys were conducted using a combination of fixed-radius point counts (FRPC) (Hutto *et al.* 1986) and fixed-width line transects (FWLT) (Conner and Dickson 1980). We counted multiple species of parrots as targets, using two skilled teams of two parrot specialists to identify location and species, and to minimise observer error rate. The teams included experts with 30 years of experience with visual and auditory identification of parrot species, and with excellent local knowledge of nesting and roosting locations. We encountered the birds along the transects with 2 km distance between tracks and 50 m of visibility at each point. We conducted the census on seven transects in Masihulan Camp and Illie Camp, on seven transects in Sasarata Camp and Pasahari, on three transects in the buffer zone of the Manusela NP at Negeri Masihulan, and one transect in Negeri Pasahari (Figure 5.1). Between each transect there was at least 2 km distance. In total, we conducted 180 FRPC transects and 180 FWLT transects.

Parrot censuses were carried out in the early mornings during a consistent time period from 07:30 h to 12:00 h, but in some locations, we spent more time (until 15:00 h) because of the difficult path and remoteness. Data were collected by two teams to estimate population size by direct count with the birds. We detected and identified the birds by means of their calls, because parrots are noisy birds and have a specifically distinct loud call (Nandika *et al.* 2013). Moreover, no other birds have similar calls to parrots in the location. The best time for observation was 30 minutes after sunrise and towards sunset as these times are the peak of activity in birds (Bibby *et al.* 2000). In cockatoos, activity begins as soon as sun rises, and they immediately start looking for food (Nandika *et al.* 2013). Competition between species of birds that have the same types of food will lead to separation of resource use and ecological niches. The FRPC data were collected for 15 minutes on each occasion, identifying all parrot species visually and by their calls. During the FWLT method we walked slowly and steadily, while detecting parrot species.

The data on confiscated parrots were collected by the Maluku office of the Natural Resource Conservation Center of the Ministry of Forestry (Balai Konservasi Sumber Daya Alam, BKSDA Maluku hereafter) from 2016 to 2018 (Setiyani and Ahmadi 2020). We also included data on birds confiscated by BKSDA Maluku in the Manusela NP and sent to the Pusat Rehabilitasi Satwa Kembali Bebas Avian Rehabilitation and Reintroduction Center Masihulan (PRS Masihulan hereafter) between 2019 and 2020. Currently, these data represent the best available measure of the recent poaching pressure on parrots in Seram Island.

5.2.3. Data analyses

Density of birds (*D*) was calculated based on the total birds observed for each species and divided by the total plot area (*A*). If the total number of birds observed is *S*, and the total number of individuals observed in each transect is *N*, then the index of species richness follows the Menhinick index (Menhinick 1964):

$$R_2 = S/\sqrt{N} \quad (1)$$

If total individuals observed of a species is *n* and the species proportion is *pi*, then the species diversity follows the Shannon-Wiener index (Pielou 1966a; Spellerberg and Fedor 2003):

$$H' = -\sum \frac{n}{N} ln \frac{n}{n} = -\sum pi \, ln \, pi \quad (2)$$

The Pielou index was used to calculate species evenness (Pielou 1966b):

$$E = H'/lnS \quad (3)$$

Species frequency (*F*) was calculated based on the total number of each species divided by the total number of birds observed in a sample plot (Engen 1974).

In order to evaluate if parrot species are poached proportionally to their abundance in the wild, we calculated the Savage selectivity index (*W*) (Manly *et al.* 2007), following a previously established protocol for the parrot trade (Romero-Vidal *et al.* 2020):

$$W = u_i/p_i \quad (4)$$

where u_i is the proportion of a given species recorded on the trade (by BKSDA Maluku and PRS Masihulan) and p_i is the proportion of the same species observed in the wild (by the current study). We tested the null hypothesis that parrot species are poached in proportion to their availability in the wild by comparing the statistics of *W* to the corresponding critical value of the chi-squared distribution after Bonferroni correction, with one degree of freedom (Romero-Vidal *et al.* 2020). We excluded *E. semilarvata* from this analysis, given that we have not observed any individuals in the wild.

5.3. Results

5.3.1. Wild parrot populations on Seram

During the surveys we recorded 530 individual parrots from 10 species. Of these, 97 individuals were observed at Masihulan Camp, 294 at Sasarata Camp, and 139 in the buffer zone of the Manusela NP (105 in Masihulan and 34 in Sasarata buffer zones; Figure 5.1). The dominant parrot species observed were *E. bornea, T. haematodus,* and *G. geoffroyi.* The

encounter rates for each species at each transect were: *E. bornea* 25.7%, 29% and 24.5%; *T. haematodus* 23%, 26.4%, and 18.7%; and *G. geoffroyi* 14.4%, 29.6%, and 17.1% respectively.

During our study, we detected *E. bornea* with the highest density (*D*) of 51.8 individuals/km^2 (ranged between 19.2–104.8 individuals/km^2; Table 5.2). We also recorded high densities for *G. geoffroyi* (39.9 individuals/km^2), *T. haematodus* (37.1 individuals/km^2), and *C. moluccensis* (10.8 individuals/km^2). The lowest density was for *L. domicella* (2.3 individuals/km^2).

Table 5.2. Parrot densities (*D* = individuals/km^2) and species evenness (*E*) at different transects conducted in the Manusela National Park, Seram, Indonesia, including Masihulan Camp (1), Sasarata Camp (2), and Manusela NP buffer zones (3). For exact locations, see Figure 5.1.

Scientific name	N	*D*			*E*		
		1	2	3	1	2	3
Alisterus amboinensis amboinensis	18	8	0.8	9.72	0.49	0.04	0.18
Cacatua moluccensis	26	10.4	1.6	15.28	0.74	0.06	0.31
Charmosyna placentis	6	-	-	8.33	-	-	0.13
Eclectus roratus roratus	41	4.8	16.8	19.44	0.36	0.52	0.33
Eos bornea rothschildi	184	19.2	104.8	40.28	1.08	1.14	0.57
Eos semilarvata	0	-	-	-	-	-	-
Geoffroyus geoffroyi rhodops	96	9.6	40.8	45.83	0.6	1.06	0.57
Lorius domicella	3	0.8	-	2.78	0.11	-	0.05
Micropsitta bruijnii bruijnii	6	-	-	8.33	-	-	0.1
Tanygnathus megalorynchos affinis	16	0.8	7.2	8.33	0.06	0.3	0.18
Trichoglossus haematodus	134	24	63.2	34.72	0.8	1.2	0.52

The transects with the highest richness (R_2) in the Masihulan Camp area were Km 20 (R_2 = 1.6) and Illie Camp (R_2 = 1.5; Figure 5.2). The highest parrot richness in the Sasarata Camp was recorded in the mangrove track (R_2 = 0.9) and Wae Mual (R_2 = 0.9; Figure 5.2). Finally, in the buffer zone of the Manusela NP the highest parrot richness was in shelter track (R_2 = 1.2) and cultivation track (R_2 = 1.1; Figure 5.2). Our data showed that the highest parrot species diversity (*H'*) is in secondary forest, ecotone areas, or in a transition area between primary forest and cultivation. The lowest parrot diversity was detected in the Masihulan Camp on Hatusaka track (*H'* = 0.9) and Illie Camp (*H'* = 0.7), and in the Sasarata Camp is in Negeri Pasahari (*H'* = 0.9; Figure 5.2).

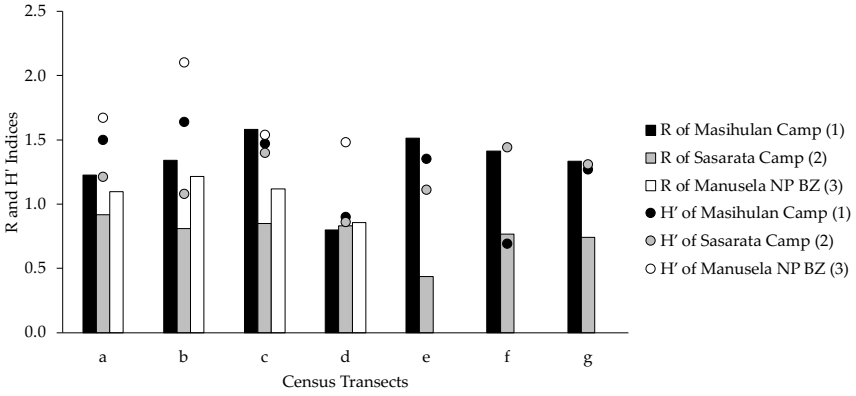

Figure 5.2. Comparison of species richness (*R*) and species diversity (*H'*) across transects. The location of each census transect is shown in Figure 5.1.

The parrot species evenness (*E*) showed relative homogeneity in the buffer zone of the Manusela NP, because no particular species dominated this area. However, in both the Masihulan and Sasarata Camp parrot species evenness revealed higher heterogeneity. The dominating parrot species in the Masihulan Camp were *E. bornea* (*E* = 1.08), *T. haematodus* (*E* = 0.8), *C. moluccensis* (*E* = 0.7), and *G. geoffroyi* (*E* = 0.6; Table 5.2). The dominated species in the Sasarata Camp were *T. haematodus* (*E* = 1.2), *E. bornea* (*E* = 1.1), and *G. geoffroyi* (*E* = 1.1; Table 5.2), while the evenness index was very low for *C. moluccensis* (*E* = 0.1). We found similar results for both *E. bornea* and *T. haematodus* that are captured in high numbers for the wildlife trade (Table 5.2).

5.3.2. Parrot trade and poaching pressure

In the past five years, BKSDA Maluku confiscated a total of 891 parrots, including 378 individuals of *E. bornea*, 216 *E. roratus*, 174 *T. haematodus*, 47 *C. moluccensis*, 45 *C. placentis*, 13 *L. domicella*, 12 *T. m. affinis*, and six *A. amboinensis*. Based on the confiscation data, the intensity of parrot poaching shows a decreasing tendency since the new government regulation (Republic of Indonesia 2018) that protects all Indonesian parrot species by law.

The selectivity index (*W*) showed significant positive poaching selection for the following four species: *C. placentis*, *E. roratus*, *L. domicella*, and *E. bornea* ($P < 0.005$; Figure 5.3). In the case of five other species (*T. haematodus*, *T. m. affinis*, *A. amboinensis*, *M. buijnii*, and *G. geoffroyi*) we observed significant negative selection for poaching ($P < 0.005$; Figure 5.3). For *C. moluccensis*, we could not reject the null hypothesis that they are poached in proportion to their availability in the wild (Figure 5.3).

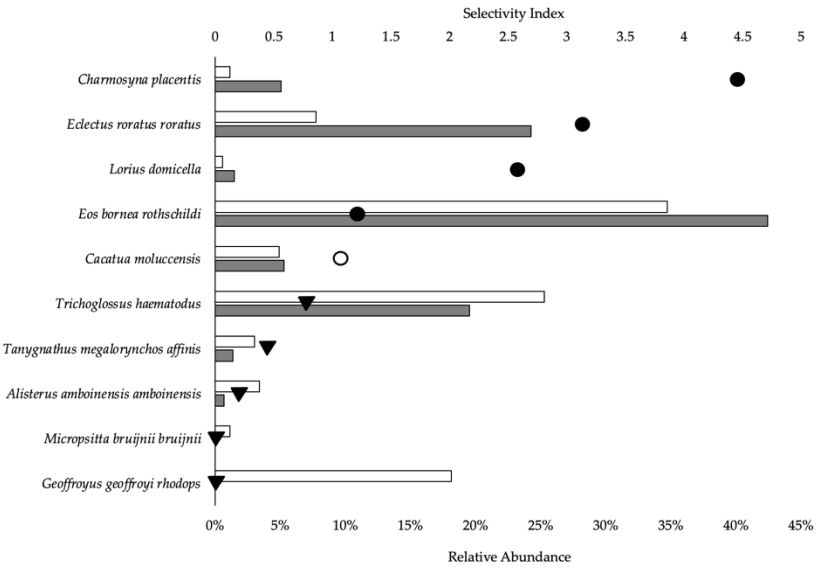

Figure 5.3. Relative abundance of parrot species in Seram, Indonesia as confiscated from the trade (dark gray bars) and observed in the wild (white bars), and selectivity index (W; black dots: significant positive selection; black triangles: significant negative selection; white dot: non-significant selection).

5.4. Discussion

5.4.1. Wild parrot populations on Seram

Seram is a highly biodiverse Indonesian island that hosts many endemic species including a high diversity of parrots. The Manusela NP is supposed to safeguard this high parrot diversity, including three species only found on this island (Table 5.1). However, poaching pressure for

the illegal trade is a significant threat to their existence both inside and outside of the national park (Setiyani and Ahmadi 2020), where law enforcement is scarce. Illegal logging and fires also occur locally but this forest damage covers only 10% of the area of Manusela NP (Bowler and Taylor 1989).

The data presented in this study are from the most detailed parrot census conducted on Seram Island in recent years. *E. bornea, T. haematodus*, and *G. geoffroyi* were found in highest frequency and not limited to high elevation. These species are locally nomadic (Coates and Bishop 2000) and their populations can extend from very small to large areas, depending on the habitat carrying capacity (Alikodra 2002). We found *M. b. bruijnii* and *C. placentis* in the lowest frequencies and showed that both species have specific habitat and food requirements. Low evenness values in species may have been related to several limiting factors such as altitude, like in the case in *E. semilarvata* and *L. domicella*, which are difficult to find ≥700 m. Both *M. bruijnii* and *C. placentis* were closely related to the presence of certain species of trees. Species evenness was significantly associated with bird density, indicating a positive relationship with disturbed habitats as well as vertical heterogeneity with high bird density (Chettri *et al.* 2005).

Open areas of lowland forest types, mangrove forests, savannas, and areas bordering cultivation had low species richness, though the figure may be influenced by the fruiting and flowering seasons. The majority of food sources were located inside forests (Mioduszewska *et al.* 2019) but parrots used cultivated and mangrove areas for foraging as well. Groups of parrots had an increasing species richness in forest types with open canopies and vertically heterogeneous vegetation structures. Frugivorous, granivorous, and nectarivorous species were closely related to disturbed areas (Chettri *et al.* 2005). The presence of frugivorous species is important because of their function to help plants with pollination and spread of seeds (Partasasmita *et al.* 2009; Tella *et al.* 2019). The dominance of cockatoos in Masihulan Camp area is closely related to the rewilding process of confiscated birds that are concentrated in the region. The existence of PRS Masihulan (Figure 5.1) possibly plays an important role in the increasing population of cockatoos in the region, and rewilded birds can be identified as they are banded.

5.4.2. Parrot trade on Seram

Poaching is one of the biggest threats faced by parrots as many people buy them as pets, generating demand for illegal trapping and smuggling (Pires *et al.* 2021). In Indonesia, BKSDA Maluku reported birds as the highest ranked smuggled taxon for trade accounting for 86% of species in Maluku, of which 96% were parrots (Setiyani and Ahmadi 2020). Between 2016 and 2018, a total of 1,135 individuals of 16 parrot species were confiscated, and about 44% came from Seram (Setiyani and Ahmadi 2020). During a bird market survey in Jakarta, three out of 13 parrot species (32%) were registered as originating from Seram, including *T. haematodus*, *C. placentis*, and *E. bornea* (Chng *et al.* 2015).

Applying the selectivity index in this study allowed us to evaluate the differences in poaching pressure suffered by the parrot species in Seram. The highest pressure was found to be on *C. placentis*, *E. roratus*, *L. domicella*, and *E. bornea* (Figure 5.3), meaning that these species were found in the trade more frequently than expected given their abundance in the wild. On the other hand, the non-significant selective pressure on *C. moluccensis* does not

indicate that poaching is not a risk for this species, but that their frequency on the trade corresponds to their abundancy in the wild. Moreover, the rewilding of confiscated individuals possibly skewed this index, as without these activities, there would have been fewer individuals observed in the wild, hence the index value would have been higher, i.e., the species positively selected. *C. moluccensis* is also hunted for traditional tribal ceremonies by the Huaulu, Naulu, and other tribes. Although birds account for only 6% of the wildlife consumed by the villagers in terms of the amount of protein (Sasaoka *et al.* 2011), wild birds are especially important for them during certain periods.

Based on non-structural observations of communities in the area including Wahai, Air Besar, Sepa, and Masohi (Figure 5.1), people keep parrots as pets, such as *E. bornea*, lorikeets, and even cockatoos (D.N. unpublished data). Almost 90% of the households in Sepa keep parrots as pets (3–4 individuals per family) and may sell the birds (Dr La Eddy, Pattimura University, unpublished data). *C. placentis* has also been observed for sale (20–30 individuals in small boxes) at Masohi (Figure 5.1). In the Manusela village, six species of parrots were caught in the forest strictly for the purpose of wildlife trade (Sasaoka 2003). However, not every parrot species is at equal risk of being traded, and there is controversy concerning the role of demand and the opportunity-based factors driving the illicit wildlife trade (Pires *et al.* 2021). The major source of income for people living in central Seram is seasonal migrant work (mainly harvesting cloves) but this income is unstable because of the fluctuation in production and the price. Hence, their dependency on wild parrots is enhanced during times of hardship caused by the decrease of their main income (Sasaoka *et al.* 2011).

In order to involve local people in parrot conservation and rehabilitation, PRS Masihulan (Figure 5.1) was established in 2004, in the buffer zone of the Manusela NP. It has been employing former parrot trappers as caretakers who are also involved directly in the monitoring of the re-wilded birds (Garai and Olah 2019). These activities have greatly affected the decrease of direct poaching by local villagers (Metz and Zimmermann 2011). An undercover investigation determined that trapping of cockatoos had essentially stopped in the close vicinity of PRS Masihulan (Metz and Nursahid 2004). For instance in 1998, the density of *C. moluccensis* was 7.9 individuals/km^2 on Seram (Kinnaird *et al.* 2003). Their density is now 20.4 individuals/km^2 near PRS Masihulan while it remained low in other parts of Manusela NP (Table 5.2). This figure is probably due to their reproductive success and the decreased impact of direct poaching around PRS Masihulan. Sadly, the poaching of *C. moluccensis* still occurs in other parts of the island, including Manusela NP. For example, in May 2020, BKSDA Maluku confiscated 10 individuals from Tomalehu (Figure 5.1), and the team of the Konservasi Kakatua Indonesia tagged two birds (*C. moluccensis* and *L. domicella*) seized by BKSDA Maluku at Waypirit Harbour (Figure 5.1). The *L. domicella* individual had been banned before and this is the second time this bird has been trapped for the illegal parrot trade. Banding is important for recording the origin and the history of the birds and also for rewilding purposes. Other methods could include genetic tagging (Olah *et al.* 2016b) that can aid to assign the provenance of the confiscated parrots (Olah *et al.* 2021a).

5.4.3. Conservation status of endemic parrot species

The result of our current analysis of direct observations combined with recent parrot confiscation data (Setiyani and Ahmadi 2020; Pires *et al.* 2021) have important implications to

the conservation status of three parrot species endemic to Seram. It is important to note that confiscations only represent a small fracture of the poached individuals, so actual figures for these species could be much higher and more worrying for their conservation status.

Cacatua moluccensis is currently considered Vulnerable (VU) on the IUCN Red List (IUCN 2021) and our analysis supports their uplisting to Endangered (EN). Their population size has been decreasing at least since 1994, based on previous IUCN evaluations (BirdLife International 2021a). Although its distribution previously covered some satellite islands of Seram, based on BirdLife data it has been declared extinct from Haruku, Saparua, and Nusa Laut islands (Figure 5.1). In Ambon, it is very difficult to find these cockatoos (D.N. pers. obs.) and they may only remain in the western part of the island. Based on abundance data in the past (7.9 individuals/km^2), their population size decreased by over 50% in some parts of the Manusela NP (currently 1.6 individuals/km^2 in Sasarata Camp), probably due to exploitation. Our selectivity analysis showed that poaching levels are consistent with their abundance in the wild (Figure 5.3). BKSDA Maluku and PRS Masihulan reported 47 confiscated individuals in the past five years in Maluku alone. Data from our direct field survey showed that the frequency of encounters was relatively low in Masihulan NP and its buffer zone (15.3% and 9.7% of all parrot individuals respectively) and that it was difficult to find them in the Sasarata area (relative frequency of 1.3%). These results support the IUCN criteria A2bd+3bd+4bd of EN (IUCN Standards and Petitions Committee 2019).

Lorius domicella is currently considered Endangered (EN) on the IUCN Red List (IUCN 2021) and our analysis supports its uplisting to Critically Endangered (CR). They have a very limited distribution only in Seram in habitats above 800 m, and their population has been decreasing at least since 1994 according to IUCN evaluation (BirdLife International 2021c). Our survey showed very low relative frequency of all parrot individuals in the Masihulan NP (1.3%) and its buffer zone (1.6%). The number of mature individuals might be below 250 with less than 50 in each subpopulation. These results support the IUCN criteria C2a(i) of CR (IUCN Standards and Petitions Committee 2019). In addition, the threat of poaching is of concern as there were 13 recorded individuals confiscated in the past five years in Maluku and our selectivity analysis showed a significant positive poaching pressure on the remining population (Figure 5.3). Hence, we recommend the urgent inclusion of the species to CITES Appendix I.

Eos semilarvata is currently listed as Near Threatened (NT) on the IUCN Red List (IUCN 2021) and our analysis supports its uplisting to Vulnerable (VU). It has a limited distribution at altitudes above 800 m in Seram, with a decreasing population size since at least 2019 based on IUCN evaluation (BirdLife International 2021b). We did not register this species during our census activity on the transects, but recorded one foraging flock of 10 individuals while in the field. Their area of occupancy is possibly less than 2,000 km^2 in 10 or fewer locations in Seram with a continuing decline, and the number of mature individuals are potentially less than 10,000 with 1,000 or less individuals in each subpopulation. These trends support the IUCN criteria B2a, B2b(ii), and C2a(i) of VU (IUCN Standards and Petitions Committee 2019). Although BKSDA Maluku confiscation data did not record this species in the past five years, 40 wild-caught birds were exported in 2019 from Soekarno Hatta airport (SEAIR 2019), indicating that the poaching activity can threaten the wild population. Hence, we also recommend the inclusion of the species to CITES Appendix I.

5.4.4. Conclusion

Manusela National Park and its buffer zone have high parrot diversity. Over the last five years confiscation data showed that nine out of the 11 parrot species of Seram Island were poached. Illegal poaching activities do not only threaten parrot populations but are very likely to contribute to forest decline given the extremely important ecological role of parrots as seed dispersers (Blanco *et al.* 2016; Tella *et al.* 2019; Hernández-Brito *et al.* 2021). The decline in parrot populations, as an apparent result of high demand on the wildlife market may lead to the extinction of some species from the island. In this context, we have highlighted arguments for the uplisting on the IUCN Red List of three parrot species. We also showed that proper management and the rehabilitation of confiscated parrots, and the inclusion of local communities into conservation efforts with environmental education campaigns can have positive effects on parrot conservation in the area. Our results contribute important information to local and international authorities and wildlife management programs that may help reduce the local parrot trade and inform future rewilding projects.

Part II. An interdisciplinary approach to macaw research in the Peruvian Amazon

6. Nest site selection of scarlet macaws in lowland Peru

6.1. Introduction

Information about nest sites and reproductive success of wild bird populations is necessary for effective conservation and management strategies (Renton *et al.* 2000). Breeding success is a crucial determinant of recruitment rates, population size and long-term population viability in many avian species (Martin and Geupel 1993). The type of nest used by birds can determine breeding success rates and other aspects of life-history (Paredes and Zavalaga 2001). Most parrots are obligate secondary cavity nesters (Monterrubio-Rico and Escalante-Pliego 2006), thus their breeding success is closely related to the availability and quality of nest hollows (Collar 1997). The number and quality of nest cavities have been shown to be limiting for various parrot species, like Eclectus Parrot *Eclectus roratus* Müller 1776 (Heinsohn 2005; Heinsohn 2008), Palm Cockatoo *Probosciger aterrimus* Gmelin 1788 (Heinsohn *et al.* 2003), Swift Parrot *Lathamus discolor* White 1790 (Stojanovic *et al.* 2012), *Amazona* parrots (White *et al.* 2005a), and Blue-throated Macaw *Ara glaucogularis* Dabbene 1921 (Hesse and Duffield 2000).

Secondary cavity nesters can be assisted by the provision of artificial nest boxes (Brightsmith 2005b). They can be placed in areas where hollow availability is low, and can be supplied in large enough numbers to facilitate statistical inference for scientific research (Major and Kendal 1996). Their characteristics usually mimic the natural nest hollows of the target species, but artificial nests can also be more amenable to experimental manipulation as they reduce variation in nesting circumstances and improve accessibility to the nest (Villard and Pärt 2004). They can be useful tools for supporting reintroduction and translocation of endangered bird species (White Jr. *et al.* 2006), and for enhancing ecotourism by increasing the numbers of nesting birds (Nycander *et al.* 1995). Nest boxes have made it easier to perform comparative and experimental field investigations, since they can facilitate the installation of electronic monitoring devices (Grenier and Beissinger 1999; White Jr and Vilella 2004). Nevertheless, concerns have been raised about the generality and applicability of data from nest box studies (Lambrechts *et al.* 2012). For instance, there have been doubts about whether experimental setups of artificial nests mirror accurately enough the natural systems they attempt to model (Major and Kendal 1996).

The family *Psittacidae* (parrots) has the highest number of threatened species of bird family (Bennett and Owens 1997). Approximately 30% of all the parrot species are threatened globally and 37% of the Neotropical parrots are threatened (IUCN 2014). Despite the increase in parrot studies in the past decades (Downs 2005; Murphy *et al.* 2007; Heinsohn 2008; Berkunsky and Reboreda 2009; Theuerkauf *et al.* 2009; Brightsmith and Villalobos 2011; Vigo *et al.* 2011; White Jr *et al.* 2012) more research is required to document basic natural history and determine the effects of processes such as deforestation, habitat fragmentation, hunting by humans for food, and trapping for the pet trade (Laurance *et al.* 2009).

Macaws (genera *Ara*, *Anodorhynchus, Cyanopsitta, Primolius, Orthopsittaca,* and *Diopsittaca*) are charismatic parrots that remain poorly understood in the wild (Forshaw 2006).

Currently 5 species of macaws are extinct, and of the remaining 17 species, 3 are critically endangered (CR), 4 endangered (EN), 2 vulnerable (VU), and 1 near threatened (NT). Many of these macaws are found in the Amazon Basin in South America, which also contains a highly diverse and complex globally important ecosystem. Even today, this region that includes 60% of the world's remaining tropical rainforest (Laurance *et al.* 2002) is little known. The large size and wide-ranging habits of macaws, together with their popularity in human society, make them suitable 'umbrella' species for conservation in the Amazon region (Roberge and Angelstam 2004).

The Tambopata Macaw Project has been studying the breeding ecology and natural history of three large macaw species (Scarlet Macaw *Ara macao macao* Linnaeus 1758*,* Reda-and-green Macaw *Ara chloropterus* Gray 1859*,* and Blue-and-yellow Macaw *Ara ararauna* Linnaeus 1758) in the southeastern Peruvian Amazon for over 20 years (Nycander *et al.* 1995; Brightsmith 2005b; Brightsmith *et al.* 2008a). In the Tambopata region of Peru *A. macao* is still found in abundance (Renton and Brightsmith 2009). This natural environment provides ideal circumstances for understanding the nest preferences of this bird, which can then be applied in other locations where their populations are declining.

In this chapter we examine the long-term nesting success of *A. macao macao* (hereafter *A. macao*) with emphasis on the effectiveness of providing the birds with artificial nest boxes. Our aims are to a) determine the natural nesting requirements and reproductive success of the breeding macaws; and b) examine the efficacy of two different types of artificial nest (wooden and PVC) and compare their success to natural nest cavities. We anticipate that data from both natural and artificial nest types will have important conservation applications by informing conservation managers seeking to assist macaw populations where nest hollows are in short supply.

6.2. Methods

6.2.1. Study area

The study was conducted in the forests surrounding the Tambopata Research Center (TRC) in southeastern Peru (13° 8.070' S, 69° 36.640' W), in the Department of Madre de Dios, in the Tambopata National Reserve (2,747 km^2), near the border of the Bahuaja-Sonene National Park (10,914 km^2). This area, which receives an average annual rainfall of about 3,300 mm (Brightsmith, 2004), is located in tropical moist forest adjacent to subtropical wet forest (Tosi 1960) and is surrounded by four main forest types: *terra firme*, floodplain, palm swamp, and a mixture of early and late successional forest. The research centre is located 880 m from a large clay lick, Collpa Colorado, that serves as an important source of sodium rich soil which macaws feed on year round, especially during breeding when they feed it to their chicks (Brightsmith *et al.* 2008b; Powell *et al.* 2009; Brightsmith *et al.* 2010; Brightsmith and Villalobos 2011). All the studied nests were located within 3 km of TRC. The apparently high density of nests is likely due to both the provisioning of artificial nests and the close proximity of the large clay lick (Brightsmith 2004; Brightsmith *et al.* 2008b). In an effort to document the breeding parameters of the normal wild population data from the nests of the hand-raised and released individuals of *A. macao* found in the study area (Nycander *et al.* 1995; Brightsmith *et al.* 2005)

were excluded from the analyses presented in this chapter. Data on these birds will be presented elsewhere.

6.2.2. Study species

The breeding of the *A. macao* in southeastern Peru takes place during the wet season (November–April). The species nests in hollows of emergent trees including ironwood tree *Dipteryx micrantha* Harms 1926 (*Fabaceae*), *Calycophyllum sp*. Candolle 1830 (*Rubiaceae*), *Hymenaea oblongifolia* Huber 1909 (*Fabaceae*), *Erythrina sp*. (*Fabaceae*), and barrigona palm *Iriartea deltoidea* Ruiz & Pavon 1978 (*Arecaceae*) (Brightsmith 2005c; Renton and Brightsmith 2009). Birds usually lay 2–4 eggs asynchronously, the incubation period is 26–28 days, and the chicks fledge around 86 ± 4 days post hatching (Forshaw 2006; Vigo *et al.* 2011). Macaws like other parrots hatch asynchronously (Stoleson and Beissinger 1997). Although 3 eggs hatch on average, in general only 1 or 2 chicks survive until fledging, mainly due to starvation of the youngest siblings (Vigo *et al.* 2011). We used The Clements Checklist of Birds of the World as source of the nomenclature of avian taxa (Clements *et al.* 2013), the IUCN RedList database for other animal taxa (IUCN 2014), and the International Plant Names Index for plants (IPNI 2012).

6.2.3. Nest monitoring

We employed data collected from November 1999 – March 2011 (12 nesting seasons) to determine factors affecting the nest use and reproductive success of wild *A. macao*. The number of natural nests varied annually as new nests were found and old nests were lost due to takeover by stinging insects or through tree fall. The first artificial macaw nests were installed at the site in the early 1990's (Nycander *et al.* 1995). The number of artificial nests monitored each year varied as new nests were added, old nests were moved, trees containing nests fell down, and old nests were abandoned by the birds. All artificial nests were hung one per tree although distance between adjacent artificial nests varied greatly: from 31 to 425 m. They were hung at an average height of 26.8 ± 5.7 m.

Wooden nest boxes (Figure 6.1A) were about 1.5 m tall and made from tropical hardwoods (*Cedrela odorata, Cedrelinga catenaeformis, Calophyllum sp.*, etc.), with an approximate weight around 70 kg. PVC nests (Figure 6.1B) were constructed from 26 to 39 cm diameter PVC pipes lined with wire mesh and weighed 35–60 kg. Both nest types were filled to a depth of 30 cm with a mixture of sawdust, wood, and sand. A single door near to the bottom of each nest allowed investigators access to eggs and young individuals. The artificial nests were fixed to tree trunks with 2.5 cm climbing webbing or 11 mm climbing rope, and they were attached both at the top and bottom to reduce swaying and spinning.

We climbed to the nests using single-rope ascending techniques (Perry 1978; Perry and Williams 1981) and lowered chicks to the ground in buckets (Nycander *et al.* 1995). Nest visits usually took 30 to 50 minutes each from arrival until departure. For the safety of the chicks and the researchers, nests were not climbed during rain or high wind. Over the course of the 12 breeding seasons we monitored a total of 26 natural, 12 wooden and 24 PVC nests. The number of nests monitored per season was 10.1 ± 3.7 SD natural, 3.1 ± 2.2 wooden and 10.3 ± 3.3 SD PVC. Nests were monitored from October to April, and those located closer to TRC were usually monitored more intensely than those further from the centre. Nests where there was no

presence of *A. macao* were climbed 1 to 2 times per week from November–January. However, where macaws were seen defending the nests, climbing frequency increased to every one to two days (on average 5 climbs before eggs were found at occupied nests). Climbing protocols changed throughout the study: from 2000–2002 we climbed every 2–3 days during incubation and from 2003–2011 once eggs were found we did not climb the nest again until the estimated hatch date to minimize the effect of human disturbance on nesting. After chicks hatched we measured them daily for the first 15 days, then two or three times per week. Climbs were increased to daily or every other day near the estimated fledging date. Overall, occupied nests were climbed an average of 28.6 (± 1.6 SE) times per season. If at least one egg was laid in a nest during the breeding season it was considered a "nesting attempt" and the nest was considered "occupied" for that season. For each nesting attempt we determined the following: 1) whether eggs were damaged, hatched, or did not hatch; and 2) whether the nest was depredated, taken over by other macaws, fell down, or successful (fledged 1 or more chicks).

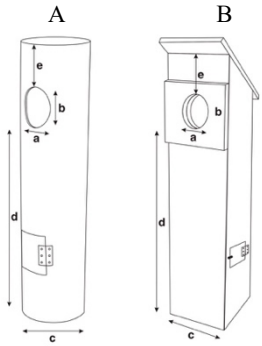

Figure 6.1. PVC (A) and wooden (B) nest designs used in the study for *A. macao*. Main measurements (and their average values) analysed were: a) horizontal (17.18 cm) and b) vertical diameter (18.40 cm) of the hole; c) inside diameter (36.15 cm); d) maximum depth (100.85 cm); and e) inside height (23.44 cm).

6.2.4. Statistical analysis

To determine the relationship between nesting success and nest characteristics, we analysed four measures of nest use and nesting success as response variables: whether eggs were laid (yes or no), whether ≥ 1 egg hatched (y/n), whether ≥ 1 chick fledged (y/n) and number of chicks fledged. We used combinations of 43 different variables depending on the analysis. The explanatory variables examined fall into four main categories: *Nest monitoring* (10) to test if the actions of the researchers had any effect on breeding success; *Presence of adult macaws* (4) to test the influence of parental behaviour on nest success; *Nest cavity characteristics* (12) to test the importance of nest measurements on reproductive success; and *Nest site characteristics* (12) to test the effects of visibility, habitat type and distance to other nests (Table 6.1).

We used a statistical modelling approach with all analyses carried out in GenStat 13.2 (Payne 2009). Whenever the data included repeated measures of the same nest over multiple years we assigned nest identity as a random effect, while the other factors were examined as fixed effects of interest.

We fitted generalized linear mixed models (GLMM) with a binary response (yes/no) to determine which factors influenced: a) whether eggs were laid; b) hatching success (one or more eggs hatched); and c) fledging success (one or more chicks fledged). We determined with linear mixed models (LMM) which factors affected the number of chicks fledged (continuous response variable), using all nests where eggs were laid. Due to the risk of over-

parameterization, the variables of interest were tested in four separate blocks: 1) nest site characteristics; 2) nest cavity characteristics; 3) macaws present at nest; and 4) the extent of researcher monitoring activity. Once the significant variables were obtained from each block, we combined these variables into a final model and reran it.

In order to determine success at the most occupied nests we performed a generalized linear model (GLM) analysis on nests that were occupied for at least 5 seasons over the study period. We used the number of years a nest was occupied as the response variable, which we compared to the total years the same nest was observed (as binomial total of the model). We started each analysis with a full model and progressively dropped non-significant terms until the most parsimonious model containing all significant terms was obtained. Unless otherwise stated, we present data as mean ± standard error.

Table 6.1. Variables used in the regression analyses for testing the nest site selection of *A. macao* in the lowlands of southeastern Peru.

Category	Variable	Description
Breeding effort	# eggs	Number of eggs in clutch
	# hatched	Number of chicks hatched
	# fledged	Number of chicks fledged
	Outcome last year	The outcome of the nest the year before: (0) no activity or no eggs, (1) eggs hatched, (2) young fledged, (3) predation, (4) fight or takeover, (5) disappeared or cracked eggs after hatch date, (6) nest flooded, (7) nest (tree) fell, (8) unknown), (9) chick found dead in nest without any sign of predation, (10) eggs broken or disappeared before hatch date
	Occupancy rate	% of all years nest was occupied (calculated only for nests monitored ≥ 5 years)
Nest monitoring	# Climbs per season	Number of times nest was climbed in the season
	before eggs	Number of climbs before eggs were laid
	1–10 days	Number of climbs during first 10 days of incubation
	11–20 days	Number of climbs during second 10 days of incubation
	21–26 days	Number of climbs during last 6 days before expected hatching
	1st week Chick Age	Number of climbs during first 7 days after first chick hatched
	1–2 weeks Chick Age	Number of climbs from day 8–14 after first chick hatched
	2–4 weeks Chick Age	Number of climbs from day 15–28 after first chick hatched
	4–8 weeks Chick Age	Number of climbs during the 2nd four weeks after first chick hatched
	8–12 weeks Chick Age	Number of climbs during the 3rd four weeks after first chick hatched
Presence of adult macaws	% birds present	Percentage of climbs macaws were present
	Min dist	The average minimal distance between the climber and the macaws
	% birds called	Percentage of climbs macaws made an alarm call at the nest
	% birds left	Percentage of climbs macaws left the nest area
Nest cavity characteristics	Nest type	(1) artificial wooden, (2) artificial PVC, (3) natural hole in tree, (4) natural palm hollow
	Nest position	Nest faces: (1) Horizontal, (2) Vertical
	# natural in tree	Number of additional natural nests in the same tree
	# artificial in tree	Number of additional artificial nests in the same tree
	Hole vert	Vertical diameter of the hole entrance (if there is more than one hole the largest one) in cm
	Hole horiz	Horizontal diameter of the hole (if there is more than one hole the largest one) in cm
	Diameter inside	Internal diameter of cavity 30 cm from entrance in cm
	Max depth	Bottom of hole to base of the nest in cm

	Inside height	Top of hole to the roof in cm
	Hole height *	Height of hole entrance from ground in cm
	Direction hole faces	The compass bearing of the hole entrance
	Vertical angle the hole faces	Positive angle faces above horizontal (+90 straight up; -90 straight down)
Nest site characteristics	Dist to 1st	Distance (m) to the nearest nest occupied by Scarlet Macaws
	Dist to 2nd	Distance (m) to the second nearest nest occupied by Scarlet Macaw
	Macaw 1st	Distance (m) to the nearest nest occupied by either Scarlet or Red-and-green Macaw
	Macaw 2nd	Distance (m) to the second nearest nest occupied by either Scarlet or Red-and-green Macaw
	Collpa (clay lick)	Distance (m) of nest from Collpa Colorado clay lick
	Habitat	(1) *terra firme* forest, (2) floodplain forest, (3) palm swamp forest, (4) successional forest
	Tree height	Total height of the tree where the nest is situated
	Tree circumference	Circumference (at 150 cm above ground or above the buttresses) of the nest tree
	Canopy major axis	Maximum width of the canopy of the nest tree
	Lowest leaf	Height of the lowest leaf (from the ground level) of the nest tree
	Canopy minor axis	The width of the canopy measured perpendicular the maximum canopy width of the nest tree
	Tree species	Species of the nest tree

* The nest height is defined as the hole height.

6.3. Results

6.3.1. Characteristics of nest trees and nest hollows

We analysed data from a total of 147 nesting attempts (n = 8 in 1999–2000, n = 9 in 2000–01, n = 15 in 2001–02, n = 12 in 2002–03, n = 10 in 2003–04, n = 11 in 2004–05, n = 14 in 2005–06, n = 12 in 2006–07, n = 14 in 2007–08, n = 15 in 2008–09, n = 8 in 2009–10, n = 19 in 2010–11) that occurred in 42 different nest sites (18 natural, 8 wooden nest boxes and 16 PVC nest boxes) over the 12 years of the study. Most of the available nests were located in floodplain forest (55%), with the remainder in upland or *terra firme* forest (28%) and successional forest (17%). The distribution of the occupied nests among habitats did not differ significantly from the distribution of available nest sites ($\chi^2_2 = 0.32$, $P = 0.850$; Figure 6.2).

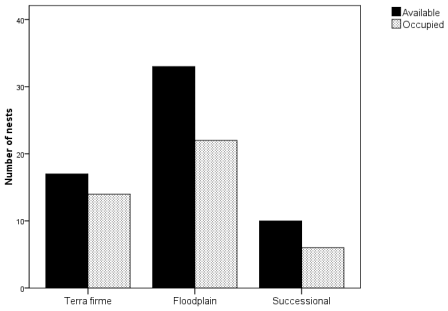

Figure 6.2. Distribution of available and occupied (natural and artificial) *A. macao* nests by habitat types in the lowlands of southeastern Peru. Differences were not significant.

Of the occupied nests, 44% successfully fledged ≥ 1 chick, 55% failed, and 1% had unknown outcome. Among the 81 nest failures, 37% were lost because the eggs were broken or disappeared before the hatch date. In 32% the eggs were cracked or failed to hatch after the anticipated hatch date. Most of these cases (18 of 26) were in PVC nests with only four in wooden nests and four in natural nests. In 15% of failed nests we found chicks which were killed by parasites, sickness, and bee or wasp attacks but without any sign of predation. For

only 7.5% of failed nests (n = 6) did we suspect loss to predators. Of these 6 predation events, 4 occurred in natural nests, one in a wooden nest and one in a PVC nest. At least 6 clutches (7.5% of failed nests) failed due to fights with other pairs of macaws over nest ownership (2 in artificial and 4 in natural nest). We suspect some of the nests where eggs were cracked or did not hatch may have also failed due to fights over nest ownership. One nest (1% of failed nests) was lost because the bottom of the PVC nest box fell off. No nests were lost due to the nesting tree falling down.

6.3.2. Determinants of reproductive success: clutch initiation

Over the 12 seasons, 37% of the occupied nests were in natural cavities and 63% were in artificial (15% wooden and 48% PVC). Nearly half (49%) of the clutches had three eggs, 12% had one egg, 25% had two eggs, and 14% had four eggs. The mean number of eggs laid per clutch was 2.70 (± 0.08 SE) with no significant difference between natural and artificial nests (GLMM $_{\text{Nest type}}$: $\chi^2_2 = 1.66$, $P = 0.441$; Table 6.2).

Table 6.2. Nest occupancy and breeding success of *A. macao* in the lowlands of southeastern Peru.

	Artificial wooden nests	Artificial PVC nests	Natural nests	Combined data for all nests
Number of occupied nests (number of available nests) [a]	22 (37)	70 (123)	55 (NA)	147
Clutch size (N) [b]	2.73 ± 0.21 (22)	2.8 ± 0.11 (69)	2.55 ± 0.13 (47)	2.7 ± 0.08 (138)
% of occupied nests that hatch [c]	73	51	67	61
% of eggs that hatched [d]	60	42	58	50
Number of chicks which hatch per successful clutch (N) [e]	2.25 ± 0.23 (16)	2.28 ± 0.16 (36)	1.97 ± 0.12 (36)	2.15 ± 0.09 (88)
% of occupied nests that fledged [f]	50	43	44	44
% of hatchlings that fledged [g]	39	52	51	49
Number of chicks which fledged per successful nest (N) [h]	1.27 ± 0.14 (11)	1.43 ± 0.09 (30)	1.52 ± 0.12 (23)	1.44 ± 0.06 (64)

[a] Number of occupied nest years over the study period (number of total nest year where available).
[b] Average number (± SE) of eggs laid in occupied nests.
[c] Percentage of occupied nests in which hatched one or more eggs.
[d] Percentage of laid eggs that hatched (Hatching success).
[e] Average number (± SE) of hatchlings for each nest which hatched one or more eggs.
[f] Percentage of occupied nests that fledged one or more chicks.
[g] Percentage of hatchlings that fledged (Fledging success).
[h] Average number (± SE) of fledglings for each nest which fledged at least one chick.

A. macao laid eggs significantly more often in nests which were successful the year before (GLMM $_{\text{Outcome last year}}$: $\chi^2_7 = 21.2$, $P = 0.030$). In total 85% of nests successful one year were re-occupied the next year, while only 41% of nests which were not successful (no activity or failed) were occupied the subsequent year. We found no significant effect on occupancy of the distance to the nearest nest occupied by the same species (Mean $_{\text{occupied nest}}$ = 192m ± 18 SE; Mean $_{\text{unoccupied nest}}$ = 193m ± 22 SE; GLMM $_{\text{Distance to nearest nest}}$: $\chi^2_1 = 0.74$, $P = 0.39$). However, we found that the distance to the second nearest nest had a significant positive effect on occupancy (Mean $_{\text{occupied nest}}$ = 321m ± 22 SE, Mean $_{\text{unoccupied nest}}$ = 375m ± 29 SE; GLMM $_{\text{Distance to second nearest nest}}$: $\chi^2_1 = 4.84$, $P = 0.029$).

The proportion of years a hollow was occupied was dependent on the nest's inside diameter (GLM $_{\text{Diameter inside}}$: $\chi^2_1 = 8.89$, $P = 0.003$). The highest occupancy rate was for nests with 40–50 cm inside diameter (69% occupancy over the period each nest was monitored), while the lowest occupancy was for nests with > 50 cm diameter (26%). All other variables tested were not significant ($\chi^2_1 < 1.84$, $P > 0.175$; Table 6.1).

6.3.3. Determinants of reproductive success: hatching success

A. macao hatching success did not differ significantly between natural and artificial nests (GLMM $_{\text{Nest type}}$: $\chi^2_2 = 1.79$, $P = 0.421$; Table 6.2). The likelihood of hatching one or more egg increased with the number of eggs in the clutch (GLMM $_{\text{Number of eggs}}$: $\chi^2_1 = 14.63$, $P < 0.001$). The mean number of eggs at nests where one or more eggs hatched was 3.04 ± 0.08 SE compared with 2.21 ± 0.14 SE at nests where no eggs hatched (ANOVA $F_{1,136} = 32.44$, $P < 0.001$). Hatching success was higher in nests with larger internal diameters (Mean $_{\text{chicks hatched}}$ = 38.5 cm ± 0.9 SE, Mean $_{\text{chicks didn't hatch}}$ = 34.4 cm ± 0.94 SE; GLMM $_{\text{Diameter inside}}$: $\chi^2_1 = 4.24$, $P = 0.048$). Hatching success was also higher in nests with a larger canopy minor axis (Mean $_{\text{chicks hatched}}$ = 22.50 m ± 0.58 SE, Mean $_{\text{chicks didn't hatch}}$ = 19.80 m ± 1.1 SE; GLMM $_{\text{Canopy minor axis}}$: $\chi^2_1 = 6.5$, $P = 0.017$). Hatching success was not related to any of the other physical characteristics of the nests or nest trees (GLMM: $\chi^2_1 < 2.63$, $P > 0.150$). However, nests where eggs hatched had adults which were more likely to be present at the nest during nest inspections (Mean $_{\text{eggs hatched}}$ = 84% birds present ± 1.3 SE; Mean $_{\text{eggs did not hatch}}$ = 66% ± 2.9 SE; GLMM $_{\text{\% of birds present}}$: $\chi^2_1 = 8.07$, $P = 0.005$). Hatching success was not related to any of the measures of nest monitoring intensity (GLMM: $\chi^2_1 < 2.65$, $P > 0.106$).

6.3.4. Determinants of reproductive success: fledging success

For nests that successfully fledged young, the mean number of fledged chicks was 1.43 ± 0.06 SE, with no significant difference between natural and artificial nests (LMM $_{\text{Nest type}}$: $\chi^2_2 = 0.51$, $P = 0.783$; Table 6.2). The number of fledglings was significantly positively related to the number of eggs laid (LMM: $\chi^2_1 = 14.9$, $P < 0.001$;

Figure 6.3). Fledging success did not vary significantly with any of the nest cavity characteristics measured (GLMM: $\chi^2_1 < 3.24$, $P > 0.078$).

Nests where chicks fledged had adults which were more likely to be present at the nest during nest inspections (Mean $_{\text{fledged}}$ = 85% ± 1.5 SE presence, N = 65; Mean $_{\text{no fledge}}$ = 70% ± 2.4 SE presence, N = 81; GLMM $_{\text{\% of birds present}}$: $\chi^2_1 = 10.22$, $P = 0.002$). The probability of fledging did not vary with the number of times nests were checked during the breeding season (GLMM: $\chi^2_1 < 1.91$, $P > 0.172$).

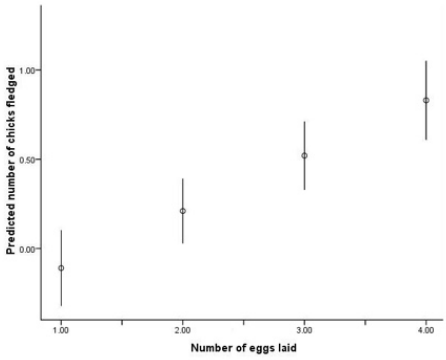

Figure 6.3. Predicted number (± SE) of fledged chicks versus the number of eggs at laying per *A. macao* nests in southeastern Peru. Data from 147 nesting attempts between 1999 and 2012. We used predictions of linear mixed modes (LMM) to build this graph.

Table 6.3. Characteristics of occupied natural and artificial *A. macao* nests in the lowlands of southeastern Peru.

Variables	Nest type	N	Minimum	Maximum	Mean (± SE)
Extra natural hollows per tree	Natural	12	0	2	1.08 ± 0.19
	Artificial	17	0	1	0.12 ± 0.08
Extra artificial nests per tree	Natural	12	0	0	0.00 ± 0.00
	Artificial	17	0	0	0.00 ± 0.00
Entrance hole vertical measurement (cm)	Natural	10	14	77	35.30 ± 6.18
	Artificial	17	14	30	18.40 ± 0.92
Entrance hole horizontal measurement (cm)	Natural	10	6	34	19.45 ± 2.60
	Artificial	17	13	37	17.18 ± 1.29
Inside diameter (cm)	Natural	9	17	66	38.56 ± 5.07
	Artificial	17	32	42	36.15 ± 0.79
Maximum depth (cm)	Natural	10	45	213	109.20 ± 20.10
	Artificial	17	14	176	100.85 ± 9.74
Inside height (cm)	Natural	10	0	155	69.60 ± 17.32
	Artificial	17	0	38	23.44 ± 2.71
Entrance hole height from the ground (m)	Natural	10	8.8	36.3	26.57 ± 2.75
	Artificial	17	19	36.1	29.34 ± 1.18
Angle the entrance hole faces	Natural	10	40	358	194.10 ± 32.09
	Artificial	17	20	340	183.18 ± 29.40
Vertical angle the entrance hole faces	Natural	11	-11	90	22.18 ± 11.50
	Artificial	17	0	0	0.00 ± 0.00
Distance to the clay lick (m)	Natural	12	450	2039	1180.50 ± 103.43
	Artificial	17	536	1927	1083.94 ± 105.40
Tree height (m)	Natural	12	28.6	58.7	44.94 ± 2.67
	Artificial	17	21.7	61.9	43.57 ± 2.75
Tree circumference (m)	Natural	12	2.8	5	3.72 ± 0.21
	Artificial	17	2	5.3	3.28 ± 0.20
Canopy major axis (m)	Natural	12	11.2	40.3	26.24 ± 2.73
	Artificial	17	15	44.9	25.28 ± 2.00
Lowest leaf height from the ground (m)	Natural	12	19.6	35.6	27.51 ± 1.56
	Artificial	17	21.2	31.2	27.13 ± 0.75
Canopy minor axis (cm)	Natural	12	2.3	32.4	18.75 ± 2.03
	Artificial	17	12	29.1	20.98 ± 1.58

6.4. Discussion

To date most reproductive information on wild *A. macao* has been based on only a few long-term studies in the field (Nycander *et al.* 1995; Vaughan *et al.* 2003; Vaughan *et al.* 2009). Our long-term data provides more insights into their reproductive biology and also allows important comparisons between natural and artificial nests, and an evaluation of the latter as a potential tool for enhancing macaw conservation.

6.4.1. Nesting preferences

A. macao are secondary cavity nesters (Renton and Brightsmith 2009) and use high and deep hollows with relatively large entrances in emergent canopy trees (*Dipteryx, Hymenaea*), isolated trees in broken canopy successional forest (*Erythrina*), and occasionally live or dead palms (*Iriartea*) (Brightsmith 2005c). During the 12 years of this study we monitored a total of 62 nests and not all were used every year. A major determinant of nest use was whether the nest had been successful in the previous year. A similar observation was made by Berkunsky & Reboreda (2009) for Blue-fronted Amazon (*Amazona aestiva* Linnaeus 1758) and by White *et al.* (2005a) for Puerto Rican Amazon (*Amazona vittata* Boddaert 1783). We found that macaws preferred to use nests with larger internal diameters, but this was the only physical characteristic that we found that affected their preference.

Our analysis occurred over a fairly small area (< 9 km^2) and this may have hampered our attempts to detect significant effects of the spatial distribution of nests. In addition, the study site was quite close to the Colorado clay lick, which serves as an important sodium source for the birds at this site (Brightsmith 2004; Brightsmith *et al.* 2008b; Brightsmith *et al.* 2010) and may have encouraged nest site clumping. However, the distance to the second nearest nest was significant with occupied nests having a closer second nearest neighbour. This variable may act as a proxy for local nest density, which may suggest that good nests are clustered in good habitat (e.g. near fruiting trees) or that the macaws prefer to breed close to each other at high density. Our anecdotal observations suggest that the latter may hold true and that there may be anti-predation benefits of nesting in close proximity. For example, neighbouring macaws were observed to respond in unison when predators (e.g. eagles, monkeys, tayras) or humans approached nests. Other parrot species are also known to nest in close proximity to each other when nest locations are available, thereby improving predator detection (Eberhard 2002).

6.4.2. Reproductive success

A. macao, like other birds, often lay more eggs than they raise, which may act as a form of insurance in case of hatching failure or other loss during incubation (Stinson 1979). The mean clutch size in this study was 2.70 (± 0.08 SE), similar to previous data for this species (Nycander *et al.* 1995; Forshaw 2006) and similar to other parrots of similar body size. The average clutch size for *A. macao*, *A. chloropterus*, and *A. ararauna* (body mass 1015–1250g) is 2.5–2.8 (Nycander *et al.* 1995), whereas in large cockatoos of the genera *Calyptorhychus, Cacatua, Lophochroa,* and *Probosciger* (275–841 g) mean clutch size ranges from 1.0, e.g. *P. aterrimus* (Murphy *et al.* 2003), to 3.3, e.g. Pink Cockatoo *Lophochroa leadbeateri* Vigors 1831 (Rowley and Chapman 1991).

Eggs hatched in 61% of occupied nests (Table 6.2), and 50% of eggs hatched successfully (hatching success). This compares to a 56% hatching success for Monk Parakeet *Myiopsitta monachus* Boddaert 1783 (Navarro *et al.* 1992), 64% for Red-tailed Black-Cockatoo *Calyptorhynchus banksii* Latham 1790 (Saunders *et al.* 1984), 72% for Red-lored Amazon *Amazona autumnalis* (Enkerlin-Hoeflich, 1995), 81% for Thick-billed Parrot *Rhynchopsitta pachyrhyncha* Swainson 1827 (Enkerlin-Hoeflich 1995), and 90% for Horned Parakeet *Eunymphicus cornutus* Gmelin 1788 (Robinet and Salas 1999). Our 61% hatching rate (% of occupied nests that produced hatchlings) is very similar to the 60% rate previously described

by Nycander *et al.* (1995) for *A. macao* in the same study site in Peru. Nearly 70% of breeding failures occurred during incubation even though this represents only one third of the total nesting period (Vigo *et al.* 2011).

Hatching success was higher in nests with larger inside diameter suggesting that females do better while incubating eggs if they have more space. In support of this result, PVC nests with smaller inside diameters also had a higher rate of apparently infertile or cracked eggs. Similar results were also reported from Costa Rica, where *A. macao* preferred to nest in tubes with larger diameters (Vaughan *et al.* 2003).

Among the occupied nests, 44% successfully fledged at least one young (Table 6.2), and 49% of the hatchlings fledged (fledging success). This is an intermediate result compared to other parrots, e.g. 27% of active *E. roratus* nests were successful (Heinsohn and Legge 2003), 22% for *P. aterrimus* (Murphy *et al.* 2003), 40% for *A. macao cyanoptera* Wiedenfeld 1995 in Guatemala (Boyd and McNab 2008). Nycander *et al.* (1995) described a higher rate of successful fledging for *A. macao* at the same Tambopata site but with a much smaller sample size: 9 of 14 (64%) occupied nests fledged one or more young. Fledging success (percentage of hatchlings that fledged) was 91% for Burrowing Parrots *Cyanoliseus patagonus* Vieillot 1818 (Masello and Quillfeldt 2002), 88% for Hyacinth Macaw *Anodorhynchus hyacinthinus* Latham 1790 (Guedes 1995), 63% for *E. cornutus* (Robinet and Salas 1999), and 50% for Pacific Parekeet *Aratinga strenua* Ridgway 1915 (Wermundsen 1998).

Adults are usually absent more from their nest during the nestling stage because parents need to obtain more food for their rapidly growing chicks and because the chicks can thermoregulate on their own (IñIgo-Elias 1996; Vaughan *et al.* 2009). Our results show that nesting success increased with the proportion of time the adults were present during nest inspections. It is unclear how this influences nesting success, but a variety of interpretations are possible. Parents that spend more time near the nest during nest inspections may be less afraid of the investigators, and by spending less time off their nests, the nest visits may not affect them. Alternatively, parents that are more efficient foragers may benefit by both bringing more food to the nest and by having more time to spend in nest attendance (Persson and Göransson 1999). This suggests that social factors, parental quality, and food availability may be important features determining reproductive success for the species. Such factors may be even more important where predation risk from diurnal predators (like *Micrastur* sp. forest-falcons) threaten macaw chicks (R. Garcia, pers. comm.).

Our research and those of others shows that brood reduction takes place in *A. macao* as in many other parrot species (Nycander *et al.* 1995; Masello and Quillfeldt 2002). Regardless of the number of chicks which hatch, *A. macao* pairs using natural hollows never fledged more than two chicks during our study. The fact that natural pairs did not fledge more than two young begs the question of why the birds continue to lay up to four eggs. Our results show that the average number of chicks fledged is greater with clutches of three and four eggs than with only two eggs. This presumably provides the selective pressure to maintain the average clutch size above 2.0.

The limitation of tree cavities on the reproductive rate of this and other large parrot species (Beissinger and Bucher 1992; Newton 1994; Heinsohn *et al.* 2003; Wiley *et al.* 2004) can result in intense conflict over nest sites. We confirmed nest loss by nest fights in only six cases. However, we did not systematically monitor nests for conflict during this study, and recent

observations suggest we may have underestimated the impact on nesting success from this source. Preliminary analysis of nest observation data suggests that increased frequency of intruding pairs of *A. macao* at nests correlates with reduced hatching success (Brightsmith & Vigo unpublished data).

Nest predation for *A. macao* in this study was relatively low with only six (4%) of the occupied nests suspected of being predated (7.5% of all failed nests). This was in spite of a diverse community of birds, reptiles, and mammals capable of taking both adults and young, and may reflect the ability of macaws to defend their nests due to their large body size of 1,015 g (Dunning 2008) and strong beaks. Other smaller parrots have higher predation rates, e.g. 45% in *E. roratus* (Heinsohn and Legge 2003) and 23% in Rose-ringed Parakeets *Psittacula krameri* Scopoli 1769 (Shwartz *et al.* 2009). Known nest predators of *A. macao* are Black Spider Monkey (*Ateles paniscus* Linnaeus 1758), Bolivian Squirrel Monkey (*Saimiri sciureus* Linnaeus 1758), White-throated Toucan (*Ramphastos tucanus cuvieri* Linnaeus 1758), Chestnut-eared Aracari (*Pteroglossus castanotis* Gould 1834), rodents including *Rattus* spp., and insects like cockroaches (Nycander *et al.* 1995). Other potential predators are Brown Capuchin Monkey (*Cebus paella* Linnaeus 1758), Tayra (*Eira barbara* Linnaeus 1758), Common Opossum (*Didelphis marsupialis* Linnaeus 1758), Crested Eagle (*Morphnus guianensis* Daudin 1800), forest-falcons (*Micrastur* spp.), and snakes including *Oxybelis fulgidus* Daudin 1803, *Leptodeira annulata* Linnaeus 1758, and *Tripanurgos compressus* Daudin 1803.

In 37% of failed nesting attempts eggs were found broken or disappeared from nests before the anticipated hatch date, and these were probably the consequences of fights for nest possession or predation. At 32% of failed nests, the eggs remained intact but failed to hatch by the hatch date. In these cases, the eggs were probably infertile, cracked during incubation, or were not incubated continuously (possibly due to nest takeover attempts in some cases). Most of these clutches occurred in PVC nests where higher internal temperatures (D.J. Brightsmith, unpublished data) and smaller internal diameters could have been important contributing factors. In 15% of failed nests, we found dead chicks probably resulting from parasites, starvation, or unknown diseases. Further and more detailed investigations are needed to better evaluate the causes of nest failures.

The asynchronous nesting of *A. macao* makes it difficult to estimate the developmental stages of nestlings (Myers and Vaughan 2004). We therefore climbed nest trees at a high frequency to determine the exact date of hatching, and to examine nestlings to determine the timing and causes of death. We did not find any significant effect of climbing rate on reproductive success at any stage of breeding. To date few studies have explicitly tested the hypothesis that researcher activities impact on reproductive success of the study species (e.g. Major 1990), although such knowledge is clearly important especially where researchers are studying the nests of species of high conservation concern.

6.4.3. Nest boxes vs. natural cavities

There were no significant differences in success rates between artificial nest boxes and natural nest hollows at any stage of reproduction. In addition, we did not observe differences between wooden boxes and PVC tube nests. Other studies of birds have found that natural nests had higher success than artificial nests [e.g. Barrow's Goldeneyes *Bucephala islandica* Gmelin

1789 (Evans *et al.* 2002), Eastern Yellow Robins *Eopsaltria australis* Shaw 1790 (Zanette 2002), or Bufflehead *Bucephala albeola* Linnaeus 1758 (Evans *et al.* 2002)]. Our results have important implications for conservation of macaws and follow other studies demonstrating the high value of artificial nests (Vaughan *et al.* 2003; White Jr. *et al.* 2006; Lambrechts *et al.* 2012). Our current designs with small modifications as outlined below could be useful for *A. macao* in other geographic locations where the status of the local population is of concern, e.g. in Costa Rica (see Vaughan *et al.* 2003), or with modifications for other macaw species. However, the occupancy of these artificial nests may be highly variable among different study sites even for the same species. Our project has also hung similar artificial nests in a tourist lodge and a local community within 60 km of TRC and none of these nests were occupied over the two nesting seasons they were monitored, probably due to a lower ratio of macaws to natural nest sites (D.J. Brightsmith, unpublished data). Further studies are needed to determine the variation of acceptance of these artificial nests.

PVC nests may be preferred by researchers as they are more durable and require less maintenance than wooden nest boxes that quickly rot in humid tropical environments. In addition, the destruction of wooden nests is hastened by the incubating female macaws which chew on the inside of the box. PVC nests are not only durable for macaws but also immune to attacks by woodpeckers, termites, bees and fungi, and are relatively light, easy to make, transport, and erect (Nycander *et al.* 1995). Considering the preferences of *A. macao* at our site, we suggest that the diameter of artificial nests should be larger than 40 cm in future applications. However, the artificial nests in this study had smaller entrance sizes than natural nest cavities (Table 6.3), and this design feature should be maintained as it may help to deter predators as found in other studies (Zanette 2002; White *et al.* 2005a).

Artificial nests can also enhance scientific research. The low side doors on the artificial nests provide easy access to eggs and chicks for the researchers facilitating morphological studies (Vigo *et al.* 2011). This is in contrast to natural nests where it can be difficult to reach to the bottom and extract the chicks. Furthermore, artificial nests also facilitate the installation of electronic monitoring devices like microphones, sensors, and cameras (Grenier and Beissinger 1999; White Jr and Vilella 2004).

In both a previous study (Nycander *et al.* 1995) and this one, many *A. macao* appeared to become accustomed to researchers climbing to check the nests. The presence of artificial nests where macaws and other species are predictable and habituated to human presence can increase the value of each bird to ecotourism (Munn 1992; Brightsmith and Bravo 2006). So in areas where macaws are protected, the use of artificial nests and other methods to attract nesting macaws can be important for ecotourism operations (Vaughan *et al.* 2005).

6.4.4. Conclusion

Even with 12 years of data we were unable to isolate the attributes of *A. macao* nest trees and holes that are best for reproductive success. There are probably many factors that the birds consider each time they choose a nest cavity. Renton & Brightsmith (2009) suggest that *A. macao* might be less able to successfully compete for high-quality nests with the sympatric *A. chloropterus* leading to greater flexibility in their choice of nest sites. Our sample of known nests is useful for describing their broad choice of nest site but clearly does not provide enough variation to isolate the factors that most affect breeding success. However, our data showed a

high rate of reoccupation of successful nests in the consecutive years and anecdotal observations suggest that many of these consecutive reoccupations were by the same pairs of birds. Others found that *Amazona* parrots re-used successful nests in consecutive breeding seasons (Enkerlin-Hoeflich 1995; Berkunsky and Reboreda 2009).

We showed that artificial and natural nests had similar reproductive parameters, suggesting that artificial nests can also contribute important data to natural history studies of species where access to natural nests is limited. This finding also supports the use of artificial nests for conservation management especially in regions where the large emergent canopy trees with the best nest hollows have been removed (e.g. due to logging) but habitat has otherwise been maintained (Munn 1992).

7. Blot fly parasitism in scarlet macaw nests

7.1. Introduction

The parasitic fly genus *Philornis* (MEINERT, 1890, Diptera, Muscidae) comprises 51 species (Skidmore 1985) and has a mainly Neotropical distribution (Carvalho and Couri 2002). Their larvae are obligate subcutaneous blood-feeding parasites of nestlings of a wide range of avian hosts (Arendt 2000; Allgayer *et al.* 2009). Larval development is rapid taking 4–6 days in furuncles with their caudal spiracles extending through the dermal openings of their avian hosts (Uhazy and Arendt 1986). *Philornis* infestations can increase bird mortality, decrease reproductive success, and affect nest site selection (Loye and Carroll 1998). They may even increase extinction risk for some avian hosts (Snyder *et al.* 1987; Fessl and Tebbich 2002). *Philornis* infestations have been noted repeatedly on parrot nestlings including macaws (Nycander *et al.* 1995; Renton 2002; Berkunsky *et al.* 2005).

The Tambopata Macaw Project has been studying the breeding ecology and natural history of large macaws (*Ara spp.*) in natural and artificial nests in the southern Peruvian Amazon for over 20 years (Nycander *et al.* 1995; Brightsmith 2005c; Brightsmith *et al.* 2008a). During nest inspections researchers found that scarlet macaw (*Ara macao*) nestlings heavily infested by bot fly larvae showed reduced survival (Nycander *et al.* 1995). Motivated by this observation, researchers at the site have opportunistically removed parasitic larvae to improve chick growth and fledging.

This situation gave rise to the following questions which guide the present study: (i) what are the overall rates of infestation, (ii) do different nest types affect levels of infestation, and (iii) what is the most suitable method of parasite removal in this particular host-parasite system?

7.2. Methods

The study was conducted in the forests surrounding the Tambopata Research Center (TRC) in southeastern Peru (13° 8.070' S, 69° 36.640' W), in the Department of Madre de Dios, in the Tambopata National Reserve. The centre is located in tropical moist forest near the boundary with subtropical wet forest (Tosi 1960) at 350 m elevation with an average annual rainfall of 3,236 mm (Brightsmith 2004). At this site scarlet macaws nest in natural hollows (Brightsmith 2005c; Renton and Brightsmith 2009) and in artificial wooden and PVC nest-boxes installed on emergent and isolated trees (Nycander *et al.* 1995).

We studied scarlet macaw nests in natural hollows, artificial PVC nests and wooden nest-boxes from November 2000 to March 2011 (12 breeding seasons). Nests were located within a 2.2 km radius of TRC. To determine the growth and health status of nestlings, we climbed to the nests using single-rope ascending techniques (Perry 1978; Perry and Williams 1981). We removed the chicks and lowered them to the ground in plastic buckets (Nycander *et al.* 1995). Once on the ground, each chick was checked visually for signs of bot flies and the number of bot flies was recorded. Chicks were also weighed and measured as part of ongoing studies (Vigo *et al.* 2011). On average, each of the 256 chicks involved in the study was handled 29.8

± 1.7 SE times during the ± 86 day period of nestling development. These visits lasted about 30 – 50 minutes. The anatomic location of bot fly infestations was recorded in 89 cases.

Three different methods of killing or removing the parasitic larvae were used over the course of the study. From 2000 – 2007 all bot fly larvae were treated with Negasunt® powder. The powder was placed liberally on the swollen area caused by the larvae. Normally only a single treatment was needed as the larvae were dead and swelling reduced by the next nest inspection 1–3 days later (D.J. Brightsmith, pers. obs.). In 2007 researchers attempted to remove the dead bot fly larvae using haemostats the day after treatment with Negasunt® powder. From 2007 – 2010 bot fly larvae were removed by holding an alcohol-soaked swab against the skin over the larvae for about 30 seconds to prevent the larva from breathing and forcing it to the surface. The swab was then removed and the veterinarian removed the larva with a haemostat. Sometimes after removing the larva an anti-parasite aerosol (Curabichera Spray) was applied. This technique required speed and experience and was often unsuccessful in the case of small larvae located deep in the skin.

Starting in 2010 we began to remove bot fly larvae using the Sawyer Extractor™ Pump Kit (a reverse syringe design device designed to extract snake venom). Larvae were removed by (1) cleaning the area around the bot with an alcohol soaked swab, (2) placing the head of the extractor over the larva, and (3) depressing the plunger of the extractor to start the suction. Usually within a few seconds small larvae were sucked completely out of the bird. Larger larvae only partially emerged from the wound but were easily grasped and removed with a haemostat. After bot fly removal the area was cleaned with an alcohol swab and covered with an antiseptic cream.

To quantify levels of infestations, we calculated prevalence as the percent of all chicks which had ≥ 1 larvae with 95% exact confidence intervals (CI). We also calculated the mean and median number of larvae per chick with ≥ 1 larvae (heretofore intensities). As parasites typically show an aggregated distribution across host individuals (Crofton 1971), we presented bias-corrected and accelerated bootstrap confidence limits (CI) around the mean and median intensities. We used Fisher's exact test and Mood's median test to compare prevalences and median intensities and present 2-sided exact *P*-values in each case. Index of discrepancy (Poulin 1993) was used to quantify skewness of parasite distribution. For statistical analysis Quantitative Parasitology 3.0 was used (Rózsa *et al.* 2000).

The analyses discussed above included multiple chicks hatched and raised in the same nest. This means that our results may be influenced by pseudo replication (treating each chick as statistically independent instead of the more conservative method of treating each different nest as statistically independent). To eliminate the effects of this pseudo replication, we pooled all chicks hatched in the same nest through all years so that we created one prevalence (±SE), mean, and median intensity of its chick pool per nest. These fully independent parameters were compared across nest types using Kruskal-Wallis Tests using GenStat 13.2. Pearson chi-square test was used to compare observed and expected bot fly infestations in nests with multiple chicks.

We tested the effects of bot fly infestation on nestling growth using growth data from 45 scarlet macaw chicks studied from 2000 – 2008 as presented in Vigo *et al.* (2011). For each chick we determined the number of bot flies recorded during the following time periods: 0–33 days (the period of fast weight gain), 34–63 days (the period of slow weight gain) and 64 days

to fledging (the period of weight loss). We used linear mixed models (LMM) of GenStat 13.2 to determine whether numbers of bot fly larvae in each of the above mentioned phases influence the (a) asymptotic size and (b) maximum growth rate and (c) age of maximum growth rate for the three biometric variables weight, wing, culmen, and tarsus.

7.3. Results

We monitored 19 natural tree cavities, 10 wooden and 19 PVC pipe boxes occupied by scarlet macaws and an average of 16.6 (±1.2 SE, range: 10–25) nesting events (laid at least 1 egg) per breeding season. We examined a total of 256 nestlings, 21.3 (± 2 SE) nestlings per breeding season (range: 10–33 chicks). In total, 372 bot flies were registered during the 12 years of the study. Bot fly larvae prevalence was 28.9% (CI: 23.4–34.9%), mean intensity was 5.03 larvae per infected chick (CI: 3.54–7.81) and median intensity was 2 (CI: 1–2) botflies per infected chick. The index of discrepancy was 0.89 indicating a rather high level of skewness, close to the theoretical maximum of 1. Larvae were most frequently located on the wings (36% of 89 reports), in open internal cavities such as ears (10%) or nares (7%), on the feet (9%), the face (7%) or the rump (7%). Other body parts affected less frequently were the head, chin, neck, legs, and upper chest (24%). Bot fly infestations occurred from the second day to the 86th day of nestlings' age with a peak time of infestation in the first month. Bot fly infestations were not randomly distributed among chicks. In the 44 cases where there were multiple chicks in bot fly infested nests multiple chicks were infested in 50% of the cases. The probability that in nests with multiple chicks more than one chick has bot fly infestation was significantly higher than expected (chi-square = 12.5, 2 d.f., $P = 0.002$).

Larval prevalence in natural nests (11%, CI: 6–19%, N = 90 nestlings monitored) was significantly lower than in wooden nest-boxes (39%, CI: 27–52%, N = 57) and PVC boxes (39%, CI: 30–48%, N = 109, Fisher's exact test: $P < 0.001$; Figure 7.1). Mean and median parasitism intensities did not differ significantly across different nest types (Bootstrap 2-sample t-test: $P_{(\text{natural vs. wooden})} = 0.219$, $P_{(\text{natural vs. PVC})} = 0.431$, $P_{(\text{PVC vs. wooden})} = 0.147$; Mood's median test for the 3 nest types: $P = 0.125$).

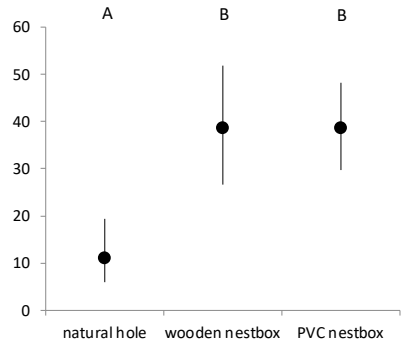

Figure 7.1. The prevalence of *Philornis* infestations of scarlet macaw chicks in natural cavities (11%, N = 90 nestlings monitored), artificial wooden nest boxes (39%, N = 57), and PVC nest boxes (39%, N = 109) in southeastern Peru. Vertical lines represent 95% confidence intervals around means (black dots). Columns labelled with different letters differ significantly (Fisher's exact test; $P < 0.001$).

When data from each nest were pooled across years, the mean of prevalence in natural nests (13%, ±5.5 SE, N = 17 nests monitored) was significantly lower than in wooden nest-boxes (46%, ±8.7 SE, N = 8) and PVC boxes (27%, ±6.4 SE, N = 12; Kruskal-Wallis statistic = 9.5, $P < 0.009$). Mean and median intensities for nestlings did not differ significantly among nest types (Kruskal-Wallis statistics < 1.9, $P > 0.39$ for all three comparisons).

Over the study period we killed or removed larvae from nestlings 188 times including repeated treatments of reinfected chicks. We attempted to remove larvae using Negasunt® Powder and haemostats (N = 27 cases), alcohol and haemostat (N = 49) and Sawyer Extractor^{TM} (N = 112). The bot fly larvae were successfully removed from nestlings in 33% with the Negasunt® method, 80% with the alcohol and haemostat method, and 100% with the Sawyer Extractor^{TM} method. The efficiency of the Sawyer Extractor^{TM} method was significantly higher than the two other methods (Fisher's exact test $P < 0.001$).

Asymptotic tarsus length was negatively correlated with the number of bot flies during the fast growth phase (0–33 days) (LMM $_{\text{bot flies 0–33 days}}$: $\chi^2_1 = 7.81$, $P = 0.008$). Asymptotic body mass was negatively correlated with the number of bot flies during the fast growth phase (LMM $_{\text{bot flies 0–33 days}}$: $\chi^2_1 = 6.64$, $P = 0.014$) and during the 0–63 day phase as well (LMM $_{\text{bot flies 0–63 days}}$: $\chi^2_1 = 6.59$, $P = 0.015$). Higher bot fly number also predicted lower weight of nestlings in these phases (LMM predictions; Figure 7.2).

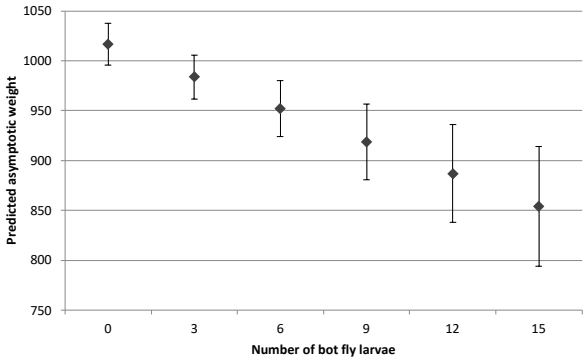

Figure 7.2. The predictions of linear mixed model (LMM) for the effects of bot fly number for asymptotic weight (±SE) of scarlet macaw nestlings during 0–63 days.

A total of 10 bot infested chicks died during the study, but only 3 were confirmed to have died due to the infestations: one died at age of 33 days due to a bot fly related ear infection, one died at 40 days old of infection after 26 larvae were detected all over its body, wings, head and nostrils, and one died at age 26 days after a single bot severed tendons in the leg and the bird was unable to stand. In some cases, we observed the natural disappearance of *Philornis* larvae before expected emergence day. We cannot exclude the possibility that adult birds may remove some larvae from their chicks.

7.4. Discussion

Artificial nests are important tools in conservation of different parrot species. By testing different types of artificial nests compared to natural ones can result better designs for the birds. In this study we compared parasite prevalence among different nests to see whether any of the nest types results in higher bot fly infestation. Parasite prevalence was significantly lower in natural nest hollows than in either artificial wooden or PVC nests. This could be the result of the material of the nest, as usually temperature in PVC nests can raise quickly and might result in higher parasite prevalence (D.J. Brightsmith, unpublished data). However, mean and median intensity did not differ significantly among nest types. The most extreme intensities in our study (63, 40 larvae per chick) were higher than those found for other Neotropical parrot chicks: 31 larvae for a hyacinth macaw (*Anodorhynchus hyacinthinus*) nestling (Guedes 1993), >15 larvae per scarlet macaw nestling (Nycander *et al.* 1995), and >25 larvae in two blue-

fronted amazon (*Amazona aestiva*) nestlings (Seixas and Mourao 2003). But are much lower than some reports for passerines: pearly-eyed thrashers (*Margarops fuscatus*) had a maximum of 220 larvae/nestling with an overall mean intensity of 37 (Arendt 1985).

Bot fly larvae are subcutaneous blood-feeders whose presence may facilitate secondary bacterial infections. However, we found little evidence of this as most infested chicks survived to fledging. In general, higher bot fly numbers during early development correlated with smaller overall chick size (weight and tarsus). We suspect that the bot flies are causing reduced chick size, but we cannot rule out the possibility that smaller nestlings get more bot flies for some other reason related to parental care or other unmeasured variable. Our findings that direct mortality caused by bot flies was uncommon agree with those from the literature, where the clearest direct impacts on chick health are from cases where the larvae invade sensitive locations such as sensory organs, respiratory pathways, mouth, or limbs (Arendt 2000).

Removing larvae may be helpful to the chicks, but also comes with a risk of injury, infection, impairment, and even death to the host if done incorrectly. For this reason, it is important for field personnel to use methods which maximize the benefits while minimizing risk. The Negasunt® Powder used contains 3% Coumaphos that kills the bot fly larvae in the bird, 2% Propoxur that repels other insects from the lesion and 5% Sulfanilamide anti-bacterial. We found no gross negative effects on the chicks. However, Coumaphos is classed as highly to very highly acutely toxic to birds if consumed (Abou-Donia *et al.* 1982; Abdelsalam 1999) and may be consumed by either by the parents or chicks. Therefore, we feel that using Negasunt® Powder should be avoided in wild birds.

The ‘alcohol and haemostat’ method reduces the risk of toxicity to the chick but it had a lower rate of success as bots that did not come to the surface of the skin were difficult to remove. The individual level of skill and veterinary training of the person using the technique also appeared to influence success. In addition, when unsuccessful, follow up attempts to remove the larvae often required incisions to remove the living or dead larvae. As a result, we do not recommend this method for extracting bot fly larvae.

By comparison, the new extractor method described here was highly efficient (100% in this study) and relatively easy for researchers of varied levels of skill and training. The age of the youngest macaw nestling we have subjected to this method was 2 days and we performed the process without complications. However, there are two concerns. When the bot is in areas where the extractor cannot get a good seal (tip of the wing, toe, etc.) suction may not be sufficient to remove the bot. In addition, the design of the extractor we used does not allow researchers to regulate the amount of suction. As a result, one must be careful when applying this method to young chicks of small-bodied species so as not to tear the skin. For this reason, researchers interested in using this technique should test it first on older individuals and monitor for bruising and skin tears before trying it on younger individuals.

Bot flies of various genera are known to infect a wide array of wild and domesticated vertebrate hosts (Milton 1996; Cogley and Cogley 2000; Angulo-Valadez *et al.* 2010) and this new extractor method should be effective on a wide range of taxa. If an extractor with variable suction levels was available, it would allow removal and collection of skin-dwelling arthropods from an even broader array of vertebrate hosts. Regardless, as presented, this technique should have broad application for veterinarians and scientists who wish to remove parasitic fly larvae quickly and easily without making incisions.

8. Developing non-invasive genetic tagging in macaws

8.1. Introduction

Genetic tagging, the technique of unique identification of individuals by their DNA profile, is now well-established (Palsboll 1999; Andreou *et al.* 2012). Genetic tagging became feasible with the development of methods allowing access to highly variable genetic markers such as codominant microsatellites, which are still the international standard for forensic analysis despite major advances in next generation sequencing methods (Bruford and Wayne 1993; Paetkau *et al.* 1995; Guichoux *et al.* 2011; Phillips *et al.* 2014). Genetic tagging was first used with invasively collected samples like skin biopsies of whales, fin clips of fishes, or ear tissues of small mammals (Palsboll *et al.* 1997; Peakall *et al.* 2006; Andreou *et al.* 2012). For non-invasive genetic tagging studies of mammals, DNA has been obtained from hair and scat samples (Taberlet and Luikart 1999; Arrendal *et al.* 2007; Ruibal *et al.* 2009; Coster *et al.* 2011). Genetic tagging can also provide data of value beyond individual identification. For example, it is standard practice to include a sex typing marker, since information about the sexes of individuals is needed for population demography or studies of sex-biased dispersal (Wright *et al.* 2005; Beck *et al.* 2008; Blackmore *et al.* 2011).

Despite its wide use in mammals, genetic tagging has not yet been widely adopted for birds (Taberlet and Luikart 1999; Segelbacher 2002; Horváth *et al.* 2005). Furthermore, even studies assessing the reliability of moulted feathers as a DNA source are scarce (Segelbacher 2002; Gebhardt *et al.* 2009), and the conclusions sometimes contradictory. Some studies have recommended avoiding the use of plucked or cut feathers due to low DNA quality of such samples (McDonald and Griffith 2011; McDonald and Griffith 2012), while others advocate their use (Katzner *et al.* 2012). Due to their degraded DNA content, feathers in museum samples are likely to be particularly problematic (Sefc *et al.* 2003). Despite the limitation of low quality and quantity of DNA, naturally moulted feathers can still provide an important source for genetic tagging when no other samples are easily available (Heinsohn *et al.* 2007; Monge *et al.* 2016). However, damaged feather samples can still present a challenge for reliable sex typing (Gebhardt and Waits 2008b), and for genetic tagging more generally in birds.

One-third of the extant parrot species are classified as threatened on the IUCN Red List (Chapter 1). Capturing parrots for genetic samples often requires a large effort due to such attributes as their high mobility, preference for the forest canopy and their often remote habitats (Masello *et al.* 2002; Heinsohn *et al.* 2007; Murphy *et al.* 2007). Despite these challenges there are some population genetic studies on parrots (Brock and White 1992; Wright and Wilkinson 2001; Heinsohn *et al.* 2007; Chan *et al.* 2008; Wenner *et al.* 2012; Masello *et al.* 2015).

Here we focus on two sympatric macaw species (Scarlet Macaw *Ara macao* and Red-and-green Macaw *Ara chloropterus*) from the lowland Peruvian Amazon, where they frequently visit 'clay licks' to supplement their dietary sodium by eating clay (Powell *et al.* 2009; Lee *et al.* 2010; Brightsmith and Villalobos 2011). Both species are considered as globally of Least Concern (IUCN 2014), and the availability of their shed feathers at clay licks make them a suitable test species for developing, validating, and applying genetic tagging for the first time

on a large sample of wild parrots. We build on previous studies that applied genetic tagging to other species (Palsboll 1999; Peakall *et al.* 2006), tested non-invasive molecular sexing in parrots (Gebhardt and Waits 2008b; Presti *et al.* 2013), demonstrated species identification in macaws (Abe *et al.* 2012), and showed reliability of feather genotyping compared to blood samples (Segelbacher 2002; Maurer *et al.* 2010).

The goal of our study was to assess the potential for non-invasive genetic tagging with 11 microsatellite loci by using moulted feathers of macaws sampled in the wild. Here we (1) evaluate the DNA quality for genetic analyses of naturally shed feathers left on the ground by macaws in tropical environmental conditions; (2) describe multilocus methods for species identification using DNA from feathers; (3) present new primers for molecular sexing on damaged feather samples; (4) validate eleven microsatellite markers for use in genetic tagging studies on large macaws; and (5) confirm that DNA from blood and feather samples yields equivalent population genetic patterns.

8.2. Methods

8.2.1. Target species and study site

The study was conducted in the lowland rainforest of Peru in the regions of Madre de Dios and Puno. The tropical moist and subtropical wet forest extends from 250 to 800 m elevation and receives on average 3,200 mm of rain per year (Tosi 1960; Brightsmith 2004). Our systematic collection of samples focused on two coexisting macaw species, the Scarlet Macaw (hereafter SCMA) and Red-and-green Macaw (hereafter RGMA). Both species have similar ecology (Brightsmith 2005a) and nest in emergent canopy trees during the rainy season (November–April) in Peru (Brightsmith 2005c).

A total of 1,263 naturally shed feathers were collected during the rainy season in the years 2009–2012. Collections were made across 10 main clay licks spread over 1,000 km of the Piedras, Heath, Tambopata, Candamo Rivers and their tributaries (Brightsmith and Munoz-Najar 2004; Brightsmith and Villalobos 2011). DNA was extracted from 886 samples (70% of the total), and 500 (40%) of these were used in the analyses of this study after the quality screening. Although the majority (84%) of these samples were collected from clay licks, some feathers were also collected in the forest, below nesting trees, and in nest hollows. Upon collection, samples were photographed with a measuring scale and stored individually in paper envelops in airtight boxes with silica gel to avoid further degradation.

To compare population genetic results between blood and feather samples in this study, we used 33 blood samples (28 SCMA and 5 RGMA) collected from captured adults and nestlings around the Tambopata Research Center (TRC; 13°8.070′S, 69°36.640′W) from both species.

8.2.2. Primer development for species-specific microsatellite markers

Potential target loci were identified from the first de novo genome assembly for the Scarlet Macaw (Seabury *et al.* 2013) using PHOBOS v3.3.12 to detect genome-wide microsatellite loci (STR). Initially, 41 autosomal di-nucleotide candidates with at least 20 repeat units were selected. We used the program iQDD v3.1 (Meglécz *et al.* 2014) to design primers for these loci with PCR product lengths of 90–300 base pairs.

M13 PCR tags were attached to the forward primers (5'–3': TGT AAA ACG ACG GCC AGT) and we amplified all loci individually. PCR products were multiplexed using different fluorescent tags (see supplementary material in Olah *et al.* 2015) and genotyped on an ABI 3130XL sequencer (Applied Biosystem) with the size standard GS500 (-250) LIZ. We used a negative and a positive control for each genotyping run. We dropped three loci that failed to amplify as a single locus.

To test the suitability of the remaining markers we genotyped a total of 86 samples that included 7 family groups. For population genetic analysis we analysed a subset of 40 unrelated individuals. We only included one sample per nest (excluding siblings) and if a sample was available from parents we excluded offspring of that pair. Consideration of the family groups confirmed mendelian inheritance, but indicated null alleles at 8 loci. A MicroChecker v2.2.3 (Van Oosterhout *et al.* 2004) analysis on the 40 sample set indicated departures from the Hardy–Weinberg Equilibrium (HWE) and null alleles at those same 8 loci. However, all of the other 30 loci fit expectation under HWE assumptions. These 30 loci exhibited 5–22 alleles, 3–14 effective alleles and expected heterozygosities of 0.669–0.930 (see table in Olah *et al.* 2015). Checks for linkage disequilibrium in GenePop v4.2 (https://genepop.curtin.edu.au) revealed that < 5% of all combinations showed potential departures from equilibrium.

We tested the cross species transferability of our new primers on a closely related species, the Red-and-green Macaw, where all but one marker resulted to be polymorphic. We also tested the primers on a distant relative, the Swift Parrot (*Lathamus discolor*, N = 12), but only seven loci were found polymorphic (2–11 alleles, 2–6 effective alleles and expected heterozygosities of 0.278–0.840).

Our panel of 30 microsatellite markers will be highly suitable for conservation genetics studies of Scarlet Macaw, other macaws, and probably other Neotropic parrot species. Furthermore, ready expansion to 38 loci is possible by re-designing primers for the addition eight loci that exhibited null alleles.

8.2.3. DNA extraction and genotyping

DNA extraction was performed using the Qiagen DNeasy Blood and Tissue kit (QIAGEN, California) following the manufacturer's instructions with some modifications to improve yield. These included longer incubation times, higher temperatures, and double elution on the spin columns in the last step, following Gebhardt *et al.* (2009). For feathers >20 mm in size DNA was extracted from the blood clot from the superior umbilicus (Horváth *et al.* 2005). The entire shaft was used as the DNA source for small feathers after cleaning the surface with 70% ethanol.

In a pilot study of 40 moulted feather samples intentionally consisting of DNA of varying quality, we screened the 30 microsatellite markers specifically designed for SCMA and also known to amplify in RGMA. From this pilot set of 30 loci, the 11 loci that yielded the highest amplification success across the trial DNA samples of lower quality were selected for this study. These 11 loci mainly amplified smaller fragment sizes (overall means of 122–284 bp). The locus SCMA 32 was found to only amplify samples of higher quality DNA. Thus, amplification success at this locus was highly correlated with amplification success at the other loci. Therefore, we used this locus to pre-select samples for the full analysis.

M13 PCR tags were attached to all forward primers (Schuelke 2000) and we amplified all loci individually. PCR products of 4 loci were multiplexed in the same lane using different fluorescent tags and genotyped on an ABI 3130XL sequencer (Applied Biosystem) with the size standard GS500 (-250) LIZ (see Table S1 in Olah *et al.* 2016b). We used a negative control for contamination check and a positive control to ensure consistent size scoring across all genotyping runs. Results were scored with Geneious version R6 (Kearse *et al.* 2012) and full genotypes were constructed. Most of the samples were genotyped once, with genotyping errors estimated from randomly selected samples (7–55 per locus) that yielded full genotype data for all 11 loci during the first scoring. This represents about 10% of the PCR reactions. Samples with 5 or more missing loci were excluded from the final analysis.

The following 11 microsatellite markers were used to construct the genotype data: SCMA 02, SCMA 09, SCMA 14, SCMA 22, SCMA 26, SCMA 27, SCMA 30, SCMA 31, SCMA 32, SCMA 33, and SCMA 34. Given that all our loci were already pre-screened for the presence of null alleles from genotyping of high quality DNA from blood, we used heterozygote deficit (homozygote excess) as an indicator of DNA quality in this study. Therefore, we tested deviations from Hardy-Weinberg equilibrium in GenePop 3.4 (Raymond and Rousset 1995) by exact probability test (Markov chain parameters were set to 100 batches with 1000 iterations per batch), and we assessed the degree of heterozygote deficit (if any). We also included blood samples that were previously genotyped during the microsatellite development for some relevant analyses.

8.2.4. Statistical analysis of feather quality

Feathers were provisionally assigned to the target species in the field based on their shape, size, and colour pattern. The size of each feather sample was derived from the photographs using ImageJ (http://imagej.nih.gov/ij/index.html). Each sample was also visually categorized by quality (good, medium, damaged) and whether it was covered by clay (yes/no). We also calculated the number of days between the collection and the DNA extraction dates for each feather. Finally, all samples were assigned a binary response variable of 1 (amplification of a fragment greater than 100 fluorescent units in the expected size range of SCMA 32) or 0 (failure to amplify). A linear logistic regression model was used to test the likely determinants of PCR amplification success at the SCMA 32 locus. Akaike information criteria (AIC) and Bayesian information criteria (BIC) were used to determine the best model containing all significant terms. Model was selected with the lowest AIC values and simultaneously having the lowest BIC values. Statistical models were computed using GenStat 13.7 (Payne 2009).

8.2.5. Species identification

A total of 14 parrot/parakeet (*Amazona*, *Pionus*, *Pionites*, *Pyrilia*, *Aratinga*, *Pyrrhura*, *Brotogeris*, *Touit*, *Forpus*) and 6 macaw species (*Ara*, *Orthopsittaca*, *Primolius*) are found in the study area, some of them with similar plumage patterns to our two target species, thus genetic species identification was crucial. Each feather was given a unique number and provisionally identified in the field. We used three independent genetics approaches for species filtering. First, we used the AgGT17 locus that was expected to provide allelic differences between our two study species (Gebhardt and Waits 2008a; Abe *et al.* 2012). However, these

earlier studies were based on less than 30 samples. In this study, we uncovered additional species-specific alleles by using a larger sample size.

In the next step we compared the identifications based on the AgGT17 locus with assignment tests based on allele frequencies of 11 other loci (Paetkau *et al.* 1995; Paetkau *et al.* 2004). We also applied a Bayesian approach with the program STRUCTURE version 2.3.4 to assign individual feather samples to species, based on their multilocus genotype (Pritchard *et al.* 2000). STRUCTURE implements the Bayesian Markov chain Monte Carlo (MCMC) method to assign individuals to *k* clusters. In order to separate clusters as species we used the no-admixture model, with independent allele frequencies among clusters. Burn-in was set to 50,000 iterations, followed by 50,000 MCMC iterations and replicated ten times for each value of *k*, from one to five. To avoid any bias in the species allocation, the AgGT17 locus was excluded from the assignment tests and STRUCTURE analysis.

By sequencing the COI gene on the mtDNA with primer pair of BirdF1/BirdR1 (Hebert *et al.* 2004), we barcoded five RGMA and ten SCMA samples further confirming the validity of our nuclear DNA methods for species identification. We used the software Geneious R6 (Kearse *et al.* 2012) to generate sequence alignments.

8.2.6. Molecular sexing

The most widely employed method for molecular sexing of birds is based on the conserved CHD gene in the avian sex chromosomes (Ellegren 1996). In this test the primers produce one amplified fragment for males and two different size fragments for females due to retroposon insertions in the females' Z chromosome (Suh *et al.* 2011). In our pilot study we tested the widely used P2/P8 primers that amplify DNA fragments between 300 and 400 bp (Griffiths *et al.* 1998) and the 2550F/2718R primers that show much better agarose gel resolution (ranging between 400 and 1000 bp) and higher confidence in sex determination on agarose assay over a wide range of bird taxa (Ong and Vellayan 2008). Both primer combinations showed very low amplification success on our moulted feather DNA in the pilot study, probably because of our more degraded DNA samples.

In order to achieve robust molecular sexing from degraded DNA, we therefore designed new primers for our target species that would yield results for small fragment size differences with capillary electrophoresis. Our assay targeted a 189 bp fragment of CHD-Z and a 215 bp fragment of the CHD-W yielding a difference of 26 bp (see Fig S1 in Olah *et al.* 2016b). The primer design was based on an alignment of CHD gene sequences of SCMA from GenBank (accession numbers: KF425691, KF412778; http://www.ncbi.nlm.nih.gov/genbank). Geneious version R6 was used to obtain the alignment and optimize primer design. The sequences of the new primers (5′ to 3′) are:

P8_SCMA_F: TGCAAAACAGGTRTCTCT
P2_SCMA_R: GAWTAAGTAGTTCAAAGCTA

We compared the new primers for macaw samples of known sex, and on blood samples previously sexed using the 2550F/2718R primers.

8.2.7. Population genetic analyses

GenAlEx 6.5 (Peakall and Smouse 2006; Peakall and Smouse 2012) was used to compute all population genetic analyses, unless otherwise stated. These calculations included allele

frequencies, observed and expected heterozygosities, probability of identity (*PI*), and probability of identity for siblings (PI_{sibs}).

The *PI* value across loci provides an estimate, under the assumptions of Hardy–Weinberg equilibrium, of the average probability that two independent samples will have the same identical genotype (Waits *et al.* 2001). It thus provides an estimate of how many loci are needed to discriminate among individuals. The theoretical estimate of the *PI* is usually lower than the observed value, hence the calculation for PI_{sibs} was introduced in forensic science (Evett and Weir 1998), to estimate the probability when full siblings occur in the dataset that share very similar alleles. To empirically confirm how many loci were needed for recovering all genotypes, we computed the genotype recovery rates by adding increasing number of loci, in order of their effective number of alleles. Lastly, we pinpointed complete genotype matches for conspecific samples in the genetic tagging analysis. We manually checked each near match for samples that only differed at 1–3 loci and resolved any scoring errors.

In order to compare between the blood samples in TRC and feathers collected in a 3 km radius around the same location, the genetic differentiation (F_{ST}) between these two groups was estimated by an analysis of molecular variance (AMOVA). Allele frequencies, observed and expected heterozygosities were calculated for blood and feather samples separately. Pairwise estimate of Shannon's Mutual Information Index was also performed (Peakall and Smouse 2012; Smouse *et al.* 2015), providing an alternative allele frequency based estimate of genetic differentiation. In order to validate that samples from clay licks (N_{SCMA} = 96 feathers) give similar population genetic results to samples from nests (N_{SCMA} = 40 blood samples and 38 feathers), we also performed an AMOVA between these two types of sampling sites for SCMA.

8.3. Results

8.3.1. Feather quality, microsatellite amplification, and population statistics

The size of feathers and the number of days between collection and DNA extraction did not significantly affect the PCR amplification success ($GLM_{Feather\ size}$: $\chi^2_1 = 1.47$, $P = 0.225$; $GLM_{Days\ since\ collection}$: $\chi^2_1 = 3.10$, $P = 0.078$). However amplification success was significantly lower for poor quality feathers ($GLM_{Feather\ quality}$: $\chi^2_2 = 108.87$, $P < 0.001$; Figure 8.1A) and when clay was present on feathers at collection ($GLM_{Clay\ on\ feather}$: $\chi^2_1 = 14.14$, $P < 0.001$; Figure 8.1B).

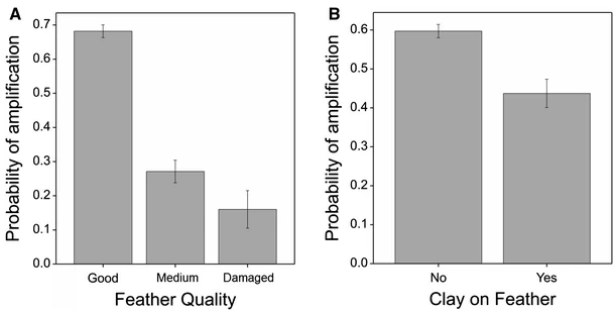

Figure 8.1. Predicted effect of significant variables from a linear logistic regression model on the probability of PCR amplification of SCMA32 locus: (A) feather quality and (B) clay on feather.

In total 500 feather samples were genotyped across the 11 loci with only 27 samples discarded from subsequent analyses because they had 5 or more missing loci. Allele frequency

and heterozygosity estimates by locus are shown in Table 8.1 for both target species. Across all loci the allele number (*N*a) ranged from 12 to 20 per locus for SCMA and from 9 to 18 for RGMA. The mean expected heterozygosity (H_E) was 0.892 for SCMA and 0.772 for RGMA. The observed heterozygosity (H_O) values across loci ranged from 0.733 to 0.908 for SCMA and from 0.553 to 0.892 for RGMA.

Table 8.1. Population statistics for microsatellite markers in non-invasive feather samples from Scarlet Macaw (*Ara macao*) and Red-and-green Macaw (*Ara chloropterus*). Presented are species, number of locus used in the analyses, locus name, number of samples (*N*), fragment size ranges, mean fragment size (MS), number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_O), expected heterozygosity (H_E), probability of departure from HWE (P_{HWE}), and probability of heterozygote deficit (P_{HED}).

# loci	Locus	Scarlet macaw (*Ara macao*)									
		N	Size range (bp)	MS (bp)	N_a	N_e	H_O	H_E	*F*	P_{HWE}	P_{HED}
6 loci	SCMA 22	131	114–160	134	19	12.1	0.908	0.918	0.01	0.221	0.412
	SCMA 32	128	175–211	192	16	10.9	0.828	0.908	0.088	0.037	0.003
	SCMA 34	132	151–189	173	17	8.5	0.803	0.882	0.09	0.018	0.005
	SCMA 33	134	174–212	193	20	11.2	0.881	0.91	0.033	0.696	0.156
	SCMA 26	130	210–240	225	14	9.4	0.808	0.894	0.096	0.002	0
	SCMA 09	132	112–136	123	12	5	0.75	0.802	0.065	0.836	0.103
9 loci	SCMA 14	131	220–252	238	14	8.8	0.733	0.886	0.173	0	0
	SCMA 30	124	206–246	229	17	9.8	0.871	0.898	0.03	0.908	0.029
	SCMA 31	108	137–169	152	16	8.7	0.861	0.885	0.027	0.235	0.197
11 loci	SCMA 02	111	268–300	284	17	12.9	0.793	0.922	0.141	0.003	0
	SCMA 27	120	209–245	226	18	11.3	0.858	0.912	0.059	0.243	0.026
	AgGT17	132	102–138	119	18	6.5	0.833	0.846	0.015	0.63	0.208
	Mean			191	16.4	9.9	0.827	0.892	0.069		
# loci	Locus	Red-and-green macaw (*Ara chloropterus*)									
		N	Size range (bp)	MS (bp)	N_a	N_e	H_O	H_E	*F*	P_{HWE}	P_{HED}
6 loci	SCMA 22	278	122–150	135	14	8.5	0.892	0.882	–0.012	0.478	0.287
	SCMA 32	279	173–199	184	11	3.1	0.631	0.677	0.069	0.005	0.001
	SCMA 34	279	157–181	169	13	5.4	0.799	0.816	0.02	0.002	0.019
	SCMA 33	280	166–190	179	10	2.4	0.575	0.586	0.019	0.571	0.269
	SCMA 26	277	222–240	231	10	5.1	0.715	0.803	0.11	0.097	0.001
	SCMA 09	280	112–132	122	11	4	0.739	0.751	0.015	0.889	0.382
9 loci	SCMA 14	273	212–238	228	9	2.4	0.553	0.59	0.063	0.124	0.103
	SCMA 30	270	206–248	230	17	4.9	0.715	0.796	0.102	0	0
	SCMA 31	220	135–165	153	12	7.5	0.836	0.867	0.036	0.89	0.197
11 loci	SCMA 02	238	260–300	283	18	5.9	0.668	0.831	0.196	0	0
	SCMA 27	267	211–245	227	17	9	0.76	0.889	0.145	0	0
	AgGT17	282	98–112	105	4	1	0.007	0.011	0.331	0.006	0.006
	Mean			187	12.9	5.3	0.717	0.772	0.091		

The average amplification success over the 11 markers was 94% for SCMA and 95% for RGMA. The lowest overall amplification success (see Table S2 in Olah *et al.* 2016b) across both species (N = 473) occurred at SCMA 31 (18.6%), SCMA 02 (14.2%), and SCMA 27 (6.3%). Scoring errors at genotype level were calculated from about 50 randomly selected samples that had no missing loci during the first scoring before the repeats. Genotyping errors occurred mainly due to allelic dropouts at the larger allele (at loci SCMA 02, SCMA 14, SCMA 26, SCMA 30, and SCMA 32), but in some cases at the smaller allele (AgGT17 and SCMA 27), and sometimes due to false alleles (SCMA 02, SCMA 14, SCMA 27, SCMA 30, and SCMA 32). The total numbers of genotyping errors were as follows: SCMA 02 (2/7 replicated samples), SCMA 27 (4/15), SCMA 14 (5/48), SCMA 32 (3/44), SCMA 30 (2/42), SCMA 26

(1/48), and AgGT17 (1/55). We found no genotyping errors at SCMA 09, SCMA 22, SCMA 31, SCMA 33, SCMA 34, and P2/P8_SCMA.

Subsequently three overlapping dataset were analysed: set (1) the 6 loci with no error and low amplification failure, set (2) combination of set (1) and 3 additional loci with some error or higher amplification failure, and set (3) combination of set (2) including two loci with both scoring error and higher amplification failure (Table 8.1). Using all 11 loci only eight repeated samples showed mismatched genotypes where 8-10 loci were adequate (see below), suggesting that genotyping errors did not affect the genetic tagging analysis. Two of these samples were confirmed siblings from the same nest.

8.3.2. Species identification

The AgGT17 locus for nuclear DNA based molecular identification of the target species amplified in all but two samples, potentially providing a technique to separate these two species. Based on 11 loci (excluding AgGT17), the STRUCTURE analysis and assignment tests (see Figure S2 in Olah *et al.* 2016b) independently allocated 18 samples into a third group probably representing a different or several different species that were not the target of this study. Most of these 18 samples also showed unusual alleles at the AgGT17 marker. After sequencing a mtDNA region of ten SCMA and five RGMA samples identified by our nuclear DNA methods, the alignments showed two different groups. After performing a nucleotide blast to NCBI GenBank (http://www.ncbi.nlm.nih.gov/genbank), the two groups matched the nucleotide sequences of our two study species correctly.

In total 142 SCMA and 313 RGMA feathers were identified and confirmed independently by the AgGT17 genetic marker, the STRUCTURE analysis, and the assignment test. In the field 28% of the feather samples were misidentified based on their colour. Thus, species ID was corrected in light of this genetic analysis.

8.3.3. Molecular sexing

The P8_SCMA_F/P2_SCMA_R primers produced two amplified fragments in females (CHD-Z and CHD-W) and one amplified fragment (CHD-Z) in males, which was easily visualized by capillary electrophoresis (see Figure S1 in Olah *et al.* 2016b). The optimized primers yielded results matching 20 blood samples of known sex for both target species. When applied to the feather DNA samples, typing was achieved for sex in 85% of SCMA samples (66 males, 55 females, 21 unknown) and 95% of RGMA samples (183 males, 114 females, 16 unknown).

8.3.4. Probability of identity and genetic tagging

The probability of identity (among siblings) analysis was calculated using the best 6 loci (Table 8.1). For SCMA the five most variable loci ($PI_{sibs(5)} = 0.002$) and for RGMA the six most variable loci ($PI_{sibs(6)} = 0.003$) were predicted to recover all unique genotypes, given the sample sizes of this study ($N_{SCMA} = 142$, $N_{RGMA} = 313$). This prediction was supported empirically, for example we did not recover more unique genotypes in feathers after the two most variable loci of SCMA (Figure 8.2A) and after the five most variable loci of RGMA (Figure 8.2B) when including the best six loci (similar results for 9 or 11 loci). We therefore used only these six microsatellite markers for subsequent genetic tagging.

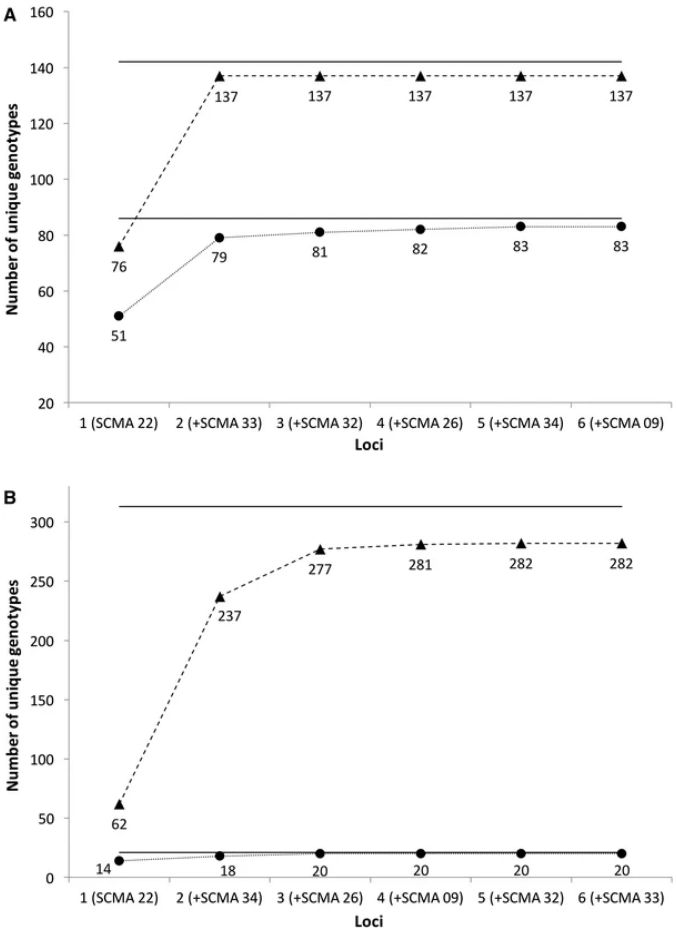

Figure 8.2. Recovery of unique multilocus genotypes for increasing combinations of loci for (A) Scarlet Macaw (*Ara macao*) and (B) Red-and-green Macaw (*Ara chloropterus*). The order of loci was defined by their number of effective alleles (from highest to lowest) for the two species respectively. Triangles (dashed line) indicate genotype recovery using only feather samples; and circles (dotted line) show recovery when using blood samples from related individuals (including parent/offspring and full siblings).

Among the 142 feather samples of SCMA we identified five complete genotype matches (total of 137 unique genotypes). When we added 86 previously genotyped SCMA blood samples we found eight additional genotype matches between blood and feather samples (Fig S3). Out of 313 RGMA feather samples there were 23 matches (total 282 unique genotypes). As expected, across both species the most frequent type of 'recapture' was in the same location from the same sampling event (15). Further matches occurred (a) among or within nests (6), (b) between nests and clay licks (6), and (c) among or within clay licks in different time (9).

8.3.5. Reliability of non-invasive feather samples

Within the 3 km vicinity of TRC we had a comparable number of blood and feather samples from both species to test whether the invasive and non-invasive samples yielded similar population genetic results. We found similar allele frequencies, observed and expected heterozygosities between blood and feather samples of SCMA (Table 8.2). The genetic distance based AMOVA with the 6 most reliable loci showed no significant differentiation between the two sample types for SCMA ($N = 73$, $F_{ST} < 0.001$, $P = 0.447$) or RGMA ($N = 23$, $F_{ST} < 0.001$, $P = 0.444$), and similar results were yielded with 9 and 11 loci. The Shannon's allele frequency-based analysis also failed to detect any significant genetic differentiation (see Table S3 in Olah *et al.* 2016b). Across all the samples we also found no significant genetic differences between samples from nests and clay licks for SCMA (AMOVA: $N = 174$, $F_{ST} < 0.001$, $F'_{ST} = 0.049$, $P = 0.326$).

Table 8.2. Population statistics for microsatellite markers on blood and feather samples from Scarlet Macaw (*Ara macao*) in TRC. Presented are number of loci used in the analysis, type of samples, number of samples (N), number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_O), expected heterozygosity (H_E), and fixation index (F). Numbers are mean values ±SE.

# locus	Type	N	N_a	N_e	H_O	H_E	F
6 loci	Blood	27.8 ± 0.1	12.1 ± 1.0	7.70 ± 0.8	0.873 ± 0.030	0.863 ± 0.012	−0.010 ± 0.025
	Feather	44.3 ± 0.2	14.1 ± 0.9	9.01 ± 1.0	0.872 ± 0.015	0.880 ± 0.015	0.008 ± 0.020
9 loci	Blood	27.8 ± 0.1	11.8 ± 0.8	7.52 ± 0.5	0.892 ± 0.021	0.862 ± 0.007	−0.033 ± 0.020
	Feather	43.1 ± 1.1	13.7 ± 0.7	8.88 ± 0.6	0.850 ± 0.023	0.881 ± 0.010	0.034 ± 0.027
11 loci	Blood	27.9 ± 0.0	12.1 ± 0.6	7.86 ± 0.5	0.898 ± 0.018	0.868 ± 0.007	−0.034 ± 0.016
	Feather	42.5 ± 1.0	14.0 ± 0.6	9.13 ± 0.5	0.841 ± 0.021	0.885 ± 0.008	0.049 ± 0.025

8.4. Discussion

We have developed, validated, and applied a genetic tagging method for feather samples collected from wild populations of two sympatric macaw species in the southeastern Peruvian Amazon. Our results demonstrate that feathers are valuable sources of DNA for genetic tagging as tested using 11 highly variable microsatellite loci.

8.4.1. Feather sampling in a tropical environment

Feathers are a promising non-invasive source of DNA but there are some conflicting views on their utility (McDonald and Griffith 2011; McDonald and Griffith 2012; McDonald and Griffith 2012). In their experimental setup with feathers of domestic goose, *Anser anser domesticus*, Vili *et al.* (2013) showed that humidity, direct sunlight, and heat have the most degrading effect on feather DNA quality. As expected, not all feathers collected from our tropical study site yielded sufficient and high enough quality DNA for molecular sexing and genetic tagging. Gebhardt *et al.* (2009) found that moulted macaw feathers at clay licks provide promising DNA samples, but they also reported a high error rate in molecular sexing of samples (Gebhardt and Waits 2008b). Here we found by logistic regression analysis that damaged feathers had significantly lower amplification success over intact feathers, consistent with other studies (Hogan *et al.* 2008; Gebhardt *et al.* 2009). Despite thoroughly washing the feathers with 70% ethanol, samples with clay still had significantly lower amplification success. In addition, clay particles appear to inhibit PCR reactions as also observed by Yankson and Steck (2009). Unlike the studies on a large grouse, *Tetrao urogallus* (Segelbacher 2002), or on large macaws, *Ara* spp. (Gebhardt *et al.* 2009), we found that the size of the feathers did not significantly affect the quality DNA yields.

For future genetic studies using feather samples in tropical environments we recommend (a) collecting only good quality and intact feathers, (b) collecting mainly clean feathers free of clay, and (c) considering feathers in a wide range of size, in order to maximize quality and quantity of DNA. If feathers are stored appropriately (e.g. in dry box with desiccant), the time interval between sample collection and DNA extraction appears to be flexible, at least over a time window of 2–5 years.

8.4.2. Species and sex identifications by moulted feathers

Correct species identification remains the critical first step when relying on feathers for population genetic analysis or genetic tagging (Rudnick *et al.* 2007). Identification of species by the morphology and colour of feather samples can be challenging, and in our study we initially misidentified almost one-third of our feather samples in the field. DNA barcoding, mainly based on mtDNA COI gene, is the standard genetic technique for species identification (Tavares and Baker 2008; Abe *et al.* 2012). However, in this study we were able to distinguish species using nuclear DNA and the same multilocus genotyping methods employed for our population genetic analyses. This minimized the need for DNA sequencing, reducing the cost of the project.

Although molecular sex typing of birds initially required a blood sample (Griffiths *et al.* 1998; Fridolfsson and Ellegren 1999) primers are now available for freshly plucked or collected feathers (Ong and Vellayan 2008; Bosnjak *et al.* 2013; Presti *et al.* 2013). Typically the molecular sexing of birds targets sex specific DNA fragments that are visualized on agarose gel electrophoresis, providing a low cost and simple laboratory assay (Miyaki *et al.* 1998; Ong and Vellayan 2008). For this technique the DNA rich avian blood with nucleated erythrocytes is usually used (Griffiths *et al.* 1998; Fridolfsson and Ellegren 1999). However, other studies have successfully applied the method to plucked feathers from captive birds (Ong and Vellayan 2008; Bosnjak *et al.* 2013). Gebhardt and Waits (2008b) even evaluated the performance of primer sets on moulted feathers of SCMA and reported high overall error rates and high dropout rates. Presti *et al.* (2013) also found that most primers did not amplify well on moulted macaw feathers and suggested the use of primers amplifying even shorter PCR fragments. With our new primers that target a shorter PCR fragment, we were able to confidently identify sexes in 84% of SCMA and 94% of RGMA moulted feather samples.

8.4.3. Genetic tagging, tracking macaws without capture

The probability of identity values calculated for siblings in our dataset of SCMA and RGMA indicated that five or six of the most variable loci were enough to recover unique genotypes for the two species respectively, given our sample sizes. We confirmed this empirically by comparing the number of unique genotypes recovered for increasing combinations of loci, including previously genotyped SCMA blood samples with many related individuals, e.g. parent/offspring and full siblings (Figure 8.2A).

Our study recovered a total of 36 genotype matches among samples, and according to the *PI* values and the genotype recovery rates we were confident that these were ‘recaptures’ of the same individuals. Recaptures found between blood and feather samples further demonstrate the feasibility of this technique. Adult SCMAs are often observed feeding their chicks with seeds mixed with clay, and crop samples of these chicks showed high content of clay (Brightsmith *et al.* 2010; Cornejo *et al.* 2011). We suspected that adult macaws visit the nearest clay licks to their nests for sodium supplementation but no evidence has been shown to confirm this (D.J. Brightsmith, pers. comm.). In the present study we found genetic evidence that juvenile SCMAs returned to their fledging site and used the nearest clay lick (e.g. feathers of fledglings from the nests Amor & Franz were recovered at the nearest clay lick to the nests in the next year).

Our ability in this study to recover individual genotypes with 5–6 strategically chosen informative markers demonstrates the potential for population and individual-based genetic studies in macaws, which can help better understand their movements in subsequent analyses. We have previously observed at least four banded breeding pairs of SCMA returning to their nesting site in subsequent breeding seasons in TRC, often re-using the same nest hollows (G. Olah, pers. obs.). In this study we were able to confirm the re-use of the same nests for breeding around TRC by the genetic tagging analysis. Berkunsky and Reboreda (2009) also showed high nest fidelity of blue-fronted parrots, *Amazona aestiva*, based on observation of banded females. This behaviour of secondary cavity nesting parrots could reflect preferences for nests associated with better characteristics. SCMA has also been showed to prefer nesting in cavities (or artificial nests) with higher previous success (Chapter 6).

We compared blood versus feather samples, and samples sourced from/around nests versus samples from clay licks, and found no genetic differentiation between these groups. These findings further show that feathers can indeed be considered as representative samples of the local population. The microsatellite markers of this study were designed from the full genome sequence of SCMA (Seabury *et al.* 2013), hence we were able to select highly variable di-nucleotide repeats for SCMA that also showed variability for the closely related RGMA. However, the mean numbers of alleles, effective alleles, observed and expected heterozygosity (Table 8.1) were lower in RGMA (cogenic species) than in the focal species (SCMA), possibly due to ascertainment bias (Ellegren *et al.* 1997; Peakall *et al.* 1998). With these possible limitations in mind, the genetic tagging technique developed for these macaws will be widely applicable to other related species of higher conservation concern. In addition, for many threatened parrots the non-invasive genetic sampling of moulted feathers may be the only available DNA source and its use can also help to address the ethical concerns of catching wild individuals.

9. Application of genetic tagging in wild macaw populations

9.1. Introduction

A high proportion of the 398 extant species of parrots (28%) are classified as threatened (IUCN 2014). The major threats faced by these birds include habitat destruction and fragmentation, poaching, and invasive alien species (Owens and Bennett 2000; Pires 2012b). Most parrots are forest dependent secondary cavity nesters, hence forest destruction decreases the availability of nesting sites and therefore reproductive success, and it can also lead to the loss of key food resources (Brightsmith 2005c; Forshaw 2006). Stochastic factors in small and fragmented parrot populations can also cause extreme population decline, for example a hurricane caused significant reduction of the critically endangered Puerto Rican Parrot, *Amazona vittata* (Wunderle Jr. 1999). Small populations may also suffer loss of genetic diversity and inbreeding depression. The reduced viability of such small populations in the face of environmental changes could consequently drive them towards extinction (Frankham *et al.* 2004). These processes were identified in the last population of Spix's macaw, *Cyanopsitta spixii*, which is now extinct in the wild (Caparroz *et al.* 2001b).

The first step towards the conservation of any species in the wild is the acquisition of information about its biology. For example, knowledge of a species' home range, population size, and dispersal patterns can be crucial for management. Tracking individual birds by satellite telemetry tags is often used to obtain information about their home range, but this technique is still limited by the number of birds that can be tagged affordably and remains most feasible for larger species (Groom *et al.* 2014; Limiñana *et al.* 2015). Traditional methods for population size estimates require individual 'tagging' of the animals for capture-mark-recapture (CMR) methods. In populations of birds this is normally achieved by capturing individuals and tagging them with leg bands, but capture/recapture of the required number of individuals for a robust population size estimate is far from straight forward for many avian, amphibian, and fish monitoring studies (White and Burnham 1999; Pollock *et al.* 2002). Dispersal patterns hold important information about the study species but they often remain undetected without genetic tools, for example as calculated by isolation-by-distance and related methods to infer the extent of dispersal (Prugnolle and de Meeus 2002; Wright *et al.* 2005; Smith *et al.* 2016).

Genetic tagging, the unique identification of individuals by their DNA profile, has proven to be a highly effective method in molecular ecology, offering a powerful tool for 'tagging' whales (Palsboll *et al.* 1997), fishes (Sekino *et al.* 2005; Andreou *et al.* 2012), amphibians (Ringler *et al.* 2015), seals (Hoffman *et al.* 2006), bears (Woods *et al.* 1999), and small mammals (Peakall *et al.* 2006; Ruibal *et al.* 2010). DNA can now be sourced non-invasively even from footprints, as it was shown for an arctic fox (Dalén *et al.* 2007). In populations of birds, non-invasive sampling of feathers is a potential source of avian DNA (Horváth *et al.* 2005; Bush *et al.* 2011). Naturally shed feathers of eagles have been used to identify and monitor adult birds individually (Rudnick *et al.* 2005). Other studies using feathers of a large grouse species, *Tetrao urogallus* have demonstrated the value of genetic tagging for estimating

local population size (Segelbacher 2002; Jacob *et al.* 2009; Mollet *et al.* 2015). Taking advantage of the new high throughput sequencing techniques, feathers and blood samples have been used from Wilson's Warbler, *Cardellina pusilla* to monitor its migration patterns by SNP data (Ruegg *et al.* 2014). However, similar techniques have not been used to estimate population size or other demographic parameters in parrots.

Here we use non-invasive genetic tagging in combination with mark-recapture modelling to infer demographic information for the Red-and-green Macaw, *Ara chloropterus*. In the Peruvian Amazon, where our study was based, these birds are abundant and visit so called clay licks (or collpas) to consume soils rich in sodium (Brightsmith 2004; Powell *et al.* 2009). However, it is not known if these clay licks act as 'magnets', in effect drawing in birds from vast areas, or whether they simply facilitate feeding aggregations of local populations. Hundreds of naturally dropped feathers are left behind at clay licks, offering excellent opportunities to obtain non-invasive DNA samples (Gebhardt *et al.* 2009). In turn, genetic tagging from these samples potentially allows one to obtain species-specific information on clay lick use by individuals and populations that are not easily captured in large numbers.

In this chapter we use more than 200 genetic samples collected from feathers at clay licks to (1) test for genetic differentiation and possible isolation-by-distance among birds using different clay licks; (2) compare the proportions of males and females using the clay licks over time and possible relatedness among groups; (3) use non-invasive genetic tagging to gain information about the local movements of the macaws; and (4) estimate the number of birds visiting these clay licks.

9.2. Methods

9.2.1. Target species and study sites

The Red-and-green Macaw has a large range over South America from southern Panama to southeastern Paraguay. They mainly reside in subtropical and tropical moist lowland and montane rainforest from sea level to 1,000 m (Forshaw 2006) and nest in hollows of emergent trees (Brightsmith 2005c; Renton and Brightsmith 2009).

Our study area was situated in lowland rainforest of the southeastern Peruvian Amazon that receives an average annual rainfall of 3,200 mm (Brightsmith 2004). Our study area was distributed in both protected (Tambopata National Reserve, Bahuaja-Sonene National Park, Los Amigos Conservation Concession) and unprotected areas (Rio Madre de Dios, Rio Las Piedras; Figure 9.1). The areas with highest human populations in the region include Puerto Maldonado and other settlements along the Inter-oceanic highway (Baraloto *et al.* 2015).

9.2.2. Sample collection

Our study area falls within the region of South America with the highest known number of clay licks that are regularly used by animals (Lee *et al.* 2010), offering an opportunity to sample moulted feathers non-invasively and repeatedly. Furthermore, many aspects of clay lick ecology have been already studied in Tambopata including the distribution of clay licks (Lee *et al.* 2010), parrot behaviour at clay licks (Burger and Gochfeld 2003; Brightsmith and Villalobos 2011), clay lick preference (Powell *et al.* 2009), soil characteristics (Brightsmith and Munoz-Najar 2004; Brightsmith *et al.* 2008b), and the effect of climate on geophagy (the

intentional consumption of soil). However, we know very little about population sizes, sex ratio, and parrot density around clay licks.

In this study we collected a total of 249 moulted contour and flight feathers from seven major clay licks (Figure 9.1) that we visited systematically (every 2–4 weeks) during each breeding (rainy) season (November–April) between 2009 and 2012. Prior to the first collection of each season we cleared the feathers of unknown age from the clay licks in order to be more confident of the date range when the feathers were left behind during the collection season. Upon collection, we stored samples individually in paper envelopes in airtight boxes with silica gel to avoid further degradation (Chapter 8). As 20 parrot species are found in the study area, we provisionally identified the feathers from our target species in the field by shape and colour, and later the species was confirmed by genetic techniques in the lab (for more details see Chapter 8).

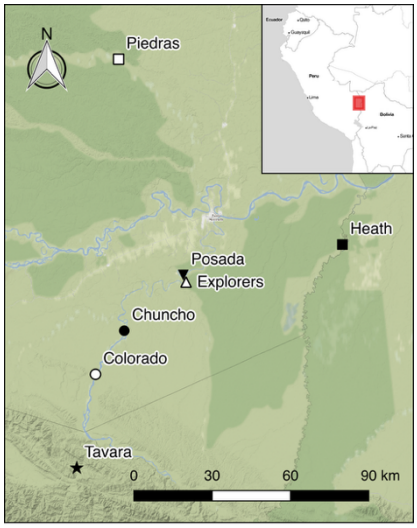

Figure 9.1. Sampling locations of moulted Red-and-green Macaw (*Ara chloropterus*) feathers at seven clay licks in southeastern Peru.

9.2.3. Genotyping for individual identification

For DNA extraction from feathers we used the Qiagen DNeasy Blood and Tissue kit (QIAGEN, California) following manufacturer instructions with modifications (Gebhardt *et al.* 2009). Based on the full genome of Scarlet Macaw, *Ara macao* (Seabury *et al.* 2013) we previously developed 30 highly variable di-nucleotide microsatellite loci for the same species and also for red-and-green macaws (Olah *et al.* 2015). We selected nine microsatellite markers (SCMA 09, SCMA 14, SCMA 22, SCMA 26, SCMA 30, SCMA 31, SCMA 32, SCMA 33, and SCMA 34) *a priori* out of our set of 30 markers, based on how well they performed (usually shorter in bp size) on more degraded DNA from feather samples (Chapter 8), and then constructed full genotypes. M13 PCR tags were attached to all forward primers and we amplified all loci individually (for more details see Chapter 8). We previously tested the nine markers for Hardy–Weinberg Equilibrium (HWE), null alleles, and for amplification failures and genotyping error rates (Chapter 8). The selected loci showed no indication of null alleles, 95% amplification success and low error rates (2.7% on average across all loci).

We also demonstrated the power of the nine loci used, with the estimates of probability of identity. Only six of these loci were required to recover unique genotypes in the sampled red-and-green macaw population ($PI_{sibs(6)} = 0.003$), hence the probability of finding exact matches

at nine loci among different individuals was extremely low. Furthermore, all of the genotype matches were manually checked locus by locus, as were all near genotype matches that differed at up to three loci. Finally, as a further check, we re-amplified all samples with full genotype matches.

9.2.4. Genetic differentiation among clay licks and isolation-by-distance

We used GenAlEx 6.502 (Peakall and Smouse 2006; Peakall and Smouse 2012) to perform the population genetic analyses, unless otherwise stated. To test for genetic differentiation among macaws using clay licks, we used the analysis of molecular variance (AMOVA) framework, with all duplicated genotypes excluded from this analysis. We defined seven 'populations' of samples *a priori*, corresponding to the seven clay licks (Tavara, Colorado, Chuncho, Explorers, Posada, Heath, and Piedras; Figure 9.1), and pooled all samples from a clay lick across the sampling intervals.

The AMOVA analysis provided estimates of overall and pairwise population genetic differentiations (F_{ST}) (Wright 1965), and their standardized [0,1] equivalents (Meirmans 2006; Meirmans and Hedrick 2011; Peakall and Smouse 2012). We performed tests for departure from the null hypothesis of no genetic differentiation by using 10,000 random permutations, and interpolated loci with missing data within each population (Peakall and Smouse 2006). We also tested isolation-by-distance across the study site by using a Mantel test (Mantel 1967) with departure from the null hypothesis of no significant relationship between genetic and geographical distances tested by 10,000 random permutations at the individual level (Smouse *et al.* 1986; Smouse and Long 1992).

9.2.5. Sex ratios and relatedness of individuals at clay licks

Molecular sexing of samples was conducted using the P8_SCMA_F/P2_SCMA_R primers, as part of the genotyping runs (Chapter 8). A G-test was performed to compare the proportion of males and females across clay licks (Peakall and Smouse 2012).

It is possible that family groups may use the same clay licks. Therefore, to test for this possibility we calculated *r* pairwise relatedness estimates (Lynch and Ritland 1999) for each pair of individuals and also the mean pairwise relatedness for each clay lick with 95% confidence intervals around the mean pairwise *r* values estimated via bootstrapping (Beck *et al.* 2008). We also used random permutation of the data set to generate a distribution for the null hypothesis of no relatedness among individuals within groups and to provide a test for significance. All bootstrapping and permutation tests were performed 1,000 times. We presented the means for the pairwise relatedness estimates and included a control group of 20 individuals from seven known family groups (nests), which were previously genotyped (Olah *et al.* 2015).

9.2.6. Genetic recaptures and population size estimates at clay licks

In our dataset we treated each genotype as a 'capture' and when an identical genotype was found we considered it a 'recapture'. By definition, the minimum time between sampling events was 14 days. If two sampling events at the same clay lick occurred within this time frame we considered them as a single sampling event. We derived estimates of population sizes at clay licks (the number of individuals visiting the same clay lick) using two capture-mark-

recapture (CMR) modelling approaches. As genetic recaptures occurred at various clay licks in different seasons, we used subsets of our genetic data for best fitting the assumptions of each model.

We used the program CAPWIRE (Miller *et al.* 2005) that allows the use of multiple detections of individuals within a single sampling session and accounts for heterogeneous feather moulting patterns among individuals. This way CAPWIRE was able to incorporate recapture events that occurred in the same sampling session while also modelling capture heterogeneity. We summarized each dataset into the total number of observations made for each individual over the sampling period (e.g., also used the total number of feathers without recaptures) and these datasets were fitted using two model types. The (1) Two Innate Rates Models (TIRMs) view populations as a mixture of two types of individuals, the seldom caught type and the often-caught type. This model type is used to estimate the probability that an individual belongs to a mixture and assigns individuals to a type. In the (2) Equal Capture Probability Model (ECM) all individuals are equally likely to be captured. A likelihood ratio test (LRT) was used to determine the most appropriate model and 95% confidence intervals about the mean were estimated with 1,000 bootstrap replicates. We used CAPWIRE to estimate the number of birds using the Posada and Explorers clay licks (pooled together as Lower Tambopata complex because of their close proximity), and the Chuncho clay lick, using pooled data over the whole study period. These sites were selected to take advantage of their high recapture events even within single sessions over the whole study period. It has been shown by simulations that population size estimates based on single-session sampling (e.g., in CAPWIRE) are as reliable as other estimates (e.g., MARK) based on multisession sampling (Petit and Valiere 2006; Puechmaille and Petit 2007).

We also used conventional closed CMR models (null M_0, temporal M_t, behavioural M_b, and heterogeneity M_h) in the program MARK 8.0 (White and Burnham 1999). Closed population models were selected *a priori* because the fundamental assumptions of demographic (births and deaths) and geographic (migration in or out) closure were reasonable for this species. We only used this model to estimate the population size at the Lower Tambopata complex, as we found the highest number of recaptures here from different sessions but within a single closed period, providing sufficient data for this analysis. Here we used data from one breeding season (December 2010–April 2011) to avoid the violation of the assumptions of closure. Deaths are likely to be minimal during this interval in this long living species (>50 years) (Brouwer *et al.* 2000). Migration was assumed to be negligible within the 5-month interval consistent with observations that individuals are using the same areas for nesting every year (D. J. Brightsmith, pers. obs.).

9.3. Results

9.3.1. Genetic differentiation among clay licks and isolation-by-distance

Across all clay licks we found that the allele number ranged from 9 to 17 per locus and the overall mean expected heterozygosity was 0.725. The observed heterozygosity values per clay lick ranged from 0.666 to 0.747 (Table 9.1). The AMOVA test attributed 1% of the variation among the seven clay licks ($F_{ST} = 0.006$, $F'_{ST} = 0.025$, $P = 0.004$). This result indicates that there were small but statistically significant genetic differences among the birds visiting these

clay licks (Table 9.1). The pairwise comparisons of F_{ST} values of clay licks showed that the Tavara and Heath clay licks were the most significantly different from other clay licks, while the Colorado and Posada clay licks were not significantly different from any others (see Table S1 in Olah *et al.* 2017a). At the individual genotype level, we found low but significant isolation-by-distance across the whole study area (Mantel test >160 km, N = 221, $r = 0.089$, $P = 0.009$).

Table 9.1. Genetic variation of Red-and-green Macaws (*Ara chloropterus*) at seven clay licks in southeastern Peru, based on nine polymorphic microsatellite loci. Number of feathers sampled ($N_{feather}$), number of unique genotypes (N), number of females (N_{Female}), males (N_{Male}), and unknown sex (N_u), number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F), population size estimates (number of individuals and 95% CI) by CAPWIRE and MARK are given. Clay licks are arranged from south to north.

Clay lick	$N_{feather}$	N	N_{Female}	N_{Male}	N_u	Na	Ne	H_O	H_E	F	*CAPWIRE*	*MARK*
Tavara	23	22	8	10	4	7.44±0.72	4.12±0.47	0.711±0.035* (1)	0.731±0.031	0.02±0.02	–	–
Colorado	13	10	2	8	–	5.88±0.56	3.47±0.61	0.688±0.061* (1)	0.652±0.044	−0.0±0.06	–	–
Chuncho	120	114	41	69	4	10.1±0.77	4.85±0.74	0.73±0.041* (1)	0.749±0.04	0.02±0.02	316 (221–486)	–
Explorers	30	18	6	9	3	6.55±0.70	4.43±0.63	0.666±0.067* (1)	0.719±0.052	0.06±0.07		
Posada	15	12	6	6	–	6.44±0.64	4.06±0.38	0.747±0.047	0.734±0.027	−0.0±0.05	89 (52–122)	84 (47–202)
Heath	23	23	7	14	2	7.55±0.91	4.44±0.48	0.685±0.047	0.75±0.029	0.08±0.04	–	–
Piedras	25	22	7	13	2	8.22±0.83	4.40±0.57	0.716±0.043* (1)	0.74±0.035	0.03±0.03	–	–
Total	249	221	77	129	15	7.46±0.31	4.25±0.21	0.706±0.018	0.725±0.014	0.02±0.01		

* Significant ($P < 0.05$) departure from HWE, with the number of loci given in parentheses.

9.3.2. Sex ratios and relatedness of individuals at clay licks

We identified 129 males and 77 females (15 samples could not be sex typed) with unique genotypes (Table 9.1). In total we found an overall significant bias towards males when the samples from all clay licks were pooled ($N_{males} = 129$, $N_{females} = 77$, $G = 13.27$, $df = 1$, $P < 0.001$). However, we detected no significant differences in sex ratios when each clay lick was considered individually ($G = 2.83$, $df = 6$, $P = 0.83$). The mean pairwise relatedness estimates did not indicate higher than expected relatedness at any clay licks, except in the control group consisting of known related individuals (Figure 9.2).

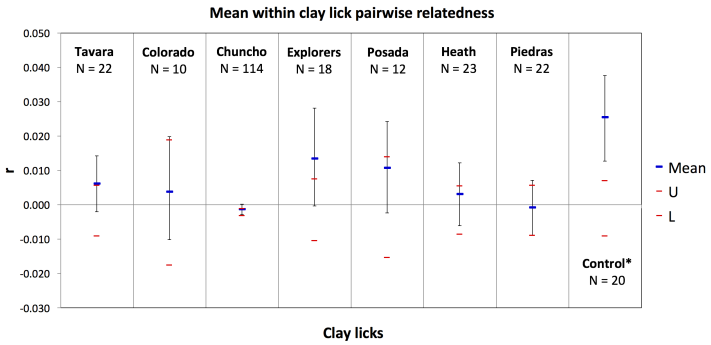

Figure 9.2. Pairwise relatedness estimates of Lynch and Ritland (1999) among clay licks of Red-and-green Macaw (*Ara chloropterus*). *Red lines* represent permuted 95% confidence intervals—upper (U) and lower (L)—around the null hypothesis of zero relatedness and error bars represent bootstrapped confidence intervals around the mean. * Control group includes known related individuals.

9.3.3. Genetic recaptures by non-invasive genetic tagging

Out of a total of 249 feather samples collected from clay licks we found 21 matching genotypes (Figure 9.3). There were 16 cases with 2 matching feathers, 3 cases with 3 matching feathers, and a further 2 cases with 4 matching feathers, resulting in 221 unique genotypes in total. 12 of the 21 genotype matches occurred in the same location and the same sampling event within the 2–4-week window (8 males and 4 females). The other nine genotype matches (six males and three females) represented recaptures between different sampling events or different clay licks (Figure 9.3).

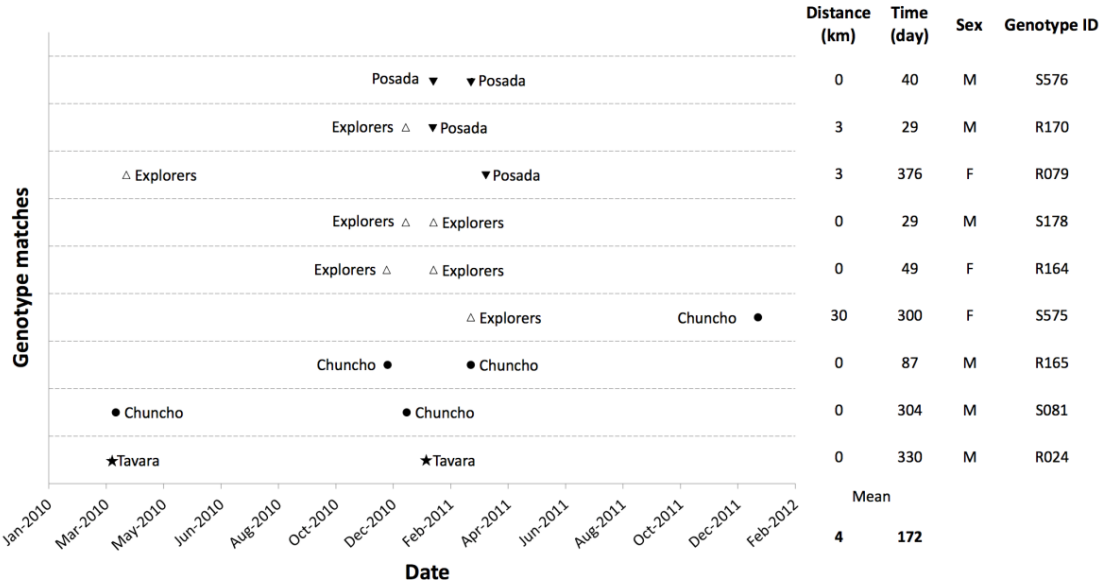

Figure 9.3. Complete genotype matches of Red-and-green Macaws (*Ara chloropterus*) in Tambopata, Peru showing recoveries in different sampling events, the distance (km) and the time interval (day) between recaptures, the sex of the bird, and genotype ID. Only those nine genotype matches are indicated that were recovered in a different location or at a different time. Symbols of the clay licks correspond to Figure 9.1.

All 21 samples were re-amplified to confirm repeatability with 19 of 21 samples giving a perfect match across all nine loci. One sample showed a one allele mismatch at two loci and another sample at one locus. The choice of nine loci therefore allowed the matching of genotypes, even allowing for some errors.

9.3.4. Population size estimates at clay licks

The temporal model (M_t) gave the most parsimonious model in MARK as determined by the Akaike information criterion (AIC). This model estimated the population size of the Lower Tambopata complex (Posada + Explorers) to be 84 individuals (95% CI: 47–202 birds; Table 9.1). For the same area CAPWIRE estimated 89 individuals (95% CI: 52–122 birds; Table 9.1). For the much larger Chuncho clay lick the CAPWIRE program provided an estimate of 316 individuals (95% CI: 221–486 birds; Table 9.1).

9.4. Discussion

In this study we used 249 red-and-green macaw feather genotypes collected over three years along more than 1,000 km of riparian habitat in the Peruvian Amazon. Using nine hypervariable microsatellite genetic markers we were able to detect 21 duplicated genotypes in the landscape. These genetic data provided novel information about the clay lick use of the enigmatic red-and-green macaw. Below we evaluate our findings, compare the outcomes with the literature and conclude with consideration of the implications for conservation.

9.4.1. Genetic differentiation among clay licks and isolation-by-distance

We found small but significant genetic differences among the seven clay licks studied. This may indicate that red-and-green macaws are not drawn completely randomly from the population when supplementing their mineral intake at clay licks. However, the AMOVA revealed only low genetic differentiation (1%) over the whole study area. These findings are broadly consistent with two other genetic studies also based on microsatellite markers of macaws but with much lower sample sizes. Marques (2010) reported genetic diversity estimates of red-and-green macaw in Brazil based on six microsatellite loci and 84 samples (H_O ranging from 0.483 to 0.902), low genetic structure (AMOVA: N = 84, 1.6% among populations, $\Phi_{ST} = 0.016$, $P = 0.120$) with possibly high gene flow. Schmidt (2013) described 0.12% of genetic variation among core breeding sites of scarlet macaw, *Ara macao cyanoptera*, in Guatemala up to 35 km apart ($F_{ST} < 0.009$, $P > 0.05$). Our population genetic results also indicated individual genotype level isolation-by-distance across large geographical scales, which could indicate some restriction on dispersal over this large scale (>160 km), or high levels of philopatry for this species.

9.4.2. Sex ratios and relatedness of individuals at clay licks

Our results suggest an overall bias towards the detection of males. Feathers were collected explicitly in the breeding season of the macaws, when females incubate the eggs and care for hatchlings, while males search for food (Nycander *et al.* 1995). This might explain the overrepresentation of males at clay licks during this period. Further support for this is provided by the observation that at clay licks we found twice as many genotype recaptures of males than females (Figure 9.3). We did not find higher than average relatedness among birds at clay licks (Figure 9.2), which indicates that family groups might visit several different clay licks. This also supports the applicability of non-invasive genetic sampling at clay licks, as it gives a good representation of the whole population and not just groups of relatives.

9.4.3. Genetic recaptures by non-invasive genetic tagging

While the low number of recaptures might not be sufficient for precise population size estimates, some interesting results were nevertheless revealed from the data. The longest period between recaptures was 376 days, and the longest distance was 30 km, with a mean distance of 4 km. Interestingly, many of the matches occurred in the same sampling event. This result could be due to the simple process of multiple shedding of feathers at the same location and time. Alternatively, as macaws in the tropics seem to shed continuously over the year (D. J. Brightsmith, pers. obs.) it more likely indicates that the same birds make multiple visits to the clay lick during our 2–4-week sampling intervals. At a nearby clay lick in the Manu Biosphere

Reserve, close-up photographs of red-and-green macaws were used to identify them by the lines of red facial feathers and beak shape (Munn 1992). Within a month period they found that 40–60% of the photos taken in different days showed same individuals.

Our genotype data confirmed and refined previously known or suspected aspects of red-and-green macaw natural history. For example, we confirmed that the macaws re-used the same clay licks (e.g. Posada, Explorers, Chuncho, Tavara), sometimes even over long periods (e.g., 330 days at Tavara clay lick; Figure 9.3). Red-and-green macaws often reuse the same nests over many years (D. J. Brightsmith, pers. obs.), so it was assumed that they would re-use the nearby clay licks too. We repeatedly recovered feathers from 20 red-and-green macaw individuals between the same or nearby clay licks within a three km range (Posada & Explorers) and once between clay licks 30 km apart (Explores & Chuncho). The lack of longer-range recaptures suggests that the macaws at clay licks are drawn from local populations. Recaptures varied from within a month up to a year (Figure 9.3).

9.4.4. Population size estimates at clay licks

Estimating population size of species in remote areas can be challenging. Capturing and later re-capturing or sighting uniquely identified macaws in a large tropical landscape is a difficult task and has not been attempted for macaws before. In our study we used individual genotypes of feathers and samples with matching genotypes to test their efficacy for CMR based population size estimates. We acknowledge that these population estimates are for the numbers of macaws visiting clay licks, which may not equate to population size estimates. Furthermore, given the very low recapture rate in our study, we only used CMR models for two clay licks with the highest number of recaptures in our dataset.

The genetic CMR methods we used provide some of the first estimates of the number of macaws using clay licks over the breeding season. Our 1-month observational data from the Chuncho clay lick with a maximum number of 109 red-and-green macaws observed in one event seems to support the magnitude of our estimate. In general, our population estimates of the number of birds using a clay lick are similar to those in Manu near Tambopata. Here photographic IDs over a month interval yielded estimates of the total number of red-and-green macaws using that clay lick falling between 241 and 282 individuals (Munn 1992).

9.4.5. Future recommendations for non-invasive genetic tagging at clay licks

The amount of information gained from feather samples seems remarkable given the tropical conditions and high likelihood that DNA will degrade quickly. However, to maximize the efficacy of genetic tagging we recommend the following sampling strategy at clay licks: (a) a pilot study should be conducted to determine the most reliable locations for feathers; (b) the focus should be on only a few sampling locations with the highest number of available samples; (c) feathers should be collected on a regular basis (e.g., every week); (d) detailed location of feathers on clay licks should be recorded to be able to differentiate depositing events of feathers with matching genotypes; (e) collected feathers should be handled as recommended by Chapter 8; and (f) a concentrated sampling effort at the most frequently used clay licks is highly desirable in order to improve population size estimates of the area.

9.4.6. Conclusion

Prior to this study we had limited insights into how red-and-green macaws use the clay licks. Here we found no evidence that red-and-green macaw communities at different clay licks consisted of related individuals. The matching genotypes detected show that individual macaws can use multiple clay licks, and that macaw communities using various clay licks can overlap in space. However, the locations of the genotype recaptures despite sampling over a large study area were highly restricted, indicating that local movements might occur more frequently. Our estimates of the number of different individuals indicate that hundreds but not thousands of macaws locally use clay licks, leading us to conclude that these communities probably represent feeding aggregations drawn from local populations. Thus, with sufficient sampling this technique can be used for population size estimates, especially for species and locations where large number of non-invasive samples are easily available. The distribution of parrot clay licks in South America supports the hypothesis of geophagy due to sodium deficient natural diet (Lee *et al.* 2010). Parrot geophagy has also been reported in Central America (Valdés-Peña *et al.* 2008), Africa (May 2001), and Papua New Guinea (Symes *et al.* 2005). These sites may offer similar sampling opportunities to our study, suggesting that the same genetic tagging techniques could be used to study clay lick use and population sizes of other species.

10. Landscape genetics as a tool to study scarlet macaw biogeography

10.1. Introduction

Dispersal is an important ecological process as it allows individuals to locate new resources, avoid competition, and avoid inbreeding depression. On a larger scale, dispersal influences spatial population dynamics, maintains genetic diversity and enables adaptation (Clobert *et al.* 2012; McDougald *et al.* 2012; Szövényi *et al.* 2012; Orsini *et al.* 2013; Smith *et al.* 2016). Flight ability is assumed to be a good proxy for dispersal (Kokko and López-Sepulcre 2006) as it often correlates with range size (Böhning-Gaese *et al.* 2006), but understanding what influences dispersal in flying species at a landscape scale is important. Both natural and man-made landscape features can impede gene flow, with roads, rivers, or mountain ridges being potentially impenetrable barriers for some species (Storfer *et al.* 2007). Direct tracking to infer dispersal across these structures is notoriously difficult (Schofield *et al.* 2013) but landscape genetics can help elucidate relationships between landscape features and gene flow (Manel *et al.* 2003; Storfer *et al.* 2007; Manel and Holderegger 2013), and identify potential dispersal barriers (Andrew *et al.* 2012).

The Amazon basin is a highly diverse and globally important ecosystem which is undergoing rapid changes such as continual development of highways and gold mining exploration at an enormous scale (Beheregaray and Caccone 2007; Asner *et al.* 2013; Baraloto *et al.* 2015; Keenan *et al.* 2015). To understand the effect of these changes on Amazonian fauna, we need baseline data about their biology and ecology in continuous, natural habitat. Macaws have been studied for decades in their natural habitat in south-eastern Peru, providing insights about their breeding biology (Brightsmith 2005c; Vigo *et al.* 2011; Olah *et al.* 2014), foraging ecology (Brightsmith 2004), parasitology (Olah *et al.* 2013), population dynamics (Lee and Marsden 2012), and conservation (Brightsmith *et al.* 2005). Nevertheless, knowledge about how natural landscape features affect their home-range and dispersal is still lacking.

Recent studies have shown contrasting results about genetic structure in macaws. For example, some studies found very low genetic differentiation among populations in continuous rainforest at scales of 100 km distance (Table 10.1), which might be expected given their large size and strong flying ability. Other studies, however, showed significant genetic differentiation between populations separated by only 80–170 km (Table 10.1). Unfortunately, these studies did not explicitly include spatial information in their genetic analyses and they had limited sample sizes mainly from nesting birds, limiting insights into the mechanisms behind these contrasting patterns. More progress can be made in understanding dispersal at landscape scales if spatial information about relevant habitat features is incorporated into landscape genetic analyses and more extensive, non-invasive samples are analysed from a wide geographic scale (Andrew *et al.* 2013).

In the Peruvian Amazon, the preferred habitat of scarlet macaws (*Ara macao*) is considered to be lowland primary rainforest, consistent with the general pattern of macaw distribution noted by Forshaw (2006) to be up to 500 m above sea level (asl). Within our study site, the foothills of the Andes Mountains rise to over 1,000 m asl and thus might act as natural barriers

to their dispersal. Scarlet macaws also prefer riverside rainforest habitats with emergent trees so they can access the canopy from the side (Britt *et al.* 2014), and habitat suitability can influence gene flow (Wang *et al.* 2008). In this study we used population genetic structure analysis and landscape resistance modelling to test whether elevation, canopy height, river system and above-ground carbon distribution (biomass) might influence dispersal in macaws.

Table 10.1. Previous population genetic studies of large macaws with varying scale and habitat among populations.

Species	Countries	Comparison	Distance (km)	Habitat between populations	*N*	# Genetic markers	F_{ST}	*P*	References
Scarlet macaw (*Ara macao cyanoptera*)	Costa Rica	Two populations	80	Montane areas to the coast of the Pacific, roads	41; 55	7	0.048	**<0.001**	Monge et al. (2016)
	Guatemala and Belize	Two populations	170	Heavily degraded landscape (roads, agriculture)	13; 61	11	0.2	**<0.001**	Schmidt (2013)
	Guatemala	Among three focal breeding areas	<35	Primary rainforest	7; 8; 16	11	<0.009	>0.05	Schmidt (2013)
Hyacinth macaw (*Anodorhynchus hyacinthinus*)	Brazil	Two populations	100	Cerrado habitat	16; 16	4	0.05	0.12	Faria et al. (2008)
	Brazil	Two pairwise populations	1600	Heavily degraded landscape (roads, agriculture, cities)	6; 16; 16	4	>0.25	**<0.001**	Faria et al. (2008)

10.2. Methods

10.2.1. Study site and target species

This study was conducted in the Tambopata/Candamo region of the south-eastern Peruvian Amazon, including two large protected areas: (1) the Tambopata National Reserve (13°8′S, 69°37′W; 2747 km^2) and (2) the Bahuaja-Sonene National Park (13°30′S, 69°47′W; 10,914 km^2). The area is tropical moist forest receiving an average annual rainfall of 3236 mm (Brightsmith 2004). Elevation gradually increases from the Lower Tambopata (lowland rainforest with average elevation of 200 m), to Upper Tambopata (lowland rainforest closer to the foothills of the Andes Mountains, 260 m), and Candamo (intermountain valley, 350 m) surrounded by foothills of the Andes (up to 1,300 m) that separate this region from Tambopata (Figure 10.1).

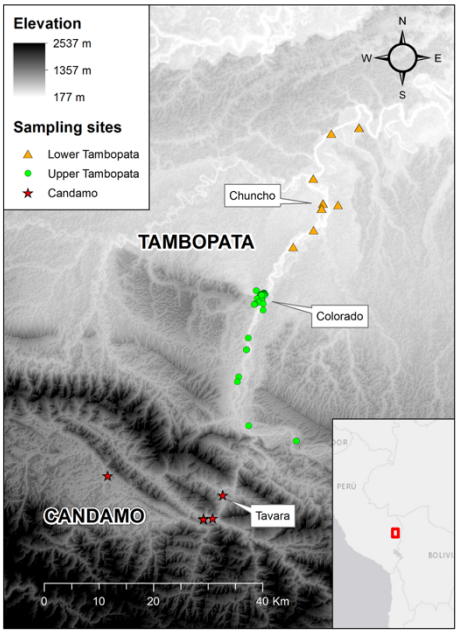

Figure 10.1. Locations of Scarlet macaw (*Ara macao*) DNA samples in the foothills of the Andes, south-eastern Peru. Sampling sites were located around three major clay licks: Chuncho (N = 26), Colorado (N = 35), and Tavara (N = 19).

The distribution of the scarlet macaw extends from Mexico to Bolivia (BirdLife International and NatureServe 2014). Although population sizes are decreasing in some regions, they are presently listed as Least Concern with an estimated global population size between 20,000 and 50,000 individuals (IUCN 2014). They are secondary cavity nesters using hollows of emergent trees (Brightsmith 2005c; Renton and Brightsmith 2009) and also occupy artificial nests in our study site (Chapter 6).

10.2.2. Sample collection and genetic markers

A total of 166 DNA samples was collected during the breeding season (November–April) each year between 2009 and 2012. We collected 126 DNA samples non-invasively by sampling naturally shed feathers from nests, around nesting and roosting sites, and from clay licks (Figure 10.1). We collected about 100 µL of blood from the jugular vein of 22 macaw nestlings and 18 adults in natural and artificial nests, accessed by using single-rope ascending techniques (Chapter 6). Blood samples were stored in 70% ethanol or on FTA paper (Whatman). When blood samples from known family units were identified, siblings or parent/offspring samples were excluded from the analyses (keeping samples of one parent whenever possible), so that only non-related individuals were included in the data set.

DNA was amplified at nine species-specific, polymorphic microsatellite markers: SCMA 09, SCMA 14, SCMA 22, SCMA 26, SCMA 30, SCMA 31, SCMA 32, SCMA 33, SCMA 34 (Olah *et al.* 2015). Genotyping errors were calculated from randomly selected samples that yielded full genotype data, and the markers selected in this study showed low or no error and low amplification failure (Chapter 8). Molecular sexing of individuals was performed using the P8_SCMA_F/P2_SCMA_R primers (Chapter 8).

10.2.3. Population-level genetic differentiation

We divided the study area into three sampling sites based on geographic location: Lower Tambopata (N = 54), Upper Tambopata (N = 82), and Candamo (N = 30), which were mainly aggregated around large clay licks (Figure 10.1). We used analysis of molecular variance (AMOVA) in GenAlEx 6.5 (Peakall and Smouse 2006; Peakall and Smouse 2012) to partition genetic variation within and among these sampling sites and to estimate overall and pairwise population genetic differentiation (F_{ST}) (Wright 1965; Excoffier *et al.* 1992; Peakall *et al.* 1995). Tests for genetic differentiation were performed by 1000 random permutations. In order to assess if there was any bias in F_{ST} estimation given the uneven sample size, we performed AMOVA on randomly subsampled sets of 30 samples from each site using the 'shuffle' function of GenAlEx. The means and standard errors were calculated across 10 replicates.

10.2.4. Individual-level population structure analysis

We used two different Markov chain Monte Carlo (MCMC) Bayesian clustering models to identify potential population genetic structure. The first was STRUCTURE 2.3.4 (Pritchard *et al.* 2000) for which we used the admixture model, correlated allele frequencies, and no location priors (Falush *et al.* 2003). Burn-in was set to 50,000 iterations, followed by 50,000 MCMC iterations and replicated 10 times for each value of the number of genetic clusters (K) from 1 to 5. We used STRUCTURE Harvester (Earl and vonHoldt 2012) to determine K (Evanno *et al.* 2005). The second analysis was conducted in GENELAND 4.0.0 (Guillot *et al.* 2005) which

includes geographical coordinates for each individual. This inclusion makes it more sensitive to weak genetic structure than the STRUCTURE analysis because spatially adjacent individuals are more likely to be in the same cluster (Guillot *et al.* 2012). We used the uncorrelated allele frequency model (Guillot *et al.* 2005) with 500,000 MCMC repetitions (saving every 100th iteration), and allowed *K* to vary between 1 and 5, with 5 independent runs. We set spatial uncertainty of coordinates to 10 km based on estimated daily movements (Munn 1992). We also used individual multilocus spatial autocorrelation analysis for each sex separately (Smouse and Peakall 1999; Peakall *et al.* 2003) to investigate potential sex-biased dispersal.

10.2.5. Individual-level landscape resistance analysis

We conducted landscape resistance analyses at two scales: (1) across the whole study area including all individuals (N = 166) and (2) across a subset of the data (N = 112) focusing on Upper Tambopata and Candamo. The latter, finer-scale analysis allowed us to specifically examine if gene flow was restricted by the foothills of the Andes and this is the main geographic barrier separating these two areas.

We developed five landscape resistance models to examine whether topography and vegetation affected macaw gene flow. The models were based on maps derived from the Carnegie Airborne Observatory (http://cao.carnegiescience.edu) using high-resolution radar and LiDAR (light detection and ranging) mapping technologies (Asner *et al.* 2012; Asner *et al.* 2014). Each was compiled on a separate raster grid with a 100 m (1 ha) resolution in ARCMAP 10.2 (ESRI). (1) The isolation-by-distance model (IBD) was a null model where each cell was given a value of one to investigate the effect of distance alone (Cushman *et al.* 2006). (2) The elevation model (Elev), based on a digital elevation model (meters above sea level), investigated the effect of topography on gene flow. (3) The tree canopy height (TCH) model considered the distribution of emergent canopy trees which are used by macaws as nests. This landscape feature might influence gene flow through an effect of habitat suitability [e.g., lower or higher gene flow in areas of preferred habitat (Smith *et al.* 2016)]. (4) The above-ground carbon distribution (ACD) model, representing biomass (Girardin *et al.* 2010) was used as a proxy for habitat complexity. Scarlet macaws in tropical rainforest prefer riverside habitats with gaps in the canopy that contain lower biomass (Britt *et al.* 2014). (5) The river distance model (Rio) was used to determine if the preferred riverside habitat of scarlet macaws acted as a dispersal corridor. This model was developed with a 500 m buffer zone at each side along the river system, with values of '1' given to the cells of the grid within this buffer zone and '100' outside of this zone, representing floodplain versus *terra firme* areas.

We used CIRCUITSCAPE 4.0.3 to generate landscape resistance values between each pair of individuals, taking into account all possible pathways (McRae and Beier 2007). To test the effect of the landscape on genetic distance, we used two different methods: Mantel and partial Mantel tests in a causal modelling framework (Cushman *et al.* 2006), and multiple regression of distance matrices (MRDM) (Legendre *et al.* 1994). These methods were implemented in the 'ecodist' library (Goslee and Urban 2007) for R (R Core Team 2013) with *P*-values based on 5,000 permutations.

Causal modelling began with simple Mantel tests of IBD and all other resistance models. We then conducted partial Mantel tests to assess the significance of relationships between

genetic distance and landscape resistance, given the spatial distance between samples (Partial 1). We assessed significance using one-tailed *P*-values for simple Mantel tests ($\alpha = 0.05$) and two-tailed *P*-values for partial Mantel tests (Goslee and Urban 2007). Elevation was an exception to this, as we predicted resistance would increase with elevation and thus report a one-tailed *P*-value for that model. When there was a significant correlation in the first partial Mantel test, we calculated the effect of the IBD model on genetic distance while controlling for the landscape resistance model (Partial 2). Where Partial 1 was significant and Partial 2 was nonsignificant, we inferred significant effects of the landscape resistance model on genetic distance, beyond the effects of geographic distance (Cushman *et al.* 2006; Smith *et al.* 2014). For MRDM, we analysed the IBD model separately and then together with each other resistance model. Thus, these models included one predictor for IBD and a maximum of two predictor variables for all other models (Smith *et al.* 2016).

We generated cumulative current maps for every pair of samples with CIRCUITSCAPE to identify areas which contribute most to connectivity between sample sites (McRae *et al.* 2013). Maps were visualized in QGIS 2.14 (https://www.qgis.org/).

10.3. Results

10.3.1. Population genetic analyses

Among the 166 scarlet macaws in our data set, the mean allele number was 15.7 and mean expected heterozygosity was 0.884 (see Table S1 in Olah *et al.* 2017c). We identified 74 males and 69 females (23 unknown) using the sexing markers. STRUCTURE and GENELAND indicated a single genetic cluster and lack of population boundaries among all individuals at both a large- and small scale. The AMOVA analysis revealed a low but significant level of genetic differentiation among the three populations ($F_{ST} = 0.005$, $P = 0.001$). The Candamo population was significantly different from the other two populations (Lower Tambopata $F_{ST} = 0.008$, $P = 0.003$; Upper Tambopata $F_{ST} = 0.008$, $P = 0.001$), driving the overall significant differentiation. Upper and Lower Tambopata populations were not significantly different ($F_{ST} = 0.001$, $P = 0.199$).

The replicated AMOVA analysis at equal sample sizes ($N = 30$) across the three populations showed very similar results (mean $F_{ST} = 0.005$, $SE = 0.0005$, 10 replicates) indicating little or no bias in estimation due to the sample size variation. The replicated pairwise population comparisons also showed similar results to the full analysis for Candamo versus Lower Tambopata (mean $F_{ST} = 0.007$, $SE = 0.0007$, 10 replicates), Candamo versus Upper Tambopata (mean $F_{ST} = 0.008$, $SE = 0.001$, 10 replicates), and Upper- versus Lower Tambopata (mean $F_{ST} = 0.002$, $SE = 0.0004$, 10 replicates).

No significant patterns of spatial genetic structure were detected by spatial autocorrelation analysis of individual-by-individual genetic distance when females and males were analysed separately, thus there was no evidence of sex-biased dispersal.

10.3.2. Landscape resistance models

There were no significant effects of the landscape resistance models at the large scale (whole study area). However, at the small scale, we found a significant effect of elevation on genetic distance between Candamo and Upper Tambopata ($r_M = 0.128$, $P = 0.02$; Table 10.2;

Figure 10.2). Both isolation-by-distance and elevation (max. 1200 m) were significant explanatories of the genetic distance in a simple Mantel test and in the MRDM analysis (Mantel $P_{IBD} = 0.021$, $P_{Elev} = 0.01$; MRDM $P_{IBD} = 0.031$, $P_{Elev} = 0.021$; Table 10.2; Figure 10.2). Isolation-by-distance was not significant when controlling for elevation in the partial Mantel test ($r_M = -0.104$, $P = 0.98$; Table 10.2), indicating that elevation, not geographic distance on its own, influences genetic distance between individuals.

Table 10.2. The effect of geographic distance (IBD) and landscape resistances (ACD, Elev, TCH, Rio) on genetic distance in Scarlet Macaw (*Ara macao*) using causal modelling and multiple regression of distance matrices (MRDM). For partial Mantel tests, genetic distance (y) was modelled as a function of x given z (y ~ x|z). Significant effects are shown in bold. All regression analyses included IBD either alone or with one other spatial predictor variable.

Scale of analysis	Mantel test type	Predictor	Mantel test		MRDM	
			r_M	P	R^2	P
Large scale (N = 166) three populations	Simple	IBD	0.023	0.201	0.001	0.385
	Simple	ACD	0.022	0.548	0.001	0.833
	Simple	Elev	0.054	0.088	0.007	0.148
	Simple	TCH	0.024	0.474	0.001	0.826
	Simple	Rio	−0.023	0.713	0.001	0.667
	Partial 1	ACD|IBD	0.004	0.944		
	Partial 1	Elev|IBD	0.080	0.063		
	Partial 1	TCH|IBD	0.006	0.914		
	Partial 1	Rio|IBD	−0.029	0.632		
	Partial 2	IBD|Elev	−0.063	0.914		
Small scale (N = 112) two populations	Simple	**IBD**	**0.080**	**0.021**	**0.006**	**0.031**
	Simple	ACD	0.064	0.144	0.007	0.280
	Simple	**Elev**	**0.110**	**0.010**	**0.023**	**0.021**
	Simple	TCH	0.071	0.080	0.007	0.276
	Simple	Rio	−0.009	0.889	0.007	0.263
	Partial 1	ACD|IBD	−0.031	0.640		
	Partial 1	**Elev|IBD**	**0.128**	**0.020**		
	Partial 1	TCH|IBD	−0.027	0.681		
	Partial 1	Rio|IBD	−0.033	0.632		
	Partial 2	IBD|Elev	−0.104	0.980		

IBD: solation by distance, ACD: aboveground carbon distribution, Elev: elevation above sea level, TCH: tree canopy height, Rio: river distance.

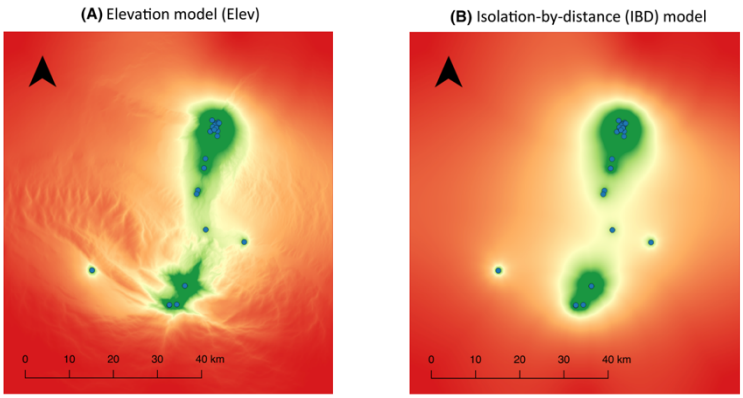

Figure 10.2. Cumulative resistance maps of CIRCUITSCAPE models for Scarlet Macaws (*Ara macao*) in the Peruvian Amazon. Colours indicate the predicted areas of conductance (green) and resistance (red) among the samples (blue circles). The analysis was based on samples from Candamo and Upper Tambopata sites.

10.4. Discussion

To our knowledge, this is the first study to combine population genetic analysis with spatially-explicit habitat information in a landscape genetic study of parrots. Our study in the Amazon rainforest habitat of scarlet macaws, where no high mountain ranges exist, suggested extensive gene flow and high dispersal ability. Nevertheless, we found evidence for some restriction of their gene flow in the foothills of the Andes Mountains indicating that high mountain ranges can impose dispersal limitations to macaws. Given the large and rapid changes occurring in the Amazon, our results provide a valuable baseline from which to compare future studies of global change in this region.

10.4.1. Isolation-by-elevation at the Andean foothills

Both of our landscape genetics analysis methods (causal modelling and MDRM) indicated significant isolation-by-elevation between two populations of macaws separated by only 20 km of mountain ridges over 1,000 m in the Andean foothills. This suggests that elevation can form a natural dispersal barrier, influencing gene flow even in large, strong flying species.

Our results about the genetic differentiation in Candamo (Table 10.2) might point towards more restricted gene flow between the birds in this valley and the two lowland populations separated by high (about 1,000 m) foothills of the Andes (Figure 10.1). A recent satellite telemetry study in Upper Tambopata (ten tagged scarlet macaws) estimated their 9-month home range to be 1,730 km^2 (Brightsmith *et al.* 2021). In that study, movements always occurred to the north-east (away from the foothills) up to 150 km but they always returned to the same area or even the same nest hollow to breed. They flew much further than the distance between Candamo and Tambopata but none of the tagged macaws were detected in Candamo, or even flew near to the foothills.

Although they fly long distances, the philopatry and nest fidelity of these macaws suggest that dispersal rarely occurs over large scales. Furthermore, our results suggest that movements of birds between the valley of Candamo and the lowland might be limited by the high elevation. Despite the significant genetic differentiation between Candamo and Tambopata, the low magnitude of F_{ST} indicates that dispersal does occur between these areas. The only obvious connection between Candamo and the lowland Tambopata is a small river which cuts through multiple parallel mountain ridges (Figure 10.1). The cumulative current maps highlight this river as a conductance funnel (Figure 10.2) potentially providing the corridor for gene flow. Although our river model showed no significant correlation itself with the genetic structure, its effect was incorporated into the elevation model as rivers also represent the lowest elevation in the landscape.

Dispersal models show that birds may evolve to avoid risky movements even if they are capable of doing so (Shaw *et al.* 2014). The dispersal of scarlet macaws from Candamo might be explained by a combination of habitats, river system, and elevation gradients. Other factors like unsuitable habitat, temperature or humidity might also influence gene flow but data were not available for analysis in the current study. In future, these data and more detailed LiDAR maps could be used to explore spatial patterns of gene flow in finer detail.

Despite parrots and macaws being noted for their capability of flying long distances over large landscapes (Faria *et al.* 2008), our landscape resistance models indicate that high

elevation might create barriers for these species. Landscape resistance has also been implied by the work of Monge *et al.* (2016) who attributed the observed genetic differentiation between two large scarlet macaw populations of Costa Rica (Table 10.1) to montane barriers rather than recent habitat fragmentation. The central cordilleras of Costa Rica and Panama ranging in elevation from 500 to 3800 m are recognized to separate the two subspecies of scarlet macaw (Schmidt 2013), further suggesting that mountains can act as geographic barriers for macaws.

Recent studies have raised concerns about using partial Mantel tests in landscape genetics because they assume linearity between distance matrices (Legendre and Fortin 2010) and disregard information about other factors (e.g., mating and dispersal) influencing gene flow (Graves *et al.* 2013). Despite problems with partial Mantel tests, simple Mantel tests are appropriate for testing isolation by distance (Guillot and Rousset 2013), so our results of IBD in scarlet macaws are unlikely to be biased. Furthermore, both causal modelling and MRDM analyses indicated similar effects of elevation on gene flow in macaws, giving us greater confidence in our findings.

10.4.2. Population structure of scarlet macaws in Tambopata

Previous studies on macaws (Table 10.1) suggested low genetic differentiation among populations living in undisturbed habitat. Our study site consists of two adjacent protected areas with a total area of more than 13,000 km^2 of primary rainforest. The Bayesian approaches could not detect any population structure in our samples from Tambopata. We identified 74 males and 69 females (23 unknown) among our samples, but we did not detect any sex-biased dispersal. In monogamous species like macaws, competition for both mates and resources is likely to affect both sexes equally, leading to predictions of equal rates of dispersal (Dobson 1982).

10.4.3. Implications for conservation

Understanding landscape effects on gene flow is important for conservation management (Segelbacher *et al.* 2010; Keller *et al.* 2015), but these patterns are still unknown for most species. Macaws still inhabit a large nationally-protected lowland rainforest in Tambopata, but the rapidly growing human population along the recently built inter-oceanic highway is a serious conservation issue in the region (Baraloto *et al.* 2015). The apparently natural population of macaws within this area has allowed us to obtain baseline data that may become useful for future conservation genetic studies, for example evaluating macaw response to human induced habitat fragmentation. The Candamo valley and its vicinity, which is also a biological hotspot, is of commercial interest for oil extraction and gold mining activities (Finer *et al.* 2008; Asner *et al.* 2013). Repeated DNA sampling over the same area could also be compared to our results in the future, to see if accelerated habitat degradation could push the species to cross previously avoided natural barriers like high mountain ridges.

10.4.4. Conclusion

In our study of scarlet macaws, we found no evidence for strong population genetic structure across the lowland of Tambopata (over 80 km). These results indicate that gene flow is extensive and correlates with the large home range of these birds. We found evidence of a single population of scarlet macaws in Tambopata, over a large protected area. However, our

study also suggests that high elevation might act as a natural barrier to gene flow, as the Candamo population situated behind mountain ridges was genetically differentiated from the other lowland populations. Intermountain rainforest valleys similar to Candamo can host other populations of scarlet macaws over their distribution range. The Candamo valley also hosts a large diversity of other species, some with much more restricted dispersal movements than large macaws. Our findings provide baseline information about natural dispersal barriers in parrots that can assist in understanding the influence of rapid anthropogenic change on their genetic population structure in the Amazon Basin.

Part III. Conservation genetics of parrots

11. Review of genetic techniques in parrot biology

11.1. Introduction

The order of parrots (Psittaciformes) contains a diverse group of species distributed mainly in tropical and subtropical regions (Kosman *et al.* 2019; Barbosa *et al.* 2021). Around one third of the nearly 400 parrot species are threatened, and they are declining faster than other comparable groups of birds, making them one of the bird orders of greatest concern (Chapter 1). The most important threats affecting parrots are anthropogenic and include agricultural expansion, the wildlife trade, logging, climate change, and invasive alien species (Chapters 1 and 2). However, the relative importance of these threats differs geographically. In the Neotropics, agriculture is the greatest threat followed by the illegal pet trade and logging (Berkunsky *et al.* 2017). In the Afrotropics, the illicit wildlife trade has the biggest impact followed by agriculture and logging (Martin *et al.* 2014; Martin *et al.* 2018), and in the regions of Oceania and Indomalaya, logging and invasive species are the most critical threats to the survival of the endemic parrot species (Chapter 3). Some species have been introduced to regions outside their natural ranges, including cities worldwide (Calzada Preston and Pruett-Jones 2021), where they may be perceived as pests (Pruett-Jones 2021b).

The discipline of genetics (using it in this review for all methods that include molecular analysis of DNA) has made a major contribution to understanding the natural world. With the advancement of new DNA sequencing technologies in the past two decades, genetic research has been revolutionised and now has a wide range of applications to the field of biology and beyond. Genetics has contributed to the study of parrots in the wild and in captivity by helping to construct precise phylogenies (Wright *et al.* 2008; Provost *et al.* 2018), tracking the history of their early diversification (Schweizer *et al.* 2010), contributing important information at the population and individual levels to help conservation efforts (Brock and White 1992; Miller *et al.* 2003; Raisin *et al.* 2012), and revealing insights into their ecology and health (Masello *et al.* 2002; Raidal and Peters 2018). Molecular genetic approaches have even been also used to further our understanding of long extinct parrot species (Johansson *et al.* 2018; Gelabert *et al.* 2020; Smith *et al.* 2021). Here, we review what has been learned through the use of different genetic methods applied to parrot conservation in past decades and in the current era of genomics. The aim of this review is to provide a comprehensive overview of this field and highlight knowledge gaps to inform future genomic research on parrots.

11.2. Short history of advances in genetic studies of parrots

The word “genetic” was used for the first time in 1819 by Hungarian nobleman Imre Festetics who formulated a number of rules of heredity (Poczai *et al.* 2014), laying the groundwork for the discovery of Mendelian genetics in the mid-19th century (Weiling 1991). However, the molecular background of these ground-breaking theories was unknown until the determination of the structure of the DNA molecule in 1953 (Watson and Crick 1953), leading to deciphering of the genetic code and the central tenets of molecular biology (Crick 1970). The invention of the polymerase chain reaction (**PCR**; see glossary in Table 11.1) in 1983

enabled the amplification of DNA and revolutionised genetic research. Even though many bird studies made use of DNA fingerprinting from the late 1980s, molecular studies of wild parrots started more slowly.

The first scientific publications on parrot genetics used karyotypes and allozymes to study the chromosomal and protein evolution of parrots at the taxonomic levels of species, family, and order (Adams *et al.* 1984; Joseph and Hope 1984; Christidis *et al.* 1991a; Christidis *et al.* 1991b). These were followed by molecular sexing with gel electrophoresis (Miyaki and Wajntal 1997). Then came the advent of mitochondrial DNA (mtDNA) analysis initially studied by enrichment and cloning, and later by sequencing of individual mitochondrial and nuclear genes (Sanger sequencing) eased by PCR technology. The initial research focus on parrots with these methods was on phylogeny and systematics (De Kloet and De Kloet 2005; Tavares *et al.* 2006; Wright *et al.* 2008) and then work increased on species-level taxonomy and phylogeographic scales (Caparroz *et al.* 2009; Smith *et al.* 2013; Rocha *et al.* 2014; Dolman and Joseph 2015).

Studies of detailed population structure and individual-based behaviour in wild populations of parrots began with the advent of DNA fingerprinting by **minisatellites**. Minisatellites (complex tandem repeat regions of DNA) were used, for instance, on the Burrowing Parrot *Cyanoliseus patagonus* (Masello *et al.* 2002), some macaw species (Nader *et al.* 1999; Caparroz *et al.* 2001a; Caparroz *et al.* 2001b), and in the Palm Cockatoo *Probosciger aterrimus* (Murphy *et al.* 2003). Later, the discovery of **microsatellite** genetic markers (simple sequence repeat) transformed the application of genetics to many biological research projects including parrot studies. The length of these markers can be measured precisely by capillary electrophoresis, providing a great advantage over the original fingerprinting methods of minisatellites visualised by gel electrophoresis. Various studies have identified and published species-specific microsatellites for parrots (Russello *et al.* 2002; Caparroz *et al.* 2003; Chan *et al.* 2005; Taylor and Parkin 2006; Russello *et al.* 2007; Afanador *et al.* 2014; Olah *et al.* 2015). This advance resulted in important tools for a wide variety of genetic research via cross-species amplification to other parrot species. Microsatellites were mainly used for fine-scale studies of individuals including family relationships. For example, Klauke *et al.* (2013; 2016) used these markers to report a cooperative breeding system, not widely known in parrots (e.g., Theuerkauf *et al.* 2009), and estimated fine-scale population structure in the recently discovered El Oro Parakeet *Pyrrhura orcesi*.

Early, or first-generation, sequencing technologies (e.g., Sanger sequencing) made it possible to read the genetic code of specific DNA sequences. Later, molecular genetic technology advanced and phased into the second- or **next-generation sequencing (NGS)** or genomics era. The massively parallel high-throughput feature (i.e., sequencing multiple fragments and individuals at once) of these new sequencing platforms pushed down the price of sequencing and sped up the process of whole **genome** sequencing. The first complete mitochondrial genome (mitogenome) of a parrot was published in 2004 for the Kākāpō *Strigops habroptila* (Harrison 2004), followed by many more (e.g., Urantowka 2014; Urantowka *et al.* 2014). The first draft of a full parrot genome, the Budgerigar *Melopsittacus undulatus*, was uploaded to the National Center for Biotechnology Information (NCBI) database in 2011 (www.ncbi.nlm.nih.gov/genome/10765). This was followed by the Puerto Rican Amazon *Amazona vittata* in 2012 (Oleksyk *et al.* 2012), and the Scarlet Macaw *Ara*

macao in 2013 (Seabury *et al.* 2013). Whole genome sequencing aided the discovery of new microsatellite markers, for example the Orange-bellied Parrot *Neophema chrysogaster* (Miller *et al.* 2013a) and the Scarlet Macaw (Olah *et al.* 2015). At the time of this publication, there are whole genomes available from second-generation technologies for 36 parrot species and complete mitochondrial genomes for 69 parrot species in the NCBI genome database (www.ncbi.nlm.nih.gov/genome). This is approximately 10% and 20% of parrot species for nuclear and mitochondrial genomes, respectively. Currently, the best available parrot genomes assembled at the chromosome level belong to the Budgerigar (61x coverage, scaffold N50 size of 104 Mb, annotated with 16,458 protein-coding genes; GenBank assembly accession: GCA_012275295.1), Kākāpō (76x, scaffold N50 = 83 Mb, 16,053 protein-coding genes; GCF_004027225.2), Blue-fronted Amazon *Amazona aestiva* (60x, scaffold N50 = 89 Mb; GCA_017639355.1), and Monk Parakeet *Myiopsitta monachus* (67x, scaffold N50 = 76 Mb; GCA_017639245.1). However, international consortia of scientists continue to sequence the genomes of many more species. The Genome 10K project aims to sequence the genomes of representatives from all genera of vertebrates (Koepfli *et al.* 2015). The B10K project (Zhang 2015; Feng *et al.* 2020) and OpenWings Project (openwings.org) aim to sequence all extant bird species and understand their evolutionary histories and relationships.

NGS opened new research pathways to genome-wide association studies aimed at understanding the underlying genetic variants determining traits (Brandies *et al.* 2019). Microsatellite, mitochondrial, and multi-locus studies have transitioned into analyses of many more (sometimes thousands) polymorphic sites of **single nucleotide polymorphisms (SNPs)**, which are found throughout the coding- and non-coding parts of the genome, giving them a further advantage over microsatellites. SNPs can be generated in several ways. In restriction-site-associated DNA sequencing (RAD-seq), one or two restriction enzymes cut the genome at enzyme-specific restriction sites, and these fragments are then barcoded, filtered, and sequenced (Elshire *et al.* 2011; Andrews *et al.* 2016). In sequence capture methods, oligonucleotide probes (baits) are designed to hybridise with specific regions of interest. These are then captured, barcoded, and enriched before sequencing (Davey *et al.* 2011). For this technique, the sequences of interest need to be known (e.g., from a complete genome of the same or related species), or the baits can be generated by other techniques such as double-digest RAD-seq (Suchan *et al.* 2016). The sequencer generated data can then be analysed in different bioinformatic pipelines (Andrews and Luikart 2014). This more comprehensive sampling of the genome has enabled more detailed examination of signatures of selection and local adaptation on the genome (Allendorf *et al.* 2010). Sequencing RNA shows which genes are being expressed (transcriptomics) and can have an important role in reintroductions by predicting the potential for local adaptation and tolerance capacity in the source population (He *et al.* 2016).

In the past decade, further advancements in genome sequencing technology have pushed the boundaries of data collection. For example, nanopore technology has enabled portable sequencers as small as a USB drive (Liang and Zhang 2015; Jain *et al.* 2016). Of great help to parrot biologists interested in conducting genetic research in the field, these sequencers were shown to work even in harsh environments (Pomerantz *et al.* 2018). Parrots are notoriously hard to capture in the wild, so making use of non-invasive sampling methods (e.g., feathers, eggshells, faeces, or even residual saliva) with the new technologies will provide further

advances (Olah *et al.* 2017b; Monge *et al.* 2020; Spitzer *et al.* 2020). Metagenomic and metabarcoding applications, where all DNA materials are extracted from environmental samples, allow bioinformatic pipelines to be used to find and match sequenced DNA of species with reference to online databases (e.g., NCBI GenBank, European Variation Archive). This way, the presence or absence of species can be detected in the environment, and abundance estimates might be derived in some cases (Andersen *et al.* 2012; Ji *et al.* 2013), although this technique needs further development. One of the major limitations of current parrot genomes is that they were produced with short-read sequencing. These short-reads make genome assembly more challenging by causing genomes to be more fragmented (with smaller scaffold sizes) and incomplete, and limit the accuracy of some downstream uses of genomic data (e.g., studying structural variants). The advent of long-read or third-generation sequencing technologies can produce reads greater than 10 Kb in length, which allow for the assembly of chromosomal-level genomes (Amarasinghe *et al.* 2020). These advances still present challenges including high-cost, specialised bioinformatic expertise, and access to high-quality genetic samples (i.e., DNA samples with high molecular weight), but these limitations are likely to be overcome in the near future.

11.3. Research fields

11.3.1. Species- and genus-level systematics and taxonomy

The species is the widely accepted default unit used for evaluating conservation status (e.g., in the IUCN Red List), hence defining species and resolving taxonomic uncertainties by genetic techniques is important for conservation (Boon *et al.* 2000). Active speciation of parrots on islands is most readily evident in Australasia, as shown by the *Eclectus roratus* and *Trichoglossus haematodus* complexes (Braun *et al.* 2016). In such cases of dynamic evolution, wider sampling and genetic data of finer resolution are often needed to resolve phylogenetic relationships (Smith *et al.* 2020). The extinction of island-endemic parrot species and replacement by invasive alien species led to loss of phylogenetic diversity, but understanding these frameworks can aid conservation strategies to restore island ecosystem function (Jackson *et al.* 2015).

In some parrots, the traditional taxonomy based on plumage might need some revision, as shown with a genetic study on amazon parrots in the Neotropics (Ribas *et al.* 2007). **Cryptic species** of parrots were suggested by genetic studies for various taxa, including the mealy amazons *Amazona* spp. (*A. farinosa*, *A. guatemalae*) in the Neotropics (Wenner *et al.* 2012) and the ground parrots *Pezoporus* spp. (*P. wallicus, P. flaviventris*) in Australia (Murphy *et al.* 2011). The need to recognize subspecies within the Mulga Parrot *Psephotellus varius*, generally considered monotypic, was also evident from phylogeographic structure either side of a well-known biogeographic barrier in southern Australia in their mitogenomic diversity and genome-wide nuclear markers (McElroy *et al.* 2018). Notably in contrast, recognition of *Amazona gomezgarzai* by Silva *et al.* (2017) has been roundly debunked by Escalante *et al.* (2018).

Defining **management units (MUs)** within species also holds important merits for conservation (Moritz 1994), however a refinement to the original definition, which was framed in terms of allele frequency differentiation, would be to define MUs with reference to the

management issue in question, such as identifying demographically independent units for population monitoring, or genetically differentiated units for mixed-source introductions. For example, a genetic study revealed cryptic diversity within the Bahama Amazon *Amazona leucocephala bahamensis* between populations living on two remote islands (Russello *et al.* 2010). A study on the Blue-fronted Amazon suggested treating its two subspecies as separate MUs (Caparroz *et al.* 2009), and a recent study argued for MU consideration for the Atlantic Forest population of the Southern Mealy Amazon *Amazona farinosa farinosa* (Hellmich *et al.* 2021). Similarly, another study on Military Macaws *Ara militaris* in Mexico proposed two MUs in the country based on genetic data (Rivera-Ortíz *et al.* 2017). In Africa, a study warned that a population of Grey Parrot *Psittacus erithacus* living on Príncipe Island, São Tome and Príncipe, should be treated as an independent MU from the continental African populations, given their evolutionary dynamics and heavy local poaching pressure (Melo and O'Ryan 2007). The Cape Parrot *Poicephalus robustus* was previously considered to comprise three subspecies until a study using multilocus DNA analyses concluded that *P. r. robustus* diverged from *P. r. suahelicus* and *P. r. fuscicollis* around 2.4 Mya (Coetzer *et al.* 2015). Accordingly, it is now usually treated as a monotypic species *P. robustus* and has been uplisted to Vulnerable by the IUCN Red List, while the other two subspecies now form the Brown-necked Parrot *P. fuscicollis* complex of Least Concern (Perrin 2012; Clements *et al.* 2019; Gill *et al.* 2024).

Evolutionarily significant units (ESUs) are independently evolving units of genetic variation (Moritz 1994). These units were proposed for the two subspecies of the Orange-fronted Parakeet *Eupsittula canicularis* in Mexico (Padilla-Jacobo *et al.* 2016). A comprehensive genetic analysis (using genome-wide SNPs and mitochondrial data) of the Red-tailed Black-Cockatoo *Calyptorhynchus banksii* identified five ESUs over their large distribution, and advised taxonomic reassessments including recognition of a new subspecies (Ewart *et al.* 2020). Distinctions between ESUs and MUs were made during a genetic assessment of Major Mitchell's Cockatoo *Lophochroa leadbeateri* (Ewart *et al.* 2021). An analysis employing mtDNA and microsatellite data failed to detect genetic evidence for the two subspecies of Kākā in New Zealand, instead it is hypothesised that phenotypic diversity was due to an adaptive latitudinal size cline consistent with Bergmann's rule (Dussex *et al.* 2015), an important consideration for possible translocation attempts. In contrast, another study using similar genetic evidence argued that the current genetic clusters of Kea should not be considered as independent conservation units because the structure evolved through very recent postglacial recolonisation processes (Dussex *et al.* 2014). In these and similar cases, appropriate taxonomic rank is debatable, but conservation and management units can be assigned where appropriate. Again, as shown with the example studies, these units of conservation can only be revealed with the help of genetic studies, which also have an ever-growing role in defining taxonomic units.

11.3.2. Conservation genetics and genomics

Conservation genetics is an interdisciplinary science dealing with the genetic factors affecting extinction risk of species and how to minimise these risks (Frankham *et al.* 2010). It is transitioning into using genomic techniques (Allendorf *et al.* 2010). In the previous section, we discussed the importance of phylogeny to conservation. Here, we provide an overview of

other major areas where the transition to genomics has contributed to the conservation of parrots.

Preventing the loss of **genetic diversity** is an essential aim of any conservation project. Genetic monitoring can provide important tools to quantify this diversity before, during, or after management efforts on threatened parrot species or populations (Chan *et al.* 2008; Faria *et al.* 2008). In small remaining populations of species, diversity can be lost due to **genetic drift**, which can override natural selection (Taylor and Parkin 2010). Intensive management restored the Echo Parakeet *Psittacula echo* population from 20 remaining individuals in 1987. Genetic research showed that re-distribution of genetic material among its populations has reduced the likelihood of losing private alleles that could otherwise be lost due to the random effect of genetic drift in small, isolated populations (Raisin *et al.* 2012). On the island of Tasmania and its own offshore islands, a study of the migratory Swift Parrot *Lathamus discolor* could not detect genetic differentiation among breeding populations in consecutive years and across multiple islands (Stojanovic *et al.* 2018). Genetic estimations were used to calculate the **effective population size** of their single, panmictic population, and after combining it with demographic data, the study calculated a potential contemporary population size as low as 300 individuals (Olah *et al.* 2021b).

Contemporary population fragmentation due to anthropogenic factors can lead to reduction in **gene flow** among the fragments resulting in genetic structure detectable via genetic testing. It is important to detect early signs of genetic fragmentation as it could lead to loss of genetic diversity and eventually to inbreeding. However, these effects take time, depending on habitat corridors, migration rates, and the mobility, dispersal, and lifespan of the species. For instance, at least a 35-year-long lag was shown between deforestation in the Brazilian Cerrado biome and changes in the genetic structure of Goias Parakeet *Pyrrhura pfrimeri* populations (Miller *et al.* 2013b), corresponding to about five generations of the species. Genetic structure was also found in the Scarlet Macaw in the highly fragmented landscape of Costa Rica (Monge *et al.* 2016). Historical population structure can also have important implications for present day conservation efforts. A broader genetic analysis of the Scarlet Macaw for instance suggested a distinct conservation unit for its Central American subspecies *A. m. cyanoptera* (Schmidt *et al.* 2020). A population genetic study on the Palm Cockatoo on Cape York Peninsula, Australia found genetic differences among the studied populations, probably due to a mountain barrier (Keighley *et al.* 2019). Incorporating this population genetic data, especially the connectivity between populations, into a **population viability analysis (PVA)** model predicted that dispersal between populations is not enough to buffer decline given their extremely low breeding success. The study concluded that Palm Cockatoos in Australia should be uplisted from Vulnerable to Endangered (Keighley *et al.* 2021).

Genetic studies can have an important role in *ex situ* conservation management of threatened species to avoid **inbreeding** and to maintain maximum genetic diversity among captive individuals. Genetic testing can accurately identify relatedness among birds, which can be useful for the mixing of breeding pairs as demonstrated by *Amazona* parrots (Ringler 2012). However, in socially monogamous species like parrots, natural mate choice can result in higher reproductive success than forced choice based solely on genetics (Ihle *et al.* 2015), as shown for Cockatiels *Nymphicus hollandicus* (Fox and Millam 2014), where pairs with higher behavioural compatibility were better parents (Spoon *et al.* 2006). The effect of **inbreeding**

depression was first explicitly studied in parrots with respect to clutch size of captive budgerigars in the 1980s (Daniell and Murray 1986). It has been used to guide the Puerto Rican Amazon recovery program through genetic fingerprinting since the 1990s (Brock and White 1992). Low levels of inbreeding were detected for the Red-tailed Amazon *Amazona brasiliensis,* indicating that more direct threats, like habitat destruction and illegal wildlife trade, should be the focus of conservation efforts (Caparroz *et al.* 2006). Genetics has also helped to identify the pedigrees of the remaining Kākāpō population *in situ* (Miller *et al.* 2003) and inform conservation strategies (Dussex *et al.* 2021). It can also detect signs of genetic adaptation to captivity, which can have negative effects on reintroduction success. A recent genetic study on wild and captive populations of Blue-throated Macaws *Ara glaucogularis* and Thick-billed Parrots *Rhynchopsitta pachyrhyncha* highlighted the need for both *in situ* and *ex situ* conservation strategies (Campos *et al.* 2021).

Establishing captive populations of endangered species is often used by conservation management programmes. However, rapid genetic adaptation to captivity (within a few generations), low founder diversity, and potential inbreeding are of concern for future recovery goals, but these have been rarely studied in parrots. A captive population of Orange-bellied Parrots was founded in 1985 and later supplemented with wild individuals. A recent study found low diversity in their toll-like receptors (TLR), partially responsible for the innate immune response and so the first line of defence against pathogens, highlighting that they might be unable to adapt to novel disease outbreaks (Morrison *et al.* 2020a). For instance, a spillover of beak and feather disease virus (BFDV) to the remaining wild population almost wiped out the entire species (Peters *et al.* 2014). The psittacine beak and feather disease (PBFD) was first reported on Red-rumped Parrots *Psephotus haematonotus* in 1907 near Adelaide, Australia (Ashby 1907). BFDV was isolated and characterised much later from cockatoos (Ritchie *et al.* 1989). PCR tests were developed for the detection of BFDV (Ypelaar *et al.* 1999; Ogawa *et al.* 2005), helping to identify cases in psittacines. A recent study provides an excellent overview of the ecology of PBFD in parrots and highlights the importance of mitigating its effects on threatened parrot species (Raidal and Peters 2018). BFDV is also an ongoing threat to many other Australian parrot species (Australian Government and Environment 2015). Another study, using SNP data of the wild and captive populations of the Orange-bellied Parrot, showed that their genetic diversity could be retained in the captive population (Morrison *et al.* 2020b), possibly improving their health for future reintroductions. Retaining diversity at the major histocompatibility complex (MHC) is also important, as it is responsible for the adaptive immune response in birds and other vertebrates (Kaspers and Schat 2012). However, the MHC has been studied in only a handful of parrot species, including the Budgerigar (Edwards *et al.* 1999), the Green-rumped Parrotlet *Forpus passerinus* (Hughes *et al.* 2008), and the Red-crowned Parakeet *Cyanoramphus novaezelandiae* (Knafler and Jamieson 2014).

Outbreeding depression occurs when distinct species hybridise or isolated populations of the same species are mixed and the results are adverse (Frankham *et al.* 2011). One proposed underlying mechanism is that species have coadapted gene complexes nearby on the same chromosomes and that recombination during hybridization disrupts their adaptive functions (Huff *et al.* 2011). Alternatively, outbreeding depression is likely to be rare and its effects restricted to the first few generations of crossing among evolutionarily diverged lineages (Ralls *et al.* 2020). Around 8% of parrot species have been recorded to hybridise in the wild (Grant

and Grant 1992) and almost half of all parrot species have been reported to hybridise in captivity (McCarthy 2006). Genetic screening of the last remaining population of the Critically Endangered Forbes' Parakeet *Cyanoramphus forbesi* helped to determine the magnitude of hybridisation with the Chatham Island Red-crowned Parakeet *C. novaezelandiae chathamensis* and to identify cryptic hybrids (Chan *et al.* 2006). A complex hybrid zone was studied involving the phenotypically distinct non-sister species Pale-headed Rosella *Platycercus adscitus* and Eastern Rosella *P. eximius*, and showed a lack of post-zygotic barriers to gene flow between these species (Shipham *et al.* 2019). The last remaining male individual of the Spix's Macaw *Cyanopsitta spixii* was breeding with a Blue-winged Macaw *Primolius maracana* and genetic sequencing showed that the resultant embryo was indeed a hybrid of the two species, but it never hatched (Miyaki *et al.* 2001).

Molecular genetic techniques can be applied in **wildlife forensic** investigations. Molecular genotyping helped Australian authorities to match DNA extracted from eggshells found in the wild to a nestling of Red-tailed Black-Cockatoo at a nearby property (White *et al.* 2012). During the investigation, forensic scientists concluded that the nestling was hatched from the eggshell recovered from a tree hollow and this led to a criminal conviction. In another case, eggs were seized from an alleged trafficker arriving in Australia. Comparing the extracted mtDNA to the genetic database of the NCBI, researchers identified several threatened parrot and cockatoo species, and the smuggler was prosecuted (Coghlan *et al.* 2012). Poachers were also arrested in Brazil intending to fly to Europe, one in 2003 with avian eggs later identified by molecular genetic techniques as of parrots and owls (Gonçalves *et al.* 2015), and another in 2018 with eggs identified as of Short-tailed Parrot *Graydidascalus brachyurus* (Formentão *et al.* 2021). Ewart *et al.* (2021) developed a forensic test with 20 nuclear SNPs for the Major Mitchell's Cockatoo and demonstrated its application for subspecies identification. A similar toolkit combining various forensic techniques was developed earlier for the Glossy Black-Cockatoo *Calyptorhynchus lathami* (Lee 2013). A set of microsatellites were developed in the Cape Parrot with sufficient discriminatory power to distinguish captive versus wild birds via parentage analyses (Coetzer *et al.* 2017), and similar markers proved to be successful in determining the geographic origin of a captive individual of Military Macaw (Rivera-Ortíz *et al.* 2021). The control regions of mtDNA of Blue-and-yellow Macaws *Ara ararauna* confiscated from the illegal wildlife trade in Brazil were sequenced and compared to reference sequences of the species, in order to find their provenance and advise on reintroduction planning (Fernandes and Caparroz 2013).

11.4. Conclusions

Our overview has shown wide application of molecular genetic- and genomic techniques for studies of parrots in their global distribution. There is increasing interest by field biologists studying parrots in incorporating genetics as part of their research agenda. Given the high proportion of threatened species in the group, and the extraordinarily high level of interest in parrots among humans (including the wildlife trade and captive breeding), one or more centralised parrot genetics laboratories, perhaps on different continents, might be advantageous for future collaborative research. This could also consolidate expertise and boost efficacy in sample collection, DNA extraction, sequencing, and genomic analysis. It would be important

to include genetics as a component of studies on parrot species with high conservation concern, as this could help to find populations with low genetic diversity and the most appropriate source populations to "rescue" them.

Recent breakthroughs in technology and consolidation of approaches will allow genetic techniques to be used more extensively in wildlife forensic investigations. The lack of validated DNA reference sequences is hindering our ability to accurately assign species identity. A focus on establishing DNA reference databases for the most traded wildlife species will assist in forensic casework. Building a baseline reference genomic database of wild parrot populations could help to determine the provenance of confiscated birds, aid rewilding and translocation projects, and resolve questions about captive or wild origins. As part of the licensing agreement to maintain some protected species in captivity, DNA samples could be taken with the explicit intention of using them to verify parentage and identity in the future (Groth *et al.* 1991; Mell and Wetherall 1992). Genetics has also been effective in disease testing. Studying the interactions between the TLR, MHC, and resistance to diseases would be important for both captive and wild parrot populations.

Choosing the correct markers for genetic analyses is very important as different conclusions might be reached without a genome-wide investigation. For instance, using RAD-seq data, Shipham *et al.* (2017) confirmed a sister relationship between the Pale-headed Rosella and Northern Rosella *Platycercus venustus*, which was previously all but overlooked based purely on mtDNA sequences in which there had been a mtDNA capture event between non-sister taxa. However, the switch from solely sequencing mtDNA regions to relatively cheap and easy SNP genotyping methods has limited the capacity for comparative studies among species as different marker panels are used, optimised for each species. Absolute metrics of genetic structure and diversity are therefore not readily comparable, so approaches that produce DNA sequences may be preferable. For example, ultraconserved elements (UCEs) that target portions of the genome that remain similar across divergent clades but contain variable sequences in the flanking regions are a common approach used in avian phylogenomic studies (Faircloth *et al.* 2012). UCEs have been used in studies on the phylogenomics of lorikeets (Smith *et al.* 2020) and historical demography of the Carolina Parakeet (Smith *et al.* 2021). There is increasing interest in applying comparative genomic techniques to conservation studies (Benestan *et al.* 2016; Garner *et al.* 2016). These are limited with current data types but perhaps the increasing use of whole genome sequencing will make independent datasets more comparable among individuals, populations, and species. This would open up interesting opportunities for questions from behavioural, conservation, and evolutionary perspectives. The field of genetics has always been at the forefront of data sharing through repositories such as GenBank, so the opportunities for comparative analyses and insights as data comparability increases are enormous. However, sequence data alone are not enough to understand genome evolution and function, and entirely new approaches, like chromosomics (Deakin *et al.* 2019) with superior bioinformatics like pangenome models (Eizenga *et al.* 2020), are needed in the future.

In conclusion, genetics has aided parrot research substantially in the past and will continue to do so as exciting new applications emerge in the advancing genomic era. We certainly encourage parrot researchers to consider implementing genetics as part of their research agenda, given the wide array of questions genetics can help to answer as demonstrated in this

review. We realise that these research projects often do not have the capacity, expertise, or funds to do genetic research. However, many commercial laboratories now provide sequencing services at ever-dropping costs, so researchers might consider using these services to generate data from their samples. For genomic data interpretation, we propose a consortium of scientists sharing their experience in conservation genomics, analysis pipelines, and mentorship of students in genetic research on parrots. This consortium could work as a specialist group within the well-established Parrot Researchers Group.

Table 11.1. Glossary of key concepts mentioned in the text.

Cryptic species	Morphologically often indistinguishable but genetically distinct species, following the evolutionary species concept.
Effective population size (N_e)	The size of the ideal, panmictic population that would experience the same loss of genetic variation, through genetic drift, as the observed population.
Gene flow	The exchange of genetic information between randomly mating populations through migration, measured in allele frequencies.
Genetic diversity	The extent of genetic variation in a population, species, or across species, measured in heterozygosity, allelic diversity, or heritability.
Genetic drift	Random changes in the genetic composition of a small population between generations. It results in loss of genetic diversity, random changes in allele frequencies, and diversification among populations.
Genome	The complete genetic material of an organism, including nuclear and mitochondrial DNA.
Inbreeding	The accumulation of deleterious mutations due to breeding among close relatives.
Inbreeding depression	Reduction in reproduction, survival, or related characters due to inbreeding.
Management units (MUs)	Populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles.
Microsatellite	A locus with a short tandem repeat DNA sequence, typically showing variable number of repeats across individuals. Consequently, they are highly informative genetic markers.
Minisatellite	Typically, between 6–100 bp section of DNA, repeated many times in a long string with no gaps between the repeats. These were the first type of DNA markers used in human identification and later in wildlife genetics.
Next generation sequencing (NGS)	Includes technologies that use short-read, massively parallel, high-throughput sequencing of the genetic material (e.g., Illumina, Ion Torrent).
Outbreeding depression	Reduction in reproductive fitness due to crossing of two populations, subspecies, or species.
Polymerase chain reaction (PCR)	A method to replicate copies (amplify) of specific segments of DNA, with thermostable Taq polymerase enzyme in a thermocycler.
Population viability analysis (PVA)	A model to predict the extinction risk of a population by using information about population size and structure, birth and death rates, risks and severity of catastrophes, levels of inbreeding depression, rate of habitat loss, etc. PVA can be used as a management tool to examine different management op-tions to recover threatened species.
Single nucleotide polymorphism (SNP)	A nucleotide site (base pair) in a DNA sequence that is polymorphic in a population and can be used as a marker to assess genetic variation within and among populations.
Wildlife forensics	Application of science to the law, including detection of illegal wildlife trade with DNA-based methods.

12. Genetics confirms extinction risk in swift parrots

12.1. Introduction

Conservation of mobile species is complicated by their variable movements because these increase the proportion of the total population potentially exposed to threatening processes (Runge *et al.* 2015). The consequences of threats may be particularly severe if they occur at critical locations where resources are limiting (Runge *et al.* 2014), and thus may act as ecological traps that increase extinction risk (Robertson *et al.* 2013). However, the degree of extinction risk may depend on whether the mobile species behaves as one or more genetically discrete subpopulations (hereafter: conservation units). The existence of multiple conservation units may reduce the likelihood of species extinction when the impact of threats is severe (Runge *et al.* 2014).

The number of conservation units for a species can depend on the scale of individual movements (Canales-Delgadillo *et al.* 2012), variation in resource availability across the geographic range (Roshier *et al.* 2008) and the spatial scale at which gene flow occurs (Haig *et al.* 2011). Whether individual movements correspond to gene flow (i.e. resulting in new breeding sites) is critical to how different conservation units may be defined. Some widespread mobile species exhibit local adaptation where food is reliable and these adaptations may assist differentiation of separate conservation units. For example, crossbills *Loxia* spp. undertake continental scale movements to exploit masting of a few food tree species in forests where seed production is unpredictable (Newton 2006). Some crossbill populations exploit more reliable food trees (e.g. trees that seed annually or are serotinous) and have evolved into distinct lineages (Benkman 1993) that can be considered separate conservation units. Reliable food enables evolutionary divergence of local crossbills from mobile populations that live in less predictable environments (Edelaar *et al.* 2012). Low resource reliability also appears to be closely linked with low population genetic structure in other comparable systems, for example irruptive owls (Marthinsen *et al.* 2009) and seed-eating passerines (Mason and Taylor 2015). Understanding genetic consequences of resource fluctuations and movement patterns may be key to defining conservation units for widespread mobile species.

We use data from a multi-year study to evaluate whether one or more conservation units exist for a mobile resource specialist. Swift parrots *Lathamus discolor* are nomadic migrants that breed in Tasmania (including two major offshore islands) and winter on the Australian mainland. The entire population moves to breed in different locations each year depending on the configuration of key resources across the potential range (Webb *et al.* 2017). Swift parrots are critically endangered, and introduced sugar gliders *Petaurus breviceps*, a small arboreal, volant marsupial, are a major cause of mortality of nests and breeding female parrots (Heinsohn *et al.* 2015). The small nest cavities preferred by swift parrots protect against native Tasmanian predators, but sugar gliders are not excluded by this passive nest defence (Stojanovic *et al.* 2017). Tasmanian offshore islands (where sugar gliders do not occur) are important population sources, whereas Tasmanian mainland sites can act as sinks for swift parrots due to predation pressure (Stojanovic *et al.* 2014).

On the basis of population-level movements of swift parrots (Webb *et al.* 2017), population viability analysis (PVA) of extinction risk have assumed that the species acts as a single panmictic conservation unit (Heinsohn *et al.* 2015), and that no island-philopatric subpopulations exist. Under these assumptions, Heinsohn *et al*. (2015) estimated that swift parrots may decline by 94% in three generations because breeding success on islands cannot offset predation mortality on the Tasmanian mainland.

This critical assumption of panmixia in the PVA of Heinsohn *et al*. (2015) has not been tested, despite the conservation significance of potential island refuges assuming population differentiation exists. Therefore, in this study we address this knowledge gap by applying population genetic analysis and simulations to answer four questions: (1) Does the species fit expectations consistent with a single panmictic conservation unit? (2) Is there genetic differentiation among populations from islands and the Tasmanian mainland? (3) Is there temporal genetic differentiation among years? (4) Is there evidence for sex-biased dispersal? We consider these results in the context of PVA of the species and evaluate the implications for the conservation of mobile species.

12.2. Methods

12.2.1. Study system and species background

This study was conducted across most of the swift parrot breeding range between 2010 and 2016 (Figure 12.1). Genetic samples for this study were not available for Maria Island or northern Tasmania, but all other sites considered by Heinsohn *et al*. (2015) were included here. Swift parrots require the co-occurrence of both food (nectar from *Eucalyptus globulus* and *Eucalyptus ovata* flowering) and nesting habitat (tree cavities) for successful breeding (Webb *et al.* 2017). Breeding occurs anywhere in Tasmania where flowering and suitable nesting habitat occur together, including two offshore islands, but the specific location of breeding varies over time depending on local resource abundance (Figure 12.2). In any given breeding season due to the variation in food availability, only a fraction of the potential range is occupied (Webb *et al.* 2017). Swift parrots move to breed where tree flowering is most abundant (Figure 12.2), and in years when food is locally unavailable, birds are absent from islands (Webb *et al.* 2017). Heinsohn *et al*. (2015) reported that the proportion of the swift parrot population that settled to breed on islands varied between 0 and 29% in any given year. Based on these high rates of movement of individuals among island and Tasmanian mainland breeding sites, Heinsohn *et al*. (2015) treated the population as a single conservation unit for the purposes of PVA.

12.2.2. Sample collection

Swift parrot nests were identified across the study area during standardized monitoring (Webb *et al.* 2014) and unstructured searches. Nests were identified using behavioural cues of swift parrots and accessed using single rope climbing techniques (Stojanovic *et al.* 2015). Nestling swift parrots were temporarily removed from their nest cavities (Stojanovic *et al.* 2015) and blood was collected using brachial venepuncture. Blood was stored on FTA paper (Whatman™). Adult swift parrots were captured during nestling provisioning and either blood (collected as above) or feathers (plucked from the flank and stored in 95% ethanol) were

collected. Tissue was taken from dead swift parrots (adult females and nestlings killed by sugar gliders, and an injured wild bird that was euthanized after entering care) and these were stored in 95% ethanol.

Our swift parrot samples were drawn from two geographic groups (referred to as 'regions' in our AMOVA analysis, see below): 'mainland' Tasmania and 'island', with spatially aggregated samples within regions treated *a priori* as 'populations'. In an additional analysis of genetic differentiation among sampling years, we defined 'temporal populations' on the basis of the year in which they were collected (i.e. 2010–2015 breeding seasons, Table 12.1).

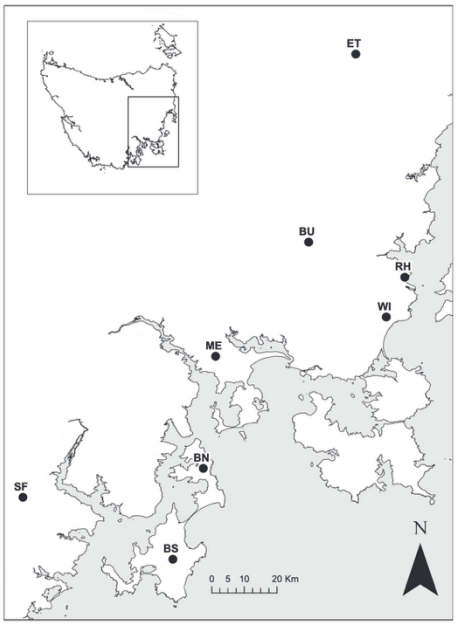

Figure 12.1. Map of the study area in Tasmania, Australia. Populations where swift parrot genetic samples were collected were: North (BN; $N = 32$ genetic samples) and South Bruny Island (BS; $N = 23$), Buckland (BU; $N = 13$), Eastern Tiers (ET; $N = 16$), Meehan Range (ME; $N = 8$), Rheban (RH; $N = 5$), Southern Forests (SF; $N = 6$), and Wielangta (WI; $N = 6$).

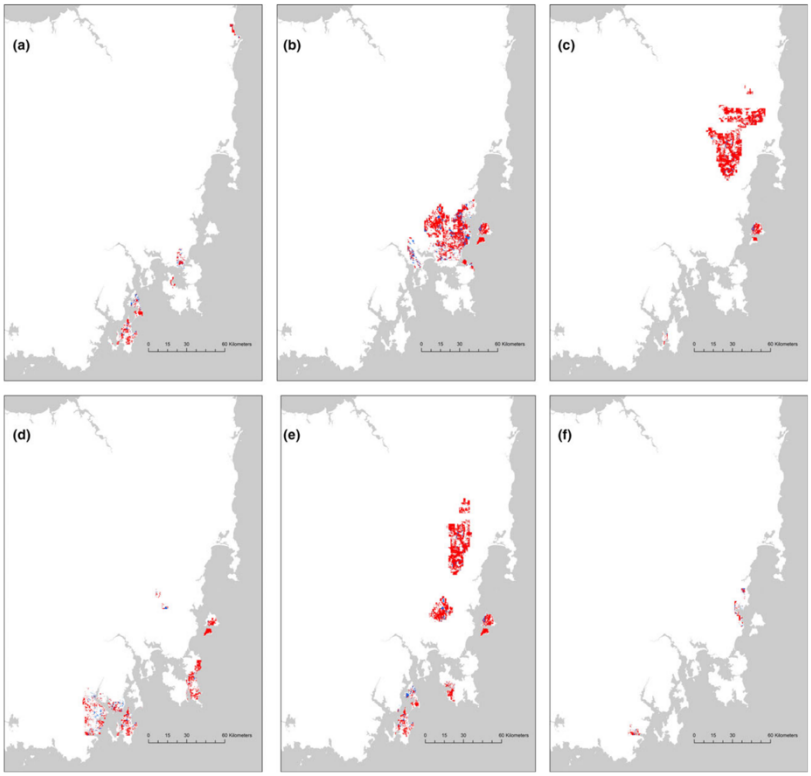

Figure 12.2. Locations and areas of habitat occupied by breeding swift parrots based on occupancy models (red = nesting habitat, blue = foraging habitat) in eastern Tasmania, Australia between (a) 2009, (b) 2010, (c) 2011, (d) 2012, (e) 2013 and (f) 2014. The location of nesting by the swift parrot population varies annually depending on where food is available, and <30% of the swift parrot population settles to breed on predator-free islands in any given year.

12.2.3. DNA extraction and microsatellite genotyping

We used two methods to extract DNA depending on the type of sample. DNA extraction from blood stored on FTA paper was performed following the standard procedure for nucleated erythrocytes (Smith and Burgoyne 2004). DNA was extracted from feather and tissue samples using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) following manufacturer instructions with some modifications (Olah *et al.* 2017c).

Using a subset of randomly selected high-quality DNA samples ($n = 30$) we screened previously described microsatellite loci ($n = 30$) that were known to be informative in other parrot species. Based on this pilot study we selected eight informative loci for further analysis (all were dinucleotide repeats): Cfor1415, Cfor2627, Cfor3031 (Chan *et al.* 2005), pCl3 (White *et al.* 2009), and SCMA 01, SCMA 04, SCMA 07, SCMA 29 (Olah *et al.* 2015).

Laboratory analysis followed Chapter 10 but briefly, M13 PCR tags were attached to all forward primers (Schuelke 2000) and we amplified all loci individually. PCR products were multiplexed in the same lane using different fluorescent tags and genotyped on an ABI 3130XL sequencer (Applied Biosystems, Foster City, CA, USA). We used water instead of DNA extract as a negative control for contamination checking and with each genotyping run, in one out of 16 capillaries we always included the same sample as positive control to ensure consistent size scoring across all genotyping runs. Results were scored using Geneious version R6 (Kearse *et al.* 2012) with full genotypes constructed across 8 loci. Approximately, 25% of the samples were repeated to estimate genotyping errors. Loci were screened for the presence of null alleles across all samples with MicroChecker 2.2.3 (Van Oosterhout *et al.* 2004). Samples with more than four missing loci were excluded from subsequent analysis.

Table 12.1. Source of swift parrot samples per region and per season.

Region/Year	2010	2011	2012	2013	2014	2015	Total per Region
North Bruny Island (BN)	–	1	–	10	–	21	32
South Bruny Island (BS)	–	2	12	4	–	5	23
Buckland (BU)	3	–	–	9	1	–	13
Eastern Tiers (ET)	–	8	1	6	1	–	16
Meehan Range (ME)	8	–	–	–	–	–	8
Rheban (RH)	–	–	–	–	5	–	5
Southern Forests (SF)	–	–	3	–	3	–	6
Wielangta (WI)	6	–	–	–	–	–	6
Total per Year	17	11	16	29	10	26	109

12.2.4. Population genetic structure analyses

We tested for deviations from Hardy–Weinberg Equilibrium in GenePop 3.4 (Raymond and Rousset 1995) using an exact probability test (Markov chain parameters were set to 100 batches with 1,000 iterations per batch). We first treated the entire sample set as a single population representative of the entire species. We also performed separate analyses for island and mainland subsets. We combined the exact *P* values using Fisher's method and report the chi-square test across all loci and populations. We used GenAlEx 6.5 (Peakall and Smouse 2006; Peakall and Smouse 2012) to calculate allele frequencies, observed and expected heterozygosities, inbreeding coefficients, G-statistics, probability of identity (*PI*), and

probability of identity for siblings (PI_{sibs}). We also used the analysis of molecular variance (AMOVA) framework offered within GenAlEx to partition genetic variation within and among *a priori* defined populations and regions (defined above). This AMOVA analysis provided estimates of overall and pairwise population genetic differentiation (F_{ST}), differentiation among regions (F_{RT}), differentiation among populations within regions (F_{SR}) (Excoffier *et al.* 1992; Peakall *et al.* 1995), and their standardized (0,1) equivalents (Meirmans 2006; Meirmans and Hedrick 2011). We performed tests for departure from the null hypothesis of no genetic differentiation using 1,000 random permutations and interpolated the missing data (Peakall and Smouse 2006).

To identify potential population genetic structure in the absence of any *a priori* grouping of the samples, we used the Markov chain Monte Carlo (MCMC) Bayesian clustering approach implemented in the program STRUCTURE 2.3.4 (Pritchard *et al.* 2000). For this analysis we ran the admixture model, with correlated allele frequencies and no location priors (Falush *et al.* 2003). Burn-in was set to 50,000 iterations, followed by 50,000 MCMC iterations replicated 10 times for each value of the number of genetic clusters (K) from 1 to 10. We used STRUCTURE Harvester (Earl and vonHoldt 2012) and the 'CorrSieve' package of R (Campana *et al.* 2011) to determine K (Evanno *et al.* 2005).

We used GenAlEx to test for isolation-by-distance across the study area using a Mantel test (Mantel 1967) based on individual genotypes. We used 10,000 random permutations at the individual level to test for departure from the null hypothesis (no spatial genetic relationships).

12.2.5. Relatedness estimates

We tested the possibility that related individuals may prefer to nest together using the '*compareestimators*' function of the '*related'* package in R (Pew *et al.* 2015) to compare the performance of four relatedness estimators simulated from the original dataset. Given similar performance, we selected the Lynch & Ritland (1999) estimator and used the '*grouprel*' function to analyse relatedness within populations using 1,000 iterations.

We used GenAlEx to calculate mean pairwise relatedness (Lynch and Ritland 1999) of each pair of individuals within our sample compared to mean relatedness among all samples, and estimated the 95% confidence interval for mean pairwise r values via bootstrapping (Beck *et al.* 2008). We used random permutation of the data to generate a distribution for the null hypothesis (no relatedness within groups) and test for significance (process performed 1,000 times). We included a control group of 15 siblings from five nests. We also used individual-focused multilocus spatial autocorrelation analysis for each sex to investigate potential sex-biased dispersal.

12.2.6. Population genetic simulations

In order to test the power of the microsatellite markers to detect potential population genetic structure ($F_{ST} > 0$ = 'genetic differentiation'), and given the constraints on the total sample size, we implemented the program POWSIM v4.1 (Ryman and Palm 2006). This program creates simulated populations of an expected divergence without the effects of migration or mutation, based on specific demographic parameters. As input we provided total allele frequencies from our study to simulate the process of genetic drift, at these starting allele frequencies, and defined effective population size(s). The program then subsamples the

simulated populations at specified generations for given sample sizes, and performs a genetic homogeneity test for equivalent allele frequencies ($F_{ST} = 0$). The proportion of significant outcomes offers an estimate of the power of a given marker set to detect genetic differentiation, given the sampling frame. We set the effective population size (N_e) to 1,000 and repeated the entire process of drift, sampling and statistical testing 10,000 times.

Using POWSIM, we first estimated the type I (α) error (i.e. falsely rejecting the null hypothesis of $F_{ST} = 0$ no differentiation) by setting drift to zero (sampling directly from the base population) and using different sample sizes (n = 10, 25, 50 and 100) from two generated populations (i.e. mainland and island). Second, we assessed the power to detect different F_{ST}values (N_e = 1000, t = 10, F_{ST} = 0.005; or N_e = 1000, t = 20, F_{ST} = 0.01) between two populations using different sample sizes (n = 10, 25, 50 and 100). We report Pearson's chi-square and Fisher's exact test, following the calculation in GENEPOP 3.4 (Raymond and Rousset 1995). In order to show the effect of allelic losses during the simulations, we report separate results from both runs where all original alleles were preserved and also runs where allelic loss had occurred (Ryman *et al.* 2006).

In a complementary simulation approach, we also used the program EASYPOP v2.0.1 (Balloux 2001) to estimate how many generations of restricted gene flow would be required to develop genetic differentiation similar to that found in our empirical dataset. We used equivalent parameter settings to POWSIM to simulate two populations (each including 250 males and 250 females, i.e. N_e = 1,000) with mating set to monogamy with 40% extra pair copulation events (as observed in the wild swift parrot populations, R. Heinsohn unpublished data) isolated over 100 generations with different migration rates (m). We used Wright's island model to simulate isolation, using seven unlinked microsatellite loci (with mutation rate of 0.0005) and five possible allelic states (matching the average effective allele number of our loci), setting maximum variability in the initial populations (randomly assigned alleles).

In the first group of models, we simulated panmixia in the first 10 generations (m = 0.75 for both males and females), before setting a different migration rate for another 100 generations (m = 0, 0.001, 0.01, 0.05 and 0.1). In the second group of models, we simulated two divergent populations with F_{ST} = 0.02 (m = 0 for 40 generations), and then set different migration rates for another 70 generations as above. We then calculated the average unbiased F_{ST} values for each simulated generation based on 100 repeats.

12.3. Results

We obtained genetic data for 109 individuals (Table 12.1), comprising 63 males and 46 females (one sample per nest).

12.3.1. Microsatellite validation

For the locus Cfor3031 the software MicroChecker indicated the presence of null alleles (estimate of Oosterhout frequency of null alleles = 0.166), hence we excluded it from further analyses. Based on repeated genotypes, across the seven loci the average scoring error was 0.5% (range: 0–2.2%), and the average allelic dropout was 12.9% (range 8.6–19.4%). Across all seven loci the allele number ranged from 3 to 20 per locus.

12.3.2. Tests for Hardy–Weinberg Equilibrium

We found no deviation from Hardy–Weinberg Equilibrium across all seven loci at the species level (Fisher's $\chi^2 = 9.55$, d.f. = 14, $P = 0.795$), or evidence for heterozygote deficit ($P = 0.326$, se = 0.029) or heterozygote excess ($P = 0.674$, se = 0.029). Only the locus Cfor3031 showed significant deviation from Hardy–Weinberg Equilibrium, but we excluded it based on the MicroChecker analysis for null alleles. We found no deviation from the Hardy–Weinberg Equilibrium when the Tasmanian mainland (Fisher's $\chi^2 = 13.83$, d.f. = 14, $P = 0.463$) and the offshore island populations (Fisher's $\chi^2 = 6.15$, d.f. = 14, $P = 0.963$) were considered separately. Mean expected heterozygosity among populations was 0.685 and observed heterozygosity values ranged from 0.42 to 0.9 (mean 0.679). The overall average fixation index was 0.007 (Table 12.2), consistent with Hardy–Weinberg Equilibrium. The five most variable loci ($PI_{sibs}(5) = 0.009$) were predicted to recover all unique genotypes, given the sample size.

Table 12.2. Population statistics for seven microsatellite markers of dinucleotide repeats for swift parrots. Column headings (from left to right) are: locus name, number of samples (N), fragment size range (bp), number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_O), expected heterozygosity (H_E), fixation index (F).

Locus	N	bp	N_a	N_e	H_O	H_E	F
Cfor1415[a]	112	204–214	6	3.221	0.652	0.689	0.055
Cfor2627[a]	111	142–180	17	6.397	0.874	0.844	−0.036
pCl3[b]	112	112–122	4	1.664	0.429	0.399	−0.074
SCMA 01[c]	110	160–206	20	10.364	0.9	0.904	0.004
SCMA 04[c]	99	251–291	17	7.054	0.848	0.858	0.011
SCMA 07[c]	106	283–301	8	2.946	0.632	0.661	0.043
SCMA 29[c]	100	220–224	3	1.787	0.42	0.441	0.047
Mean			10.7	4.776	0.679	0.685	0.007

Species of origin for the genetic markers were: [a]*Cyanoramphus forbesi*, [b]*Calyptorhynchus latirostris*, and [c]*Ara macao*.

12.3.3. Genetic differentiation in space and time

We failed to detect any evidence of population genetic structure in swift parrots. The genetic distance based AMOVA analysis partitioned only 0.17% of genetic variation between the mainland versus island regions ($F_{RT} = 0.002$, $F'_{RT} = 0.006$, $P = 0.258$) and 0.22% among populations within these regions ($F_{SR} = 0.002$, $F'_{SR} = 0.007$, $P = 0.325$). Furthermore, no significant differences in F_{ST} were detected for any pairwise comparison between the populations (AMOVA: $F_{ST} = 0.004$, $P = 0.175$), or over time (AMOVA: 0.37% among years, $F_{ST} = 0.004$, $F'_{ST} = 0.013$, $P = 0.201$). Using alternative allele frequency-based G-statistics analysis also failed to detect any significant genetic differentiation (overall $G_{ST} = 0.002$, $P = 0.333$; overall $G''_{ST} = 0.009$, $P = 0.332$),

The STRUCTURE analysis outcome was consistent with the AMOVA and G-statistics analyses, indicating a single genetic cluster and no detectable population boundaries (see supporting information in Stojanovic *et al.* 2018). Finally, at the level of individual genotypes, no significant isolation-by-distance was detected across the study (Mantel test >180 km, $N = 109$, $r = 0.048$, $P = 0.096$), nor within mainland Tasmania (Mantel test >180 km, $N = 54$, $r = 0.362$, $P = 0.362$).

12.3.4. Relatedness and sex-biased dispersal

Mean pairwise relatedness estimates (Lynch and Ritland 1999) did not indicate higher than expected relatedness at any nesting site in either the 'related' or in the GenAlEx analyses. We also did not detect significant local individual-by-individual spatial genetic structure when females and males were considered separately (Figure 12.3), indicating no evidence for a strong sex-biased dispersal pattern (Banks and Peakall 2012).

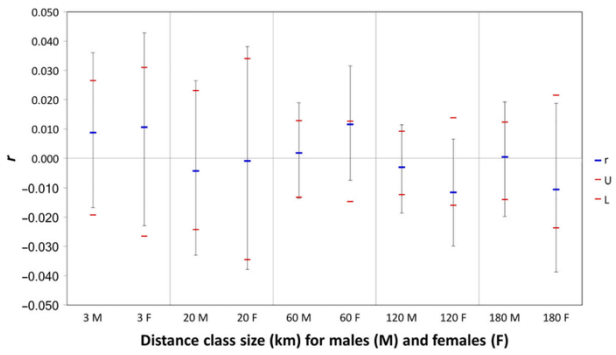

Figure 12.3. Sex-specific spatial genetic autocorrelation analysis for swift parrots comparing correlations for females versus males across study sites. The figure shows pairwise genetic distance (rc) for increasing distance class sizes (blue), 95% confidence intervals (red) about the null hypothesis of a random distribution and 95% confidence error bars about rc as determined by bootstrapping (black).

12.3.5. Population genetic simulations

The PowSim results confirmed that risk of type I errors was below 5% across the full range of sample sizes that characterized this study (10–100). For our sample of individuals from mainland and island populations respectively, the simulation results indicated almost complete power (96%) to detect genetic differentiation if the real F_{ST} was 0.01 and reasonable power (67%) to detect differentiation if the real F_{ST} was 0.005.

The EasyPop simulations confirmed that genetic differentiation develops quickly in the absence of any gene flow, as expected (even under the one-migrant-per-generation scenario with $m = 0.001$). With extensive gene flow of $m = 0.1$ per generation, a stable and low level of differentiation developed and persisted at $F_{ST} \sim 0.001$. Conversely, when we started the EasyPop simulation at $F_{ST} \sim 0.02$, differentiation declined to a similar low level of $F_{ST} \sim 0.001$ at $m = 0.1$ per generation. Under more restricted gene flow of $m \geq 0.01$, F_{ST} exceeded 0.01 in the later generations of the simulation (see supporting information in Stojanovic *et al.* 2018).

12.4. Discussion

In this study, we present the first population genetic data for the critically endangered swift parrot. PVAs predict swift parrot population decline by 94% over just three generations (Heinsohn *et al.* 2015), prompting recent revision of the species conservation status (BirdLife International 2016). The assumptions underlying PVAs must be rigorously tested, particularly given the implications of Heinsohn *et al*. (2015) for swift parrot conservation and forest management. Here, we confirm a key assumption of Heinsohn *et al*. (2015), that is, the entire swift parrot population is a single, panmictic conservation unit and that isolated subpopulations do not exist on predator-free islands.

Our multifaceted population genetic analysis, which included robust tests for Hardy–Weinberg Equilibrium, and multiple tests for population genetic structure including AMOVA, G-statistics, Structure analysis and Mantel tests, found congruent and highly consistent results.

No significant deviation from Hardy–Weinberg Equilibrium expectations was detected at either the species or regional levels (island vs. mainland), consistent with a very low average inbreeding coefficient ($F'_{SR} = 0.007$). We found no evidence for any significant population genetic structure across geographically defined regions, or among populations defined by space or time. Neither was any isolation-by-distance detected at the individual genotype level.

Our PowSim simulation confirmed that despite the challenges of collecting enough genetic material from a critically endangered nomadic population, we had adequate power to detect genetic structure ($F_{ST} > 0$) if it existed between islands and the Tasmanian mainland. However, the simulation drift process used in our method is not intended to replicate the true demography of the studied population, but to simulate their evolutionary history by randomly distributing the original alleles in a biologically reasonable way (Ryman *et al.* 2006).

We found that F_{ST} was not significantly different from zero for swift parrots (mean $F_{SR} = 0.002$, $P < 0.325$). The EasyPop simulations indicated that high levels of contemporary gene flow best explain the observed lack of genetic differentiation. For example, at migration rates of $m = 0.01$ or less, genetic differentiation developed quickly in the simulations and reached a stable $F_{ST} > 0.01$. Similarly, when starting the simulations at $F_{ST} = 0.02$, differentiation quickly declined at higher migration rates ($m = 0.01$ or more). We note that the high migration rates associated with the lower levels of differentiation are at least an order of magnitude higher than one migrant per generation ($m = 0.001$). The one migrant per generation rule of conservation genetics is considered a minimum level of gene flow to avoid loss of heterozygosity among populations (Wang 2004). However, our simulations support the well-known caveat that one migrant per generation does not imply panmixia of different populations (Mills and Allendorf 1996).

We conclude that our genetic findings collectively and consistently support the null hypothesis of a randomly mating, single panmictic swift parrot population. These genetic findings support predictions based on the monitoring of landscape scale fluctuation in food availability and population settlement patterns by swift parrots (Webb *et al.* 2014; Webb *et al.* 2017). Those studies showed major fluctuation in the proportion of the swift parrot population that settled in island or Tasmanian mainland breeding habitats in any given year, as a consequence of annual variation in food availability (Figure 12.2). For example, in some years breeding mainly occurs on the Tasmanian mainland (Figure 12.2b, c, f) with few or no birds breeding on islands. In other years both the mainland and islands are used (Figure 12.2e), whereas in some year breeding is almost exclusively on islands (Figure 12.2a, d). Such variable patterns of breeding indicate extensive gene flow among these habitats, consistent with the genetic results.

Our findings contrast with studies of other parrots, which typically show some degree of significant population genetic structure over space (White *et al.* 2014), but not always (Wright and Wilkinson 2001; Wright *et al.* 2005). However, our results are broadly consistent with the few other genetic studies of mobile resource specialists, for example comparable nectarivores (Kvistad *et al.* 2015), owls (Marthinsen *et al.* 2009) and seed eating passerines (Questiau *et al.* 1999), where population genetic structure is limited or absent. Given the ecological similarities between our study system and those of other mobile resource specialists, swift parrots may be a good model species for understanding how resource fluctuation and encounters with threatening processes impact mobile populations more generally.

Our study highlights the potential population-level consequences of spatial overlap between unpredictable resource pulses (Webb *et al.* 2017) and a widespread predator (Stojanovic *et al.* 2014). Predator-free islands support <30% of the swift parrot population in a given year (Heinsohn *et al.* 2015), but our study shows that these birds do not comprise a genetically isolated subpopulation. When food availability is limited on islands, most swift parrots settle on the Tasmanian mainland (Figure 12.2) where, at some locations, sugar glider nest predation rates can be as high as 100% (Stojanovic *et al.* 2014). When mobile species operate as a single conservation unit, localized threats may, given enough time, impact the entire population (Runge *et al.* 2014). Therefore, it follows that conservation action in predator infested mainland habitats will be critical to prevent extinction of swift parrots. This must include limiting deforestation in breeding habitat (which may be related to predation severity), augmenting nesting habitat on islands, and protecting parrot nests in sugar glider infested forests.

For mobile species in unpredictable environments, lack of isolated subpopulations and few conservation units increases overall extinction risk when resources overlap with threats. Our results support the argument that in addition to targeting conservation action for mobile species at broad spatial scales (Cottee-Jones *et al.* 2016), conservation efforts for mobile species should focus on reducing the likelihood that resources could attract animals into areas where threatening processes occur (Runge *et al.* 2014). Where this is unavoidable due to low habitat availability, targeted conservation action at critical sites may be necessary to alleviate population level impacts of threatening processes.

13. Applying genetic tools to study the breeding system of the swift parrot

13.1. Introduction

Anthropogenic threats to wild populations may impact differentially on individuals, biasing mortality in relation to age, size or sex (Boukal *et al.* 2008; Garcia *et al.* 2012). When such mortality is sex-biased, the adult sex ratio (ASR) may become skewed with potentially severe consequences for population stability (Boukal *et al.* 2008). Although theory suggests that the impacts on individuals and populations of fluctuations in the ASR of undisturbed wild populations are buffered by higher intra-sexual competition in the abundant sex (Greenwood 1980; Clutton-Brock *et al.* 2002), empirical studies have shown that increased male bias can lead to suppressed survival and reproduction in females. Such negative effects on females were demonstrated experimentally in common lizards (*Lacerta vivipara*) which had more injuries, higher mortality and fewer offspring when ASRs were male-biased (Le Galliard *et al.* 2005). However, other studies have failed to find such effects on female fitness or demography in spite of clear and sometimes dramatic evidence that harassment of females increases when the ASR is male-biased (Ewen *et al.* 2011).

Greater competition by males for females due to biased ASRs may also lead to changes in the mating system, primarily from monogamy to polyandry. Social polyandry is the rarest of avian mating systems and falls into two distinct categories depending on whether the females mate sequentially with single males who then care for the clutch alone (classical polyandry), or with multiple males who care for the clutch together (cooperative polyandry) (Faaborg and Patterson 1981). Whereas classical polyandry is a fixed mating system for a small proportion of bird species, cooperative polyandry appears to be more flexible within species, occurring when either females or the resources they need for nesting are scarce, which may then lead to males sharing females (Hartley and Davies 1997). Even large, long-lived taxa that are normally monogamous can change to cooperative polyandry when the ASR becomes dramatically male-biased (Carrete *et al.* 2006; Heinsohn *et al.* 2007; Janssen *et al.* 2008). In addition, many avian species exhibit genetic but not social polyandry as a result of extra-pair copulations by females (Westneat and Stewart 2003).

Polyandry may increase both intra-sexual conflict for mating opportunities and inter-sexual conflict such that males and females have differing optimal outcomes, for example, in the amount of male care of offspring (Kokko and Jennions 2012) and may lead to male adaptations that are harmful to females (Arnqvist and Rowe 2013). In Seychelles magpie robins (*Copsychus sechellarum*), intraspecific conflict was shown to slow down population recovery, whereas the addition of an extra male to breeding pairs of bearded vultures, *Gypaetus barbatus*, showed that males can behave in their own reproductive interests at the expense of females who suffered lower breeding success in trios (Carrete *et al.* 2006). Although theoretical models and some empirical research suggest that inter-sexual conflict may become especially harmful as male bias in the ASR increases, there are still few examples that consider the full life-history consequences and the impact on population growth and viability (Holman and Kokko 2013).

Male-biased adult sex ratios are the norm in birds (Donald 2007), yet most bird species remain socially monogamous, and polyandry when it occurs is usually genetic rather than social (Gowaty and Black 1996). Parrots as a taxon are considered to be mostly socially and genetically monogamous (Toft and Wright 2015) but have been shown in a few circumstances to adopt cooperative polyandry when females have limited breeding opportunities placing further constraints on males (Ekstrom *et al.* 2007; Heinsohn *et al.* 2007). In this chapter, we outline a revealing case of a parrot species that appears to have adopted high rates of genetic polyandry under recent circumstances where anthropogenic influences have dramatically altered the ASR in favour of males. Introduced sugar gliders (*Petaurus breviceps*) kill breeding female swift parrots (*Lathamus discolor*) in their nest hollows, usually while they are incubating eggs, across breeding sites in Tasmania (Stojanovic *et al.* 2014). Breeding males have not been observed to suffer additional mortality from sugar gliders. The birds are nomadic and gain a limited reprieve from sugar glider predation in occasional years when ephemeral food resources allow them to nest on predator free islands (Webb *et al.* 2014) but the mean annual mortality of adult females is none the less extremely high at over 50% per year. We have demonstrated via population viability analysis (PVA) that the swift parrot population is in dramatic decline from the impact of predation alone with a projected decrease of over 90% in 16 years (Heinsohn *et al.* 2015).

Here, we use data from a 6-year study to test the prediction that biases in the swift parrot ASR created by sex-specific predation push the mating system from monogamy towards genetic polyandry, and that genetic polyandry in turn entails negative consequences for reproductive success and population viability (Holman and Kokko 2013). Our analysis provides an important demonstration that, together with the direct impacts of increased mortality on adult females and nestlings, biases induced in the ASR can have further negative impacts on long-term population viability via costs associated with increased rates of polyandrous mating.

13.2. Methods

13.2.1. Study system

Swift parrots are a socially monogamous, migratory species that breeds along the eastern seaboard of the large island of Tasmania off southern Australia, and two smaller islands (Bruny and Maria) close to the east coast of Tasmania (Forshaw 2006). They require overlap of ephemeral nectar food resources (flowering *Eucalyptus globulus* and *E. ovata*) and nesting habitat (tree cavities in old growth forest) for successful breeding (Webb *et al.* 2017). Swift parrots are nomadic within their breeding range to the extent that breeding may occur anywhere in eastern Tasmania where an appropriate combination of habitats occurs each year (Figure 12.1). However, in any given breeding season, only a fraction of the broader breeding range is occupied depending on where food is available (Webb *et al.* 2017). A recent study confirmed a lack of population genetic structure in swift parrots with the whole population likely to move between breeding locations each year (Stojanovic *et al.* 2018; Stojanovic *et al.* 2019). The present study was conducted across a range of forest types over most of the breeding range between 2010 and 2016 (Figure 12.1).

Swift parrots lay a clutch of three to (rarely) six eggs. Females perform all incubation and care of nestlings up to 10 days after hatching; however, males make large contributions to feeding nestlings after this time. Extra-pair males have been observed courtship feeding the breeding female but these are often chased aggressively from the nest area by the pair male (unpublished data).

Nesting swift parrots suffer intense predation by sugar gliders (Stojanovic *et al.* 2014). Sugar gliders are native to continental Australia, but were introduced to Tasmania as early as the 19th century (Campbell *et al.* 2018). Importantly, sugar gliders are now present at all swift parrot breeding sites thus far monitored on the main island of Tasmania, although rates of predation on breeding females vary considerably. They are absent from Bruny and Maria Islands where the swift parrots sometimes breed (Figure 12.1).

13.2.2. Genetic sample collection

DNA was analysed for 371 nestlings from 85 nests that had more than one nestling over six breeding seasons. Genetic samples were not available for Maria Island or Devonport, but all other sites considered by Heinsohn *et al.* (2015) were included in this study. Swift parrot nests were identified across the study area during standardised monitoring (Webb *et al.* 2014). Nests were identified using behavioural cues of swift parrots and accessed using single rope climbing techniques (Stojanovic *et al.* 2015). Nestling swift parrots were temporarily removed from their nest cavities (Stojanovic *et al.* 2015), and blood was collected using brachial venepuncture. Blood was stored on FTA paper (Whatman™).

13.2.3. DNA extraction and microsatellite genotyping

DNA extraction from blood stored on FTA paper was performed following the standard procedure for nucleated erythrocytes (Smith and Burgoyne 2004). We used seven microsatellite loci previously used for swift parrots: Cfor1415, Cfor2627 (Chan *et al.* 2005), pCl3 (White *et al.* 2009), and SCMA 01, SCMA 04, SCMA 07, SCMA 29 (Olah *et al.* 2015). Laboratory analysis followed Chapter 8. Briefly, M13 PCR tags were attached to all forward primers (Schuelke 2000) and all loci were amplified individually. PCR products were multiplexed in the same lane using different fluorescent tags and genotyped on an ABI 3130XL sequencer (Applied Biosystem). We used a negative control for contamination checking and a positive control to ensure consistent size scoring across all genotyping runs. Results were scored using geneious version r6 (Kearse *et al.* 2012) with 112 full genotypes constructed across seven loci. Approximately, 25% of the samples were repeated to estimate genotyping errors. Loci were screened for the presence of null alleles across all samples with microchecker 2.2.3 (Van Oosterhout *et al.* 2004).

13.2.4. Genetic relatedness classification and detection of multiple paternity

For classification of relatedness, we used a subset of the total samples ($N = 291$) that contained only nestlings with a maximum of one missing locus and with at least two siblings per nest. We followed the two-program congruency approach described in Turjeman *et al.* (2016) to determine relationships among nestlings. First, we used the software program ml-relate (Kalinowski *et al.* 2006) to determine the most likely pairwise relationships. Then, we used the program colony 2 (Jones and Wang 2010) to confirm or discard relationship

classifications. We used the following relationship categories for pairwise relatedness between siblings in each software: full-siblings (FS), half-siblings (HS), unrelated (U), not full-siblings (NFS; where "full-siblings" relationship could be rejected but differentiation between the categories of "half-siblings" and "unrelated" could not be made) and non-conclusive (NC) cases where conclusions could not be reached. For both software programs, we used the settings described in (Turjeman *et al.* 2016). When ML-RELATE and COLONY 2 did not give the same results, we used the following rules: (a) when ML-RELATE showed an NFS relationship and COLONY 2 showed a HS, we accepted HS; (b) when ML-RELATE showed NC, we accepted the COLONY 2 result. We classified nests as FS (if all sibling pairs had FS relationships) or HS (if at least one sibling pair had a HS or NFS relationship). Nests with more than 50% NC relationships were not classified.

We also looked for extra cases of multiple paternity that were not detected by the relatedness analysis above. We used the number of different alleles within families, and looked for cases where the number of alleles exceeded the maximum possible under a scenario of single paternity. These included instances where all individuals were heterozygous and the number of different alleles exceeds four, or one nestling was homozygous and the number of different alleles exceeds three. We used Fisher's exact test comparing FS nests to any other categories to see whether swift parrots deviate significantly from genetic monogamy (allowing a 1% of EPC of all copulations).

13.2.5. Adult sex ratios, reproductive success and population viability analyses

Following the methods of Stojanovic *et al.* (2014) using the program mark (White and Burnham 1999), we compiled mortality rates of nesting females due to predation by sugar gliders for seven regions shown in Figure 12.1 (north and south Bruny Island were combined into one region for this purpose). We measured fledging success for all monitored nests as the number of nestlings expected to fledge as of the last nest inspection.

We modified previously published population viability analyses (Heinsohn *et al.* 2015) using vortex 10 (Lacy and Pollak 2014) to estimate (a) the population-wide ASR at the beginning of each breeding season, and (b) the long-term impact on population size of monogamous versus polyandrous breeding. We used the settings of the preferred model from our previous analysis, see Model 2 and Table 1 in Heinsohn *et al.* (2015), as these comprise a realistic portrayal of the population including the mean proportion of the birds that nested at high predation sites (on mainland Tasmania) versus low predation sites on offshore islands.

To estimate the population-wide ASR for each year of the study, we used Model 2 in Heinsohn *et al.* (2015) to estimate the number of adult (2 years old and over) males and females remaining at the end of each breeding season (i.e., after predation on nesting females). We used these values to estimate the population-wide proportion of adult males at the start of the next breeding season from 2010 until 2015. The published PVAs (Heinsohn *et al.* 2015) used mean predation rates on adult females of 56.4% over a number of years in a largely deterministic model. However here, we used the mortality rates specific to each year, calculated from the proportion of the parrot population that nested in predator infested habitat, to determine changes to male and female numbers, and hence annual variations in the ASR, more precisely. Annual adult female mortality rates, including background mortality and that caused by sugar

gliders, calculated for use in the models were as follows: 2010, 56.4%; 2011, 58.5%; 2012, 61.7%; 2013, 52.4%; 2014, 53.0%; and 2015, 61.7%.

We used Generalised Linear Models in the statistical package Genstat (12th Edition) (Payne 2009) to analyse spatial and temporal factors affecting the frequency of shared paternity, and the impact of skewed adult sex ratios and shared paternity on reproductive success. Nests were assigned a binary response (multiple paternity = yes, single paternity = no) and analysed in a GLM with binomial link function. The number of fledglings produced at each nest was analysed with a GLM using a Poisson link function. The number of eggs/nestlings was included as a variate in all models. Time of season was tested and controlled for in all analyses by including as a variate the number of days since the first breeding attempt by any bird within the same season. Nest hollows were not known to be re-used within or between seasons so were only used once in each analysis.

We constructed three new PVA models to isolate the impact on the population projection of increased levels of shared paternity associated with higher mortality of females. We kept the high predation rate on adult females and other settings, including a starting population of 2158 individuals, and other values used in Model 2 of Heinsohn *et al.* (Heinsohn *et al.* 2015)but adjusted population-wide reproductive success to three levels. Model A explored population size after 16 years (3 generations) if shared paternity occurred at the lowest rate observed in this study (33%) and consequently the population enjoyed higher breeding success. Model B examined the final population size if shared paternity occurred at the mean levels observed in this study (50.5%). Model C predicted final population size if shared paternity occurred at the highest rate recorded in our study (95%).

13.3. Results

13.3.1. Population genetics, relatedness and mating system

The total number of alleles per locus ranged between 3 and 20, mean observed heterozygosity was 0.68, while the expected heterozygosity value was 0.683 (Table 13.1). The variability of all seven microsatellite loci was predicted to recover all unique genotypes even among siblings, over our large sample of individuals ($PI_{sibs(7)} = 0.002$, $N = 94–111$, Table 13.1).

Table 13.1. Summary of microsatellite diversity showing the number of alleles (N_a), effective number of different alleles (N_e), observed heterozygosity (H_O), expected heterozygosity (H_E), fixation index (F), probability of identity (PI) and probability of identity for siblings (PI_{sibs}).

Locus	N_{Tot}	N_{Sub}	N_a	N_e	H_O	H_E	F	PI	PI_{sibs}
CI3	349	111	5	1.6	0.369	0.395	0.065	0.431	0.660
C1415	346	110	6	3.1	0.636	0.681	0.065	0.154	0.448
SCMA04	310	98	17	6.5	0.867	0.847	−0.024	0.036	0.336
C2627	350	111	17	7.0	0.892	0.857	−0.041	0.035	0.330
SCMA01	346	108	20	10.2	0.870	0.902	0.035	0.017	0.303
SCMA07	331	104	8	2.8	0.644	0.651	0.011	0.149	0.462
SCMA29	304	94	3	1.8	0.404	0.448	0.098	0.392	0.624
Over all loci								8.4E-08	2.9E-03
Mean			10.9	4.8	0.669	0.683	0.030		
SE			2.6	1.2	0.083	0.076	0.019		

We analysed a total of 374 pairwise relationships between siblings and found 264 (70.6%) FS and 74 (19.8%) HS relationships, while in 36 (9.6%) cases conclusions could not be reached. Out of the total 85 nests used for this analysis, in 82 cases (96.5%), we successfully classified at least 50% of the siblings per nest. Among these resolved nests, 60% ($N = 49$) contained only full-siblings, while 40% ($N = 33$) contained at least one half-sibling relationship. We reconfirmed seven cases, and found ten extra cases, of multiple paternity using the number of different alleles within families, bringing the number of nests with multiple paternity to 43/85 (50.5%). The proportion of nests with at least one half-sibling was significantly higher than expected under a monogamous breeding strategy (Fisher's exact test, $P < 0.001$).

13.3.2. Adult sex ratios, frequency of multiple paternity and impact on reproductive success

The modelled trajectories over the study for adult males and females, and the resulting ASR expressed as proportion of males, are shown in Figure 13.1. The estimated proportion of males in the adult population at the start of each breeding season varied little, ranging from 0.73 to 0.75.

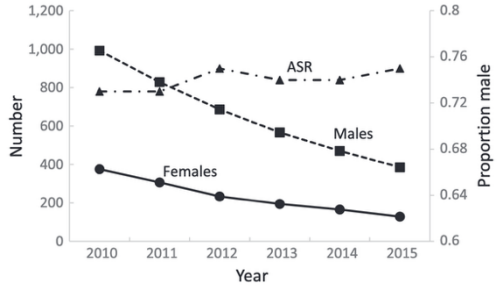

Figure 13.1. The modelled number of adult (2+) males (squares) and females (circles) (left axis) in each of the 6 years in our study and the resulting proportion of adult males (ASR, triangles) (right axis).

Rates of multiple paternity varied significantly across years ($\chi^2_5 = 2.81$, $p = 0.015$) but were not significantly affected by the number of nestlings in the brood (range = 2–5, $\chi^2_1 = 0.54$) or timing of breeding within the season ($\chi^2_1 = 2.08$). There was no significant effect of the limited range of population-wide ASRs reported above on the likelihood of multiple paternity ($\chi^2_1 = 0.94$). However, swift parrots settled to breed in different areas within and between seasons over the study (Webb *et al.* 2017), and multiple paternity increased significantly at sites where there was higher predation on nesting females ($\chi^2_1 = 4.26$, $p = 0.039$, Figure 13.2A). This suggests that local changes to the ASR, caused by loss of adult females to predators while nesting, were a determinant of whether polyandrous mating occurred at the remaining nests. The predation rates on breeding females at seven breeding sites used in this analysis, calculated using the program mark (Stojanovic *et al.* 2014), are given in the caption to Figure 13.2. There were no significant interactions between any of the variables presented above ($0.150 < p < 0.980$).

Clutch size did not differ significantly across sites ($\chi^2_1 = 1.67$). However, fewer fledglings were produced at unpredated nests as the site-specific predation rate on adult females increased ($\chi^2_1 = 4.63$, $p = 0.031$, Figure 13.2B), suggesting that local differences in the ASR caused by loss of adult females to predators while nesting, were a determinant of nest success. There was no significant difference in number of fledglings at single and multiple paternity nests ($\chi^2_1 = 1.90$), or between years ($\chi^2_1 = 2.01$).

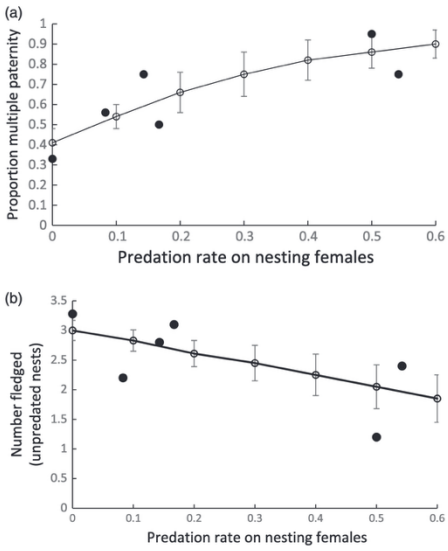

Figure 13.2. (A) observed (closed circles) and predicted proportion (open circles) ± *SE* of nests with multiple paternity against rates of predation on nesting adult females. (B) Mean number of fledglings (closed circles) and number predicted (open circles) ± *SE* for unpredated nests against the site-specific rate of predation on nesting females (NB two sites with predation rate of 0.5, Rheban and Southern Forests, are presented as one value in both A and B). The predation rates on breeding females at seven breeding sites used in this analysis, calculated using the program mark, were as follows: Bruny Island (0, $n = 56$), Buckland (0.08, $n = 19$), Meehan Range (0.14, $n = 9$), Wielangta (0.17, $n = 7$), Rheban (0.50, $n = 6$), Southern Forests (0.50, $n = 16$), and Eastern Tiers (0.54, $n = 29$).

13.3.3. Impact of shared paternity on population viability

Predicted final population sizes differed significantly between the three modelled PVA scenarios ($p < 0.001$) demonstrating the impact on population size of lower reproductive success associated with shared paternity (Figure 13.3). Model A, using the reproductive success when rates of shared paternity were lowest, predicted that the swift parrot population would decline by 89.4% over three generations. This compares with a population reduction of 92.1% under mean rates of shared paternity (Model B) and 94.9% if shared paternity is at its highest level observed in this study (Model C).

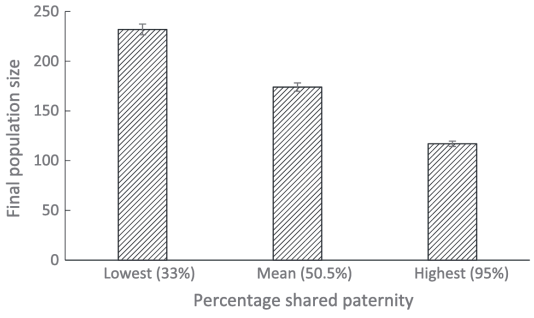

Figure 13.3. Simulated population size (± s/e) after 16 years (3 generations) if swift parrots exhibit the lowest observed rate (33%) of shared paternity (Model A: population decrease = 89.4%), the mean rate (50.5%) of shared paternity reported here, (Model B: population decrease = 92.1%), and the highest rate observed in this study (95%), (Model C: population decrease = 94.9%).

13.4. Discussion

Critically endangered swift parrots are in steep population decline due to the impact of an introduced predator, the sugar glider, that preys on nesting females and their offspring (Stojanovic *et al.* 2014; Heinsohn *et al.* 2015). In addition to the direct impact on the remaining population, this study suggests that the strong male bias in the adult sex ratio due to sex-specific predation has further ramifications for individual fitness and population viability. Our results show that swift parrots have an unusually high (50.5% of nests) yet variable rate of shared paternity (genetic polyandry) compared to most parrot species (Toft and Wright 2015). Although we do not know the extent of shared paternity prior to the introduction of the predator, it is likely that the consistently male-biased ASR (≥73% male) further promotes this form of mating in this species (Emlen and Oring 1977). Results over our 6-year study support this

contention by showing that rates of shared paternity are higher for highly mobile swift parrots when they breed in regions with higher predation on nesting females, and hence with more (within season) male-biased ASRs. Here, we discuss likely causes of the high rate of shared paternity compared to other parrot species, and how the anthropogenically induced sex ratio bias in swift parrots affords an unusual opportunity to isolate the costs of genetic polyandry on individual fitness and population viability (Holman and Kokko 2013).

Our finding that paternity was shared at 50.5% of swift parrot nests adds to a small number of studies that challenge the traditional view that parrots are largely monogamous (Toft and Wright 2015). Studies of parrot mating systems using molecular techniques are still few but now include one species with 100% genetic monogamy in the burrowing parrots, *Cyanoliseus patagonus* (Masello *et al.* 2002), and other socially monogamous species with both modest, e.g. the green-rumped parrotlets, *Forpus passerinus* with 14% nests (Beissinger 2008), and higher rates of shared paternity, e.g. monk parakeets, *Myiopsitta monachus* with 40% nests (Martínez *et al.* 2013). A small number of parrot species are also known to have more extreme social and genetic mating systems including lek promiscuity, e.g. kakapos, *Strigops habroptilus* (Merton *et al.* 1984) and cooperative polyandry and polygynandry (Ekstrom *et al.* 2007; Heinsohn *et al.* 2007). The growing body of evidence suggests that parrots as a taxon may display a similar range and frequency of social and genetic mating systems to that seen in Passerines, the bird order now known to be most closely related to parrots (Jarvis *et al.* 2014). However, the parrot species thus far targeted for molecular analysis of parentage may be skewed towards the more unusual species, and further studies of socially monogamous parrots are required.

The more extreme mating systems found amongst parrots may help in interpreting the causes of high rates of shared paternity in swift parrots. *Eclectus* parrots (*Eclectus roratus*) for example breed polyandrously because limited availability of nest hollows places severe restrictions on the availability of breeding females, and encourages males to share mates, albeit with some conflict (Heinsohn *et al.* 2007; Heinsohn 2008). In swift parrots, genetic polyandry increases at breeding sites where female mortality is higher, suggesting that unpaired males target already paired females more when the local ASR becomes more male-biased. It should be noted that swift parrots have not been observed either to live in stable groups as happens in *Eclectus* parrots, or for the males to form coalitions to maximise their chances at achieving mating success (Hartley and Davies 1997). Instead, social monogamy prevails with the socially paired male aggressively chasing other males away from the nest, even though the females have been observed to accept food surreptitiously from the interlopers and to mate with them (unpublished data). Thus, unlike *Eclectus* parrots and other species, e.g., dunnocks, *Prunella modularis* (Davies and Quinn 1992), biases in the ASR appear to promote genetic but not necessarily social polyandry in swift parrots.

The diminished reproductive success of swift parrots when the ASR is male-biased may be driven by heightened inter-locus sexual conflict, or differences between the sexes in optimal breeding behaviour (Holman and Kokko 2013). The increase in shared paternity seen in these conditions does not increase reproductive success for females, as occurs for example in dunnocks (Davies and Quinn 1992), and instead appears to incur a cost to both females and pair males. The remaining females who have avoided predation lose some reproductive success as the ASR becomes more biased (Figure 13.2B), but pair males suffer greater losses if they

also share paternity. Our anecdotal observations suggest that lower reproductive success may be due to greater harassment by additional unpaired males that attempt to courtship feed, and mate with, nesting females. These interlopers are met with frequent aggression by the resident males who chase them from the nest area and may cause important losses of time and energy for the resident pair. Under this scenario, it is possible that females could accept extra mates without (or with less) cost to their reproduction but resident males, who have more to lose, behave in ways that protect their own optimum outcome at the expense of females. Bearded vulture trios also suffer lower breeding success than pairs but a major difference is that the males seem to co-exist more peacefully (Carrete *et al.* 2006).

Our study also offers rare insight into how increasing rates of shared paternity, in this case probably driven by biased ASRs, affect population viability. We partitioned the components of predicted population decline in swift parrots due to direct predation from those due to lowered breeding success when the ASR becomes more biased. This analysis predicts that the population of swift parrots will decrease by an additional 2.7% over 16 years due to the impact of lower reproductive success when shared paternity is at 50.5%, and that the decrease could be as much as 5.5% if shared paternity occurs at the highest rates recorded. It is important to note that we do not know the natural rate of shared paternity which may have been much lower before the advent of high sex–specific predation by sugar gliders. Reproductive success may have been even higher in the past if the ASR was more balanced and shared paternity was lower. Population growth rates in other threatened species have also been compromised by biased adult sex ratios but these studies have not evaluated the impact on long-term population trends due to a biased ASR (e.g., lower breeding success) beyond the simple lack of females as mates (Steifetten and Dale 2006; Gilroy and Lockwood 2012).

Studies have rarely tested for a link between the degree of polyandry and how this may affect population trajectories (Holman and Kokko 2013). Within-sex conflict has been implicated in slower population growth rates in Seychelles magpie robins (López-Sepulcre *et al.* 2009), and as discussed above, individual fitness and population growth may both decrease in bearded vultures when unmated males join established pairs to breed cooperatively (Carrete *et al.* 2006). However in hihi, *Notiomystis cincta*, extreme harassment by males of females under highly skewed ASRs appears not to reduce female survival or breeding success (Ewen *et al.* 2011). In their major review of the consequences of polyandry for population viability, Holman and Kokko (2013) stress that there may be no visible demographic consequences of polyandrous mating if females go on producing more progeny than can survive. Both positive and negative effects of polyandry on demographic parameters may only become apparent once birth and death rates are modified by environmental change. The plummet towards extinction of swift parrots due to an introduced sex-specific predator may offer the necessary circumstances for elucidating the impact of sexual conflict and increased genetic polyandry on individual fitness and population viability. Our study adds to a growing body of studies showing that anthropogenic threats to wild populations may impact differentially on individuals and have further, less obvious, consequences for threatened species (Boukal *et al.* 2008; Garcia *et al.* 2012). In the case of swift parrots, measures to limit the impact of sugar gliders (Stojanovic *et al.* 2018; Stojanovic *et al.* 2019) should improve population growth both by limiting female mortality and increasing reproductive rates via higher rates of monogamy.

14. Genetic estimation of effective population size

14.1. Introduction

Conservation management of declining populations is heavily reliant on estimating population size. Genetic techniques may be used with high reliability to detect small population sizes if enough samples can be collected (Frankham *et al.* 2010). If detected early, intervention to increase population size may be implemented successfully (Legault *et al.* 2013). Reliable estimates of the effective population size (N_e) may be used to provide an early warning for conservation managers of the risks to genetic viability of small populations (Luikart *et al.* 2010). If intervention is not possible or is implemented too late, then Allee effects (inverse density dependence) may arise and exacerbate extinction risk in some small populations (Stephens and Sutherland 1999). However, in some cases, populations have survived at small size with low genetic diversity, demonstrating that genetic factors are not the sole reasons leading to extinction (Reed 2010). Still, for species with small, spatially aggregating populations, estimating N_e may be especially important because once genetic diversity is lost, it may be impossible to restore.

There are multiple approaches for estimating N_e using genetic techniques; however, the underlying assumptions and data requirements for these approaches can sometimes be challenging to meet. For example, species that are cryptic, or occur at low densities or in challenging environments may severely curtail opportunities to collect genetic material. Furthermore, collection of samples from within overlapping generations may be unavoidable (e.g., in long-lived species), which violates key assumptions of some genetic approaches for estimating population size (Luikart *et al.* 2010). Similar challenges exist when siblings are overrepresented within a batch of genetic samples (which may occur in species where nests/litters are more easily sampled than free-ranging adults). However, recent advances in analytical techniques have aimed to overcome some of these challenges, opening the opportunity for estimating N_e of age-structured, long-lived populations (Waples *et al.* 2014). Evaluating the most appropriate analytical approach for the available genetic data is not straightforward, and conservation scientists may be faced with multiple analytical options for estimating N_e from opportunistically collected genetic samples. Given this uncertainty, comparing different approaches for estimating N_e of imperfect genetic data is of high value to conservation practitioners.

Swift parrots *Lathamus discolor* are a good model to evaluate different approaches for estimating N_e because they exist as a single, nomadic, panmictic population (Stojanovic *et al.* 2018). Threatened by an introduced predator (Stojanovic *et al.* 2014) and severe deforestation (Webb *et al.* 2018), swift parrots are predicted to decline by 95% within three generations, making them critically endangered (Heinsohn *et al.* 2015). Estimates of swift parrot population size are mostly based on expert opinion (Garnett *et al.* 2011) and the true contemporary population size is unknown. Genetic samples have been collected from swift parrots to address other research questions (Stojanovic *et al.* 2018; Heinsohn *et al.* 2019). However, these were taken primarily from siblings within nests found during routine monitoring and thus present

analytical challenges for the estimation of N_e. In this study, we (1) employ three widely used genetic techniques to compare N_e estimates; (2) consider the ecology of our model species by factoring in available information about the mating system and predation rates; and (3) predict that the three methods should yield comparably low estimates of N_e given predicted population declines (Heinsohn *et al.* 2015) and severe habitat limitation due to deforestation (Webb *et al.* 2018; Vergara-Tabares *et al.* 2020).

14.2. Methods

Sample collection and DNA extraction followed Stojanovic *et al*. (2018) and the samples from that study are included here. Nestlings (n = 310) from 93 nests were sampled between 2010 and 2015. On average, 17 (range = 9–32) nests were sampled per year (mean brood size = 3.3). At nests found during standardized monitoring (Webb *et al.* 2014), we collected blood, feathers, or tissue (from dead birds) from 165 male, 142 female, and three unknown sex nestlings, plus 31 additional adults (22 males and 9 females). We extracted DNA from blood following standard procedure for nucleated erythrocytes (Smith and Burgoyne 2004), and from feather/tissue using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) with some protocol modifications (Chapter 8). We used seven microsatellite loci (Stojanovic *et al.* 2018) to construct full genotypes of 341 samples and the 2550F/2718R primers (Fridolfsson and Ellegren 1999) for the molecular sexing. The seven microsatellite loci used in this study were exhaustively tested with POWSIM power analysis previously, demonstrating the risk of type I errors to be below 5% and almost complete power (96%) for detecting genetic differentiation at F_{ST} = 0.01 (Stojanovic *et al.* 2018). We have also shown that these same markers have enough power to recover all unique genotypes even among siblings ($PI_{sibs(7)}$ = 0.002) over a large sample of individuals (Heinsohn *et al.* 2019).

We used three different methods to estimate the effective population size and we calculated genetic estimates of N_e ($\hat{N}_e$) or both $\hat{N}_e$ and the effective number of breeders ($\hat{N}_b$) depending on the method (Table 14.1). We first estimated N_e using the modified temporal method based on variance (Jorde 2012) and the linkage disequilibrium (LD) method based on linkage (Waples 2006). N_e was first estimated using each method individually and then we combined different estimates of N_e within and across methods (Waples and Do 2010). Since our study system contained overlapping generations, we also estimated N_b each year using the LD method and the sibship assignment method (Wang 2009). We caution that our estimates of N_b are biased by the number of nests accessible for DNA sampling in any given year (due to challenges in accessing nests in structurally unsound trees, more nests are detected than can be sampled for genetic material).

The modified temporal method (Table 14.1) applies the theory of standard temporal method of discrete generations (Jorde and Ryman 1995), but with modifications to account for overlapping generations (Jorde 2012). We used the software GONe (Coombs *et al.* 2012) with all 341 samples divided into five spatially discrete subpopulations by breeding season. Plan 1 of the software was used (i.e., samples taken after reproduction or non-lethally before reproduction) and an estimated maximum census size of 2,158 (Garnett *et al.* 2011) was used for the calculations. We ran two models using life tables according to two different scenarios: (1) equilibrium and (2) predation by sugar gliders. For these scenarios, we used previously

published demographic values (Heinsohn *et al.* 2015): (i) nine age classes, (ii) age at first reproduction is 2 years old, (iii) average clutch size of 3.14 in equilibrium and 1.87 in predation scenario, (iv) 45% juvenile mortality, and (v) 29.4% adult mortality (for both males and females) in equilibrium while 29.4% adult male and 56.4% adult female mortalities in predation scenario. Clutch sizes were automatically rescaled to result in population lambda = 1 (Coombs *et al.* 2012). GONe computed a correction factor "C" and generation times for each model. We report the mean genetic drift estimator Fs (Jorde and Ryman 2007) over all intervals per model with 95% confidence intervals (CIs).

Table 14.1. Methods used to estimate the effective populations size (N_e) and the effective number of breeders (N_b).

Method	Estimates	Software	Specifications and key assumptions	Demographic information used	References
Modified temporal	$\hat{N}_e$	GONe	Accounts for overlapping generations using life tables; closed populations.	Demographic data from Heinsohn *et al.* (2015) for equilibrium and predation scenarios.	Jorde (2012); Coombs, Letcher & Nislow (2012)
Linkage disequilibrium (LD)	$\hat{N}_e$ & $\hat{N}_b$	NeEstimator 2.1	Selective neutrality; discrete generations; closed populations.	Adjusted estimates by AgeNe using demographic parameters from Heinsohn *et al.* (2015).	Waples (2006); Do *et al.* (2014); Waples, Do & Chopelet (2011)
Sibship assignment	$\hat{N}_b$	COLONY 2	Calculates maximum likelihoods by the frequencies of full- and half-sib dyads; discrete generations; closed populations.	Estimated demographic parameters from the genotypes of a single cohort.	Wang (2009); Jones & Wang (2010)

For the LD method (Table 14.1), we used the software NeEstimator v2.1 (Do *et al.* 2014) with a threshold frequency of 0.01 for screening out rare alleles, assumed random mating (Heinsohn *et al.* 2019), and calculated 95% CIs for $\hat{N}_e$ by a jackknife-across-samples method (Jones *et al.* 2016). To estimate N_e, we used adult samples ($n = 31$) and the pooled juvenile samples ($n = 310$). We also calculated $\hat{N}_b$ using juvenile samples from each year ($n_{2010} = 54$, $n_{2011} = 24$, $n_{2012} = 57$, $n_{2013} = 72$, $n_{2014} = 21$, $n_{2015} = 82$). We calculated the relationships among the total census population size (N), adult census size (N_A), N_e, and N_b with the software AgeNe (Waples *et al.* 2011), using the same demographic parameters and scenarios as in the previous model, with Poisson factor = 1 and the number of newborns each year (N_1) = 93 in equilibrium and 23 in predation scenario estimated from empirical data (Stojanovic, unpublished data). In AgeNe, we used the following demographic information (Heinsohn *et al.* 2015): AL (adult life span) = 8, α (age at maturity) = 2, and CVf (coefficient of variation of mean number of offspring for adult life span) = 0. We calculated three adjusted values (following Table 3 of Waples *et al.* 2014) using (1) true N_b/N_e from AgeNe; (2) AL and α; and (3) AL, α, and CVf. $\hat{N}_e$ of this method is most strongly affected by N_e in parental generation but also influenced by N_e in the previous three generations.

The sibship assignment method is a hybrid approach (Table 14.1), estimating demographic parameters from the genotypes of a single cohort, which are then used to estimate N_b based on the frequencies of full- and half-sib dyads in the sample (Wang 2009). We used the software COLONY 2 (Jones and Wang 2010) with the following parameters based on default options of the manual and our previously published findings: (i) no inbreeding (Stojanovic *et al.* 2018),

(ii) social monogamy, i.e. monogamous males and polygamous females (Heinsohn *et al.* 2019), (iii) full sibship size scaled down by the full likelihood method (default), (iv) one "very long" run (recommended), and (v) "high" precision for full likelihood (default). We provided the known maternal sibships (siblings from a single nest were recorded during sampling) and ran the estimations using the same juvenile sample set described for the LD method to estimate N_b.

14.3. Results

Genetic estimates of N_e derived from the modified temporal and LD methods are presented in Table 14.2A. Using the modified temporal method, the equilibrium scenario produced a N_e estimate only slightly higher than the scenario that included predation by sugar gliders (Table 14.2A).

Table 14.2. Estimates of (A) effective population size (N_e); and (B) effective number of breeders (N_b) of swift parrot (*Lathamus discolor*) with 95% confidence intervals (in parentheses). Methods used are the modified temporal, linkage disequilibrium (LD), and sibship assignment (SA) methods. We include adjusted LD estimates using the following adjustments: $\hat{N}_{e1\text{ and }b1}$ = true N_b/N_e from AgeNe; $\hat{N}_{e2\text{ and }b2}$ = AL and α; $\hat{N}_{e3\text{ and }b3}$ = AL, α, and CVf (see methods). Unweighted harmonic means (HM) were calculated for N_b estimates using the SA results and the corresponding adjusted LD estimates.

(A)

	Modified temporal method		Linkage disequilibrium method			
	$\hat{N}_e$			Adjusted LD estimates		
Samples	Equilibrium	Predation	$\hat{N}_e$	$\hat{N}_{e1}$	$\hat{N}_{e2}$	$\hat{N}_{e3}$
Adults	-	-	471 (25–∞)	502	392	396
Nestlings	-	-	126 (86–195)	135	105	106
All	46 (31–64)	44 (30–62)	131 (104–169)	140	109	110

(B)

		LD method	Adjusted LD estimates			SA method	HM of LD and SA methods		
Year	Sample size	$\hat{N}_b$	$\hat{N}_{b1}$	$\hat{N}_{b2}$	$\hat{N}_{b3}$	$\hat{N}_b$	$\hat{N}_{b1}$	$\hat{N}_{b2}$	$\hat{N}_{b3}$
2010	54	34 (19–72)	36	44	45	37 (23–59)	36	40	41
2011	24	10 (5–20)	10	13	13	30 (18–56)	15	18	18
2012	57	28 (19–42)	30	36	38	34 (22–55)	32	35	36
2013	72	44 (30–72)	47	58	60	55 (36–82)	51	56	57
2014	21	37 (12–∞)	39	48	50	29 (17–61)	33	36	37
2015	82	37 (24–59)	39	48	50	54 (36–80)	45	51	52

Demographic estimates for the equilibrium and predation scenarios in AgeNe were very similar in some respects but not others. Using equal number of newborns (N_1) for both scenarios, in predation the total census population size (N) was reduced by 14%, adult census size (N_A) by 22%, N_e by 13%, and N_b by 29%. When we used empirical N_1 values (75% reduction under predation), the N was reduced by 79%, N_A by 81%, N_e by 78%, and N_b by 83% under the predation scenario. The N_e/N_A ratio increased by 13% under predation. The N_e/N ratio was 0.52 in equilibrium scenario and increased only by 2% (to 0.53) in predation. We used $N_e/N = 0.5$ to calculate total census population size from the different estimates (Figure 14.1).

The LD method calculated the average $\hat{N}_e$ of previous generations for adults and pooled juveniles (Table 14.2A), and $\hat{N}_b$ when calculated for each year using juvenile samples only (Table 14.2B). The adjusted LD estimates of N_e fall into the range of 392–502 for adult samples, and into the range of 105–135 for nestling samples (Table 14.2A). The adjusted

N_b estimates were much lower, ranging from 10 to 60 across years, showing some fluctuations among the years (Table 14.2B). Following calculation of Waples & Do (2010), the weighted harmonic mean of $\hat{N}_b$ was 32 over the study period (although we caution that this is an underestimate because not all nests were accessible for sampling each year).

The sibship assignment method calculated $\hat{N}_b$ as ranging from 29 to 55 over the study period (Table 14.2B). We also calculated the unweighted harmonic means of the adjusted LD and sibship assignment methods (Table 14.2B), as they have roughly comparable precision (Waples and Do 2010).

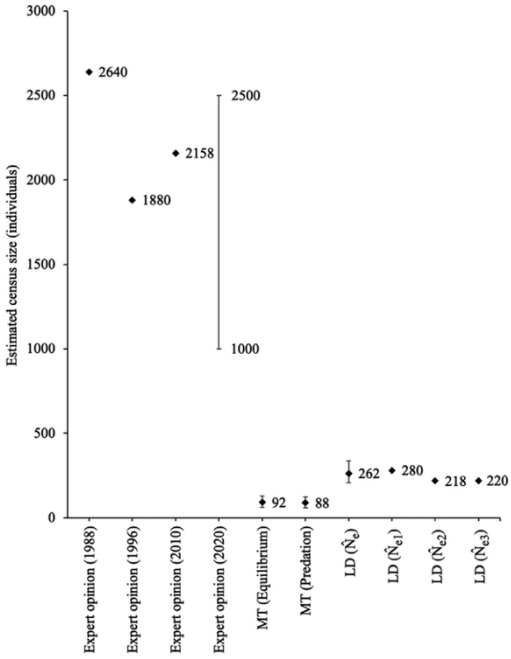

Figure 14.1. Total census population size estimates of the swift parrot (*Lathamus discolor*) based on previous expert opinions (Brown 1989; Brereton 1996; Garnett *et al.* 2011; BirdLife Australia 2020) and the current study. Expert opinions from 1988 and 1996 were multiplied by 2 as they reported breeding pairs originally. Genetic estimates of census size are based on results in Table 14.2A (MT = modified temporal, LD = linkage disequilibrium methods) and $N_e/N = 0.5$ calculated by AgeNe. Confidence intervals are displayed where available.

14.4. Discussion

Our results reveal that swift parrots have a worryingly small $\hat{N}_e$ and support the critically endangered status of the species. These findings highlight the need for urgent conservation action to halt further population decline. Cumulative impacts of severe deforestation (Webb *et al.* 2018) and predation (Heinsohn *et al.* 2015) likely contributed to a small contemporary N_e, which results in loss of evolutionary potential and increased vulnerability to genetic stochasticity (Frankham *et al.* 2010). The "50/500 rule" in conservation genetics says that N_e should not fall below 50 to minimize short-term problems related to inbreeding and should remain above 500 in the long term to maintain sufficient evolutionary potential (Franklin and Frankham 1998). With some qualifications, this rule still has a useful place in conservation biology (Jamieson and Allendorf 2012), while others even recommended that this rule should be changed to 100/1,000 (Frankham *et al.* 2014). In either case, our results indicate that low population size may be an emerging threat for swift parrots. Using the most realistic predation scenario for the study species, the modified temporal method estimated N_e of swift parrots at 44, which indicates that the species might not be able to maintain sufficient evolutionary potential even in the short term. Unfortunately, the species is a monotypic genus (Provost *et al.* 2018), and options like genetic rescue via hybridization with related species are not available to conservation managers. The small population sizes we found may make swift

parrots particularly vulnerable to the emergence of Allee effects, i.e. the decrease of their population growth rate in parallel to the decline of their population size (Crates *et al.* 2017). In the case of the critically endangered orange-bellied parrot *Neophema chrysogaster*, establishment of a captive population temporarily decreased genetic diversity of the wild population (Morrison *et al.* 2020b), and release of captive bred birds to the wild has not corrected their population decline (Stojanovic *et al.* 2020). For swift parrots, cessation of deforestation of breeding and foraging habitat may stop exacerbating habitat limitation and predation (Stojanovic *et al.* 2014), which is an important step for halting further reduction of the wild population size.

14.4.1. The timespan of estimates

It is important to note that different methods provide complementary information and one is not necessarily better than others. We estimate that N_e of earlier generations than those sampled (i.e., before 2010) may have reached 500 for adults and 105–135 for combined data from nestlings (Table 14.2A). Since our adult swift parrot samples reflect N_e of earlier generations than those of nestlings, the drop in estimated N_e between adult and juvenile samples may be evidence for steep population decline (Heinsohn *et al.* 2015). Temporal methods estimate the harmonic mean of $\hat{N}_e$ between years sampled. In the current study, this is between 2010 and 2015 and of a magnitude well below 100 (Table 14.2A). A study on the endangered black-fronted tern *Chlidonias albostriatus* showed that a combination of $\hat{N}_e$ and $\hat{N}_b$ is more appropriate assessing the status of threatened mobile and migratory species than solely $\hat{N}_e$ when using mixed-age samples (Schlesselmann and Robertson 2020).

N_b estimates the effective number of adults that bred each year to produce the cohorts of nestlings sampled (Waples *et al.* 2018). We found substantial overlap among the annual estimates of N_b and their associated confidence intervals using the LD and sibship assignment methods with even lower values (all < 60; Table 14.2B). The number of nests accessible for sample collection varied each year depending on local habitat availability (Webb *et al.* 2014; Webb *et al.* 2019), thus fluctuation in $\hat{N}_b$ was likely an artifact of sampling effort and the fraction of nests that could be reached by a climber in a given year. These considerations, and that all siblings per nest were included in the analysis, influenced the random sampling assumption of the estimators (Waples and Anderson 2017). We suspect that this downwardly biased $\hat{N}_b$ and $\hat{N}_e$ (Jorde 2012).

14.4.2. Equilibrium versus predation scenarios

Demographic $\hat{N}_e$ and $\hat{N}_b$ both decreased in the predation scenario, but N also decreased under predation modulating the change in the N_e/N ratio. If mortality was increased uniformly across both sexes, the expectation is that both N_e and N would decline but N_e/N would increase because N decreased more than N_e (Kuparinen *et al.* 2016). In our predation model, however, mortality only increased for adult females, which skewed the sex ratio and reduced the N_e/N ratio. In the modelled scenario, the two factors approximately cancelled each other out, so there was only a 2% change in the N_e/N ratio, when N is the total population. However, because only adult females had a reduction in N, adult census size (N_A) declined by a greater percentage. As a consequence, the reduction in abundance was proportionally larger for adults (81% reduction of N_A compared to 79% reduction of N when using empirical data), and the N_e/N_A ratio

increased under predation. Shortage of females may curb the number of males that breed. However, female swift parrots often mate with more than one male (Heinsohn *et al.* 2019), so we predicted that this is a case of "social monogamy," where one male helps raise the chicks, but more than one male contributes genetically.

14.4.3. Effects of genetic markers and source of samples

We previously demonstrated that our handful of microsatellite loci had adequate power to differentiate populations (Stojanovic *et al.* 2018) and even individual swift parrots (Heinsohn *et al.* 2019). Using these seven loci for genotyping our samples, the probability that we find two identical genotypes in a population including siblings is 1 in 500 (Heinsohn *et al.* 2019). Hence, our presented estimates can be considered as reliable and more molecular data would have not necessarily enabled better estimates of N_e. A recent study on the population dynamics of a critically endangered Australian songbird used over 500 SNP loci (Crates *et al.* 2019a), without being able to obtain a more precise N_e estimate with the same methods tested here. Although microsatellites are sometimes considered outdated markers in modern genomics (Allendorf *et al.* 2010), if they are carefully selected and tested for the species of interest, even a small number of them can provide reliable population size estimations.

Our source data (comprising few adults but many juvenile siblings) are typical of many conservation projects, where opportunistic collection of DNA samples during monitoring may violate some assumptions of some analytical approaches. However, as for many species, detailed demographic data derived from ecological studies were available to provide important context for our genetic analyses. By employing three commonly used analytical procedures, and using available samples (and subsets), we provide an important comparison of the range of estimates that may be derived using imperfect but commonly available genetic data. Combining multiple consecutive cohort samples in the LD method was shown to downwardly bias $\hat{N}_e$ and $\hat{N}_b$ in the great tit (Waples *et al.* 2014). Although the modified temporal method accounted for overlapping generations, we believe (based on assumptions of short generation time in our model species) that the generational overlaps led to underestimation of the real N_e. Samples from more widely spaced generations could provide much more precise estimates for the temporal method (Jorde and Ryman 1995). However, achieving such a sampling scheme is unrealistic for this and many threatened species (Grimm *et al.* 2016), particularly if ongoing declines in population size and available habitats limit opportunities to collect genetic material from increasingly scarce animals.

Importantly, we show that reliable population estimates can be derived from juvenile samples only. This is important because juveniles may sometimes be easier to sample than adults. For studies where samples from adults are unavailable, we recommend that the LD or the sibship assignment method be utilized (Waples and Anderson 2017). The results of this approach can then be treated as minimum estimates of N_e, and still yield important population data for conservation management.

14.4.4. Expert opinions versus genetic estimators

In conservation management, knowing the census population size (N) of a species or population may be more practical for implementing conservation action, than simply using $\hat{N}_e$ alone. Since the habitat use of the swift parrot varies over time (Webb *et al.* 2014), it is hard

to produce a reliable population size estimate based on traditional census methods (Figure 14.1). Breeding population size was estimated at 1,320 breeding pairs in 1988 (Brown 1989) and 940 in 1996 (Brereton 1996). In 2010, a survey estimated a total of 2,158 birds, including immatures, and based on this information, the total population was assessed to comprise 2,000 mature birds (Garnett *et al.* 2011). Today, BirdLife estimates the population size as between 1,000 and 2,500 individuals (BirdLife Australia 2020). According to our population viability analysis (Heinsohn *et al.* 2015), which was based on these probably optimistic assumptions, the swift parrot population size in 2020 was predicted to be around 1,000 birds or below. Estimates of the N_e/N ratio in other species (Frankham 1995; Luikart *et al.* 2010) showed that N_e is often 10–20% of the census population size, while our study estimated that N_e is much closer to the census N (~ 50%). The mean $\hat{N}_e$ of the adjusted LD estimates (based on all samples) was 120 in the current study (Table 14.2A). Hence, we can interpolate a minimum potential contemporary population size between 60 and 338 individual swift parrots across methods (Figure 14.1). This estimated N is much lower than previously published estimates derived from expert elicitations.

Expert opinions are often used in conservation prioritizations (Carter *et al.* 2000; Keith *et al.* 2011; Martin *et al.* 2012) but they can also fail to account for the true conservation status of species (Charney 2012; Reed *et al.* 2017). If opinions overestimate the true population size of a species, as shown in this study, they can be detrimental to species conservation. When assumptions are met, genetic methods can provide a more objective method to estimate N_e and interpolate N across taxa. The stark contrast between our results and previous expert estimates of population size underscores the risk to effective conservation when lack of empirical evidence results in over-reliance on opinion for deciding the status of species perceived to be at risk. Our study shows that experts can make overly conservative population estimates based on weak evidence and that this may create a false sense of security about a species' conservation status for decades. This may be especially apparent for species like the swift parrots that are iconic and often observed by the public. We hope our study encourages others to more carefully scrutinize population estimates (and conservation status listings) of species where empirical data (either direct counts or population genetics) are lacking.

14.4.5. Conclusion

We show that it is possible to detect a small population size despite occurrence of siblings and overlapping generations of a critically endangered parrot species. Our study has important implications for other threatened species with unknown population sizes and demonstrates that even a few microsatellite markers can be enough to derive reasonable estimates of effective population sizes if they have adequate resolution. However, the resolution of markers should be tested beforehand. Based on our results, we recommend using the adjusted LD method on pooled juvenile samples to estimate contemporary N_e and the harmonic mean of the LD and sibship assignment methods to estimate N_b. Conservation planning depends on reliable estimates of population size, and we caution against over-reliance on expert opinions of population size to assess the conservation status of a species when it is feasible to obtain empirical estimates of key population parameters.

15. Comparing genetic markers for estimating population size

15.1. Introduction

Conservation practitioners are increasingly embracing the application of genetic tools and techniques (Frankham *et al.* 2010; Hendricks *et al.* 2018; Schweizer *et al.* 2021). However, molecular ecology is a fast-moving field, and non-specialists are faced with a bewildering array of marker types and analytical techniques that can be applied in different ways to answer the same questions. For example, microsatellites or short tandem repeats (STRs) have been the marker of choice for most population genetic studies for the last few decades (Parker *et al.* 1998; Olah *et al.* 2021a), but more recently single nucleotide polymorphisms (SNPs) have become increasingly popular due to the increased genomic coverage, low genotyping error, and lower costs / faster turnaround associated with sequencing them in a massively parallel fashion (Anderson and Garza 2006). Identifying a suitable marker is the first step for a conservation genetics project, and navigating the choice may be daunting for a non-specialist (Allendorf *et al.* 2010; Benestan *et al.* 2016). This is especially so in context of threatened species management, where estimates of population genetic parameters may determine which intervention approaches are applied.

A recent review of differences between marker types found that for most studies where the exact same individuals or populations were sequenced using both microsatellites and SNPs, population heterozygosity (H_O and/or H_E) estimates are usually lower when SNPs are used, reflecting differences in methodology and approach between marker types (Sunde *et al.* 2020). Furthermore, fine scale patterns of genetic structure were more detectible and discerned in greater detail when using SNPs than microsatellites (Sunde *et al.* 2020). Highly polymorphic microsatellite markers are especially useful for threatened species where genetic variation has been greatly reduced (Hauser *et al.* 2021). On one hand, up-front expenses to design and validate microsatellite markers for each species are often high, whereas prior genetic information is not required for many SNP methods. On the other hand, microsatellite markers are often applicable across closely related species with some ascertainment bias (Gebhardt and Waits 2008a; Olah *et al.* 2015), and rapidly accumulating genomic data makes development of new markers faster and cost-effective for many species (Abdelkrim *et al.* 2009). Still, studies that compare effective population size (N_e) estimates between marker types remain relatively scarce (Athrey *et al.* 2018; Lemopoulos *et al.* 2019). Understanding the extent to which these patterns are representative across taxa is important both for studies of molecular ecology in general, as well as for species conservation where genetic information is used for planning.

In this study, we use the Critically Endangered swift parrot *Lathamus discolor* as a model to contrast estimates of N_e, observed and expected heterozygosity (H_O and H_E), the inbreeding coefficient (F_{IS}), and spatial structure using different, putatively neutral marker types on the exact same individuals. We previously used seven polymorphic microsatellite markers to show this nomadic species is panmictic and lacks spatial structure across its Tasmanian breeding range (Stojanovic *et al.* 2018) and to estimate N_e (Chapter 14). Here, we obtain SNPs for the same set of individual parrots to quantify the impact of marker type on the accuracy and

precision of estimates. We then recalculate H_O, H_E, F_{IS}, N_e, census population size (N), and spatial structure using SNP loci with an additional four years of data to evaluate the impact of larger sample sizes on these estimates. Finally, we assess the effect of the number of SNP markers on these estimates.

15.2. Methods

All DNA samples were collected with approval from the Australian National University Animal Ethics and Experimentation Committee, and under scientific licenses from the Tasmanian Government. Our earlier studies comprised a sample of total 341 swift parrots: 310 nestlings from 93 nests and 31 adults collected between 2010 and 2015 (Stojanovic *et al.* 2018; Olah *et al.* 2021b; Olah and Stojanovic 2024). These were added to an additional four years of data (2016–2019; n = 457 parrots) and provided to Diversity Arrays Technology Pty. Ltd. (DArT; Canberra, Australia) for DNA extraction, sexing, and SNP genotyping using DartSeq™ protocols, combining complexity reduction based on restriction fragments from enzyme pairs, with next generation sequencing (NGS) technology (Jaccoud *et al.* 2001; Kilian *et al.* 2012). They successful genotyped 782 individuals, including nestlings from 231 nests. We filtered SNPs using the '*dartR'* package in R (Gruber *et al.* 2018; R Core Team 2025), based on a 0.99 reproducibility threshold, retaining one variant per sequence tag, variants without missing data (call rate threshold of 1), and minimum minor allele frequency of 3%.

All analyses were repeated on two data subsets: (1) 324 individual parrots included in our previous analysis (Chapter 14) for which both microsatellites and SNPs were available – hereafter called the 'comparison' dataset, and (2) the 'full' dataset of 781 individuals. The comparison dataset highlights the effect of marker type on the magnitude and precision of each parameter, whereas the full dataset updates each parameter using SNPs on a larger sample of parrots. The comparison dataset is slightly smaller than that of our previous studies because we excluded 17 individual swift parrots from the earlier work whose SNP genotyping failed.

We calculated H_O, H_E, and F_{IS} in GenAlEx v.6.5 (Peakall and Smouse 2012). It has been suggested that at least 2–11 times the number of SNP loci may be required for comparable resolution in genetic differentiation to microsatellites (Kalinowski 2002; Morin *et al.* 2004; Haasl and Payseur 2011). To test the importance of the number of SNPs on the accuracy and precision of estimates, we calculated the focal parameters for the full filtered SNP data set as well as a random subset of 100 SNPs. We visualised the relative position of individual samples grouped by *a priori* locations in the genetic space by conducting a principal coordinate analysis (PCoA) in GenAlEx v.6.5 (Peakall and Smouse 2012). We examined genetic structure/admixture among individual genotypes first by Principal Component Analyses (PCA) and then by sparse non-negative matrix factorization (snmf) algorithms, both implemented in the *'LEA'* package in R (Frichot and François 2015). The 'snmf' function computes ancestries of each individual in chains of given clusters (K = 1–7 with 20 repetitions each). It estimates an entropy criterion that evaluates the quality of fit of the statistical model to the original data using a cross-validation technique where we blinded 10% of the data. Low cross-entropy values reflect the number of most likely ancestral populations that best explains the genotypic data. We kept the alpha parameter at the default. We also explored how the number of SNP loci influenced the patterns of genetic structure and repeated the admixture analysis on randomly

selected subsets of 100, 500, 1,000, and 2,000 SNPs in the full dataset. Finally, we investigated any potential structure in the datasets by implementing discriminant analysis of principal components (DAPC) using the *'adegenet'* package in R (Jombart and Ahmed 2011). This approach is sensitive to fine genetic differences among populations and identifies clusters where the within-group component of variation is minimized compared to between-group variation. First, we used the *'find.clusters'* algorithm (K = 1–10) retaining all principal components (PCs) and then obtained the number of PCs for DAPC by cross-validation. We selected the lowest number of components with the greatest power of discrimination (60 for microsatellites, 100 for SNPs in the comparison dataset, and 200 for SNPs in the full dataset).

We followed our earlier approach (Chapter 14) and used the linkage disequilibrium (LD) method (Waples 2006; Waples and Do 2010; Waples 2024) implemented in the software NeEstimator v2.1 (Do *et al.* 2014) to estimate N_e. Briefly, we used a threshold frequency of 0.02, assumed polygamy, and calculated 95% CIs for N_e by a jackknife-across-samples method (Jones *et al.* 2016). We calculated adjusted values of N_e (see formula in Table 3 of Waples *et al.* 2014) by using AL (adult life span = 8 years), α (age at maturity = 2 year), and CVf (Coefficient of variation of mean number of offspring for adult life span = 0). We used previously published data on life history parameters (Chapter 14) to calculate the rate of N_e / census population size (N) using the software AgeNe (Waples *et al.* 2011). We also calculated the weighted harmonic mean of N_e estimates based on microsatellites and SNPs, using the effective df for weights (Waples and Do 2010). We calculated N_e and N estimates on samples in the comparison dataset (n = 324, of which n_{adults} = 29, $n_{nestlings}$ = 295) and the full dataset (n = 781, of which n_{adults} = 44, $n_{nestlings}$ = 737).

15.3. Results

The fully updated sample comprised 358 females, 438 males, and 14 swift parrots of unknown sex (Olah and Stojanovic 2024). Filtering left us with 3,761 SNP loci for analysis. We report H_O, H_E, and F_{IS} for the comparison and full datasets in Table 15.1. There was a large difference in estimates of heterozygosity between the two marker types, with microsatellites producing much higher absolute values. In contrast, the difference between markers in F_{IS} values was smaller, given the confidence intervals around the estimates. SNPs produced comparably high confidence results regardless of the data or marker subset used, although using fewer SNP loci resulted in lower F_{IS} values (Table 15.1).

Table 15.1. Estimates of H_O, H_E, and F_{IS} with standard errors in parentheses for the comparison (n = 324) and full (n = 781) datasets of swift parrots *Lathamus discolor*.

Dataset	Marker type	Number of markers	H_O	H_E	F_{IS}
Comparison (2010–2015)	Microsatellites	7	0.678 (±0.075)	0.681 (±0.073)	0.007 (±0.012)
	SNPs	100	0.254 (±0.014)	0.258 (±0.014)	0.018 (±0.009)
	SNPs	3761	0.257 (±0.002)	0.263 (±0.002)	0.025 (±0.002)
Full (2010–2019)	SNPs	100	0.252 (±0.014)	0.256 (±0.014)	0.018 (±0.007)
	SNPs	3761	0.257 (±0.002)	0.263 (±0.002)	0.027 (±0.001)

Population genetic structure could not be found regardless of marker type using PCoA, PCA, or the *'find.clusters'* algorithm (see supplementary in Olah *et al.* 2024). Admixture analysis could not detect any genetic structure (Figure 15.1) regardless of the number of SNP loci used. However, when six *a priori* swift parrot collection locations were used, DAPC successfully parsed out some of the locations using the SNP but not the microsatellite dataset (Figure 15.2).

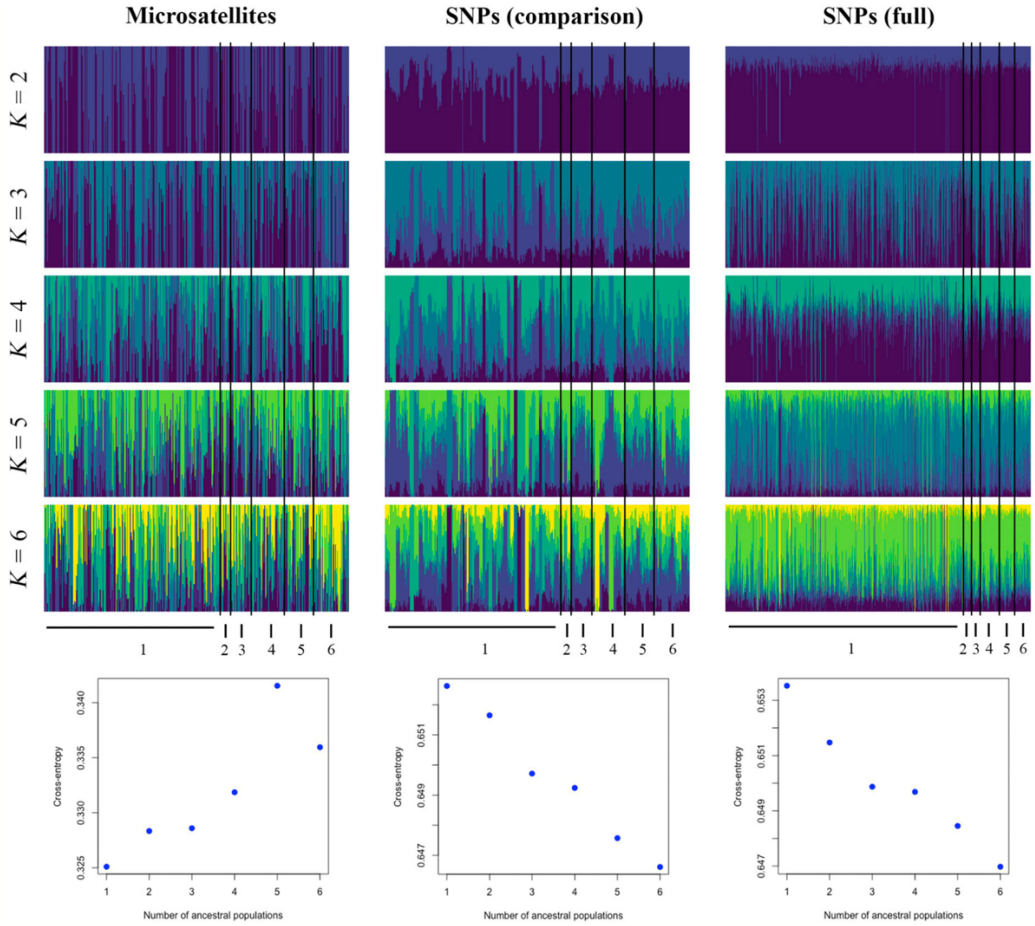

Figure 15.1. Admixture analyses with sparse non-negative matrix factorization (SNMF) on the microsatellite and SNP datasets of swift parrots *Lathamus discolor*. Displayed are the individual ancestry coefficients for $K = 2$–6 clusters on top and the cross-entropy plots below. Collection locations in Tasmania demark Bruny Island (1), Southern Forests (2), Meehan Range (3), Wielangta & Rheban (4), Buckland (5), and Eastern Tiers (6). The microsatellite and the comparison SNP datasets are based on the same 324 individuals (collected between 2010 and 2015), and the full SNP dataset is based on a total of 781 samples (spanning from 2010 to 2019).

Estimates of N_e are reported in Table 15.2 for the comparison and full datasets. For the comparison dataset, relative to microsatellites, SNPs improved the precision of N_e estimates. The accuracy of estimates derived from microsatellites and SNPs was comparable, but the microsatellites consistently resulted in slightly lower estimates of N_e for nestling, adult, or pooled samples. Neither marker could calculate finite upper confidence limits for adult parrots and estimates between markers and datasets varied considerably. Based on the full dataset,

estimates of N_e were nearly double the weighted harmonic mean of the comparison dataset for all samples (i.e. nestlings + adults, Table 15.2). Based on the adjusted N_e for the full dataset when all samples were pooled, we estimate the census population size of swift parrots was 498 individuals (CI: 469–530) over the decade when the samples were collected. When results from the total number of SNPs were compared to those from just 100 randomly selected SNP markers, N_e estimates were similar. This was not the case for the adult samples in the comparison dataset, where N_e estimates for the total SNPs were almost sixfold larger than that for the 100-SNP-subset (see supplementary in Olah *et al.* 2024).

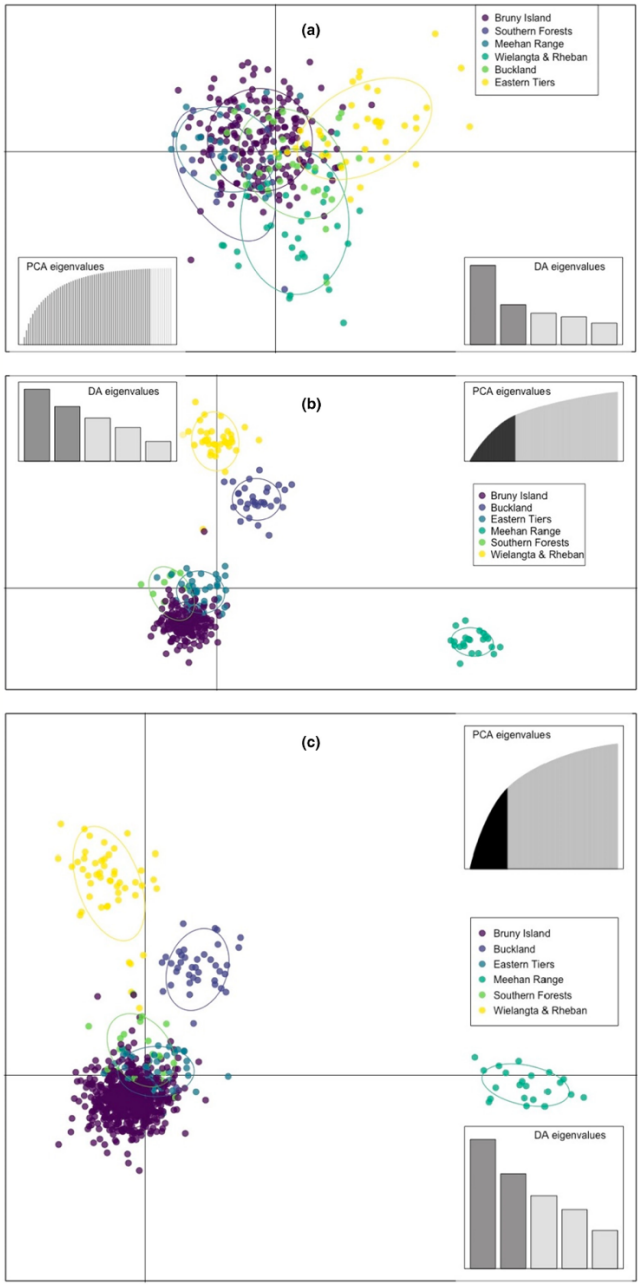

Figure 15.2. Grouping of swift parrot *Lathamus discolor* samples by discriminant analysis of principal components (DAPC) based on (a) microsatellite data, (b) SNP data from the same individuals ($n = 324$), and (c) SNP data from the full dataset ($n = 781$). Colours show collection locations.

Table 15.2. Estimates of effective population size (N_e) calculated with the linkage disequilibrium (LD) method for (1) a comparison dataset of $n = 324$ swift parrots genotyped using seven microsatellites (msat) and 3761 single nucleotide polymorphisms (SNPs), and (2) a full dataset of $n = 781$ individuals (between 2010 and 2019) based on the 3,761 SNPs only.

	Comparison dataset						Full dataset (SNPs only)			
Age	*n*	N_e (msat)	N_e (SNPs)	N_e (HMw)	N_e (HMw adj)	*N* (adj)	*n*	N_e	N_e (adj)	*N* (adj)
Adults	29	371 (23–∞)	1838 (455–∞)	1805 (419–∞)	1516 (352–∞)	2861 (664–∞)	44	193 (77–∞)	162 (65–∞)	306 (122–∞)
Nestlings	295	127 (66–301)	162 (149–177)	162 (147–177)	136 (124–149)	256 (234–281)	737	319 (302–339)	268 (253–284)	506 (478–537)
All	324	125 (67–270)	159 (146–173)	159 (144–174)	133 (121–146)	251 (229–276)	781	314 (296–334)	264 (249–281)	498 (469–530)

Note: We calculated the weighted harmonic mean (HMw) of the two estimates of N_e in the comparison dataset, the adjusted (adj) N_e, and census population size (N) for adults, nestlings, and all samples. Parentheses show 95% CIs calculated by a jackknife-across-samples method.

15.4. Discussion

For calculating common population genetic diversity metrics, SNPs had narrower confidence limits compared to microsatellites when applied to the exact same individual swift parrots (Table 15.1). For effective population size, the estimates made with SNPs fell within the confidence limits of the estimates based on only seven microsatellites, when nestlings or all samples were considered (Table 15.2). Using only microsatellite markers, we could not detect any genetic structure here (Figure 15.1) or in our previous study (Stojanovic *et al.* 2018), which is unsurprising due to the nomadism of the swift parrot (Webb *et al.* 2014). SNPs, however, were shown to recover finer population structure in the same individuals when compared to equivalent microsatellite data in fish (Jeffries *et al.* 2016), amphibians (Camacho-Sanchez *et al.* 2020), and bats (Dufresnes *et al.* 2023). Here, only DAPC on the SNP dataset including *a priori* collection locations and many PCs meaningfully grouped the samples (Figure 15.2), although broader structure was not detected in the admixture analyses. Thus, both markers were useful, but consideration must be given to the level of resolution needed for a particular application and the underlying population genetic structure (Rosenberg *et al.* 2003; Thalamuthu *et al.* 2005).

The number of markers should also be taken into consideration. Other comparative studies have used more microsatellites (n = 15 (Weinman *et al.* 2015), 111 (Layton *et al.* 2020), 328 (Liu *et al.* 2005)) or fewer SNPs (n = 960 (Tokarska *et al.* 2009), 300 (Glover *et al.* 2010), 38 (Dann *et al.* 2013)), so we also tested random subsets of our SNP profile to be more comparable with our microsatellites. The outcomes of these smaller subsets were similar to the full SNP dataset (Table 15.1 and Figure 15.1). It has been shown with admixture analysis simulations that even if gene flow exists between populations, adding more markers should considerably raise power to detect structure (Haasl and Payseur 2011). A study on two groups of a threatened fish could only find signs of introgression when using SNPs but not microsatellite data for the same individuals (Bradbury *et al.* 2015). In our admixture analyses, we could not detect population structure in the swift parrot with 100 or over 3,000 SNPs. However, sampling from admixed populations might be misleading, as the signal of population structure can be diluted after several generations since admixture (Haasl and Payseur 2011). Nevertheless, the DAPC analyses were able to identify some weak structure with the full SNP dataset and even with only 100 SNPs (Figure 15.2).

Both marker types estimated a lower H_O than H_E. As expected, SNPs and microsatellites were less comparable for the absolute values of H_O, H_E, and F_{IS}. Microsatellite markers are deliberately designed to target highly polymorphic regions of the genome, whereas the SNPs used in this study are not. Consequently, SNPs produced lower H_O and H_E estimates, and higher estimates of F_{IS} than microsatellites. This is consistent with other studies (Liu *et al.* 2017; Hauser *et al.* 2021), reflecting the design of the markers and the biased sampling of the genome. H_O and H_E are expected to be higher for multi-allelic loci like microsatellites, even if we must assume that this heterozygosity is underestimated because of homoplasy (although homoplasy is also likely to affect biallelic markers). Thus, F_{ST} and F_{IS} estimates are generally smaller for microsatellites (Liu *et al.* 2017; Crates *et al.* 2019b; Tereba and Konecka 2020). The lower heterozygosity in SNPs however, can influence their power in paternity exclusion analyses (Morin *et al.* 2004), which are important tools in threatened species conservation (Heinsohn *et al.* 2019). SNP derived estimates of H_O, H_E, and F_{IS} were robust to sample size, producing nearly identical estimates for the comparison and full datasets.

N_e estimates were higher for the full dataset (from 2010 to 2019) than the smaller comparison data (Table 15.2) – whether this is a biological effect or just a sampling artefact is unclear. When only adult swift parrots were considered (29 in the comparison and 44 in the full dataset), estimates between markers and datasets varied considerably (Table 15.2). When all SNPs were used with the scarce adult samples in the comparison dataset, it resulted in higher values of estimates compared to the 100-SNP-subset. Such upward bias could emerge, for instance, when using just one sibling per nest and biasing the sampling towards more unrelated individuals (Waples and Anderson 2017), but in our case we did not take this approach. In the full dataset however, using solely adult samples depressed the N_e estimates, possibly due to non-random sampling because some families must have been over-represented in the adult samples. The bioinformatic processing of SNPs could also be responsible for these discrepancies (O'Leary *et al.* 2018). Regardless, in accordance with previously published simulations and empirical data (Waples and Anderson 2017), we caution against only using a small number of adult samples of unknown pedigrees or purging sibling samples from the dataset when estimating N_e.

Swift parrots are critically endangered, and their conservation status hinges on estimations of population size, number of subpopulations, and trends in both over time and space (Webb *et al.* 2020). Thus, it is crucial to know if different markers yield results divergent enough to alter the interpretation of their conservation needs. Our earlier results of low N_e, using a handful of microsatellite markers (Chapter 14) were subject to debate within the swift parrot conservation community, and estimates of population size derived from expert opinion prevail in published conservation assessments (Webb *et al.* 2020). Although there are clear methodological artefacts on the precision of estimates shown here, microsatellites and SNPs are reassuringly comparable when larger number (nestlings) of samples are used (Table 15.2), supporting our earlier conclusions that the species has a smaller population than previously assumed.

Previous studies comparing microsatellite and SNP markers were conducted on plants and fish (Bradbury *et al.* 2015; Jeffries *et al.* 2016; Lemopoulos *et al.* 2019; Sunde *et al.* 2020), amphibians (Camacho-Sanchez *et al.* 2020), birds (Zimmerman *et al.* 2020; Hauser *et al.* 2021), and mammals (López-Bao *et al.* 2020; Szatmári *et al.* 2021; Dufresnes *et al.* 2023).

These found similar results regarding heterozygosity estimates to our current study, but mainly focused on detecting genetic structure among populations (e.g. Jeffries *et al.* 2016) or relatedness among individuals (e.g. Lemopoulos *et al.* 2019). Our work contributes to a growing number of studies that contrast the application of different markers to the exact same set of individuals, and our results are comparable to other taxa (Bradbury *et al.* 2015; López-Bao *et al.* 2020; Hauser *et al.* 2021; Szatmári *et al.* 2021; Dufresnes *et al.* 2023). We advance knowledge further by comparing effective population size estimates between markers in threatened wildlife. The rapid advances in genetic tools, including genomics (Brandies *et al.* 2019), are likely to yield further detailed insights into the genetic traits of populations (Chapter 11). Practitioners can be confident that a shift toward SNPs in the general literature does not necessarily make existing knowledge from microsatellites obsolete for conservation purposes.

Summary

Introduction

This thesis, based on my published research, provided a comprehensive overview of the conservation challenges facing the avian order Psittaciformes and highlighted my significant contributions to this field over nearly two decades. The work was unified by a narrative that progressed from a broad analysis of global threats to the development and application of specific, innovative conservation solutions. It represented a creative, interdisciplinary approach to research, connecting diverse data types to generate new knowledge about conservation genetics in the context of interacting environmental and anthropogenic threats.

The thesis was structured to tell a compelling story. It began in Part I, by defining the global scope of the problem. This section examined the multifaceted threats that have made parrots the most threatened bird order, using comparative and regional analyses to identify key ecological, socio-economic, and human factors driving their decline. These chapters established the critical context for the research that followed, demonstrating the urgent need for robust conservation strategies.

The thesis then transitioned to Part II, showcasing my direct engagement with these conservation challenges in the field. Here, the research shifted from broad-scale analyses to a detailed, hands-on investigation of wild macaw populations, combining field ecology with pioneering genetic methods to address specific ecological questions and conservation needs.

Finally, Part III synthesized the advanced technological and analytical core of my work. This section highlighted my contributions to developing and applying cutting-edge genetic tools to assess population viability and inform conservation management, demonstrating how innovative science could be directly translated into applied conservation actions.

In essence, this thesis culminated in a unified body of work that not only diagnosed the plight of parrots but also provided concrete, scientifically grounded tools and strategies to ensure their long-term survival. The following sections will detail the key findings from each of these parts and outline how my research collectively advanced the field of conservation biology.

Summary of key findings

This thesis provided a comprehensive overview of the research and findings presented in each of its three parts, demonstrating a clear progression from problem to solution. The collective work generated significant new knowledge by diagnosing the primary threats to parrots, applying interdisciplinary methods in a field setting, and developing and evaluating cutting-edge genetic tools for conservation. Here, I also respond my initial research questions.

Part I: Extinction risk in parrots

The research in this part provided a critical diagnosis of the threats driving parrot decline globally.

What biological, socioeconomic, and anthropogenic factors drive the extinction risk of parrots on a global and regional scale?

a) *What are the global and regional patterns of parrot extinction risk, and what intrinsic biological and socioeconomic traits are associated with higher vulnerability?*

   My work confirmed that parrots are more threatened on average than comparable bird groups, with 28% of extant species classified as threatened under IUCN criteria. The analyses modelled the factors associated with this extinction risk, revealing that species with small historical distribution sizes, large bodies, long generation times, and forest dependency are most likely to be threatened. The work also highlighted that extinct parrot species were often island endemics. Our research further demonstrated that socio-economic factors, such as a country's urbanization and per capita GDP, have correlated with extinction risk.

b) *How do global habitat destruction trends and illegal wildlife trade dynamics contribute to parrot extinction, particularly in biodiversity hotspots?*

   In Oceania, we found that invasive species posed an especially severe threat, while in Indonesia, our work applied a criminological model to show that demand- and opportunity-based factors are key drivers of the illegal parrot trade. The findings provided a robust framework for analysing wildlife trade and highlighted the need for conservation initiatives in regions where they are lacking, with a focus on areas like New Caledonia, New Zealand, and Australia, and especially the vast Indonesian archipelago. We ultimately established that protected areas alone are insufficient to combat these negative trends and that the future of parrots depends on guiding conservation policies in key hotspots.

Part II: An interdisciplinary approach to macaw research

This section applied an interdisciplinary approach to address specific research and conservation questions in the Peruvian Amazon.

How can an interdisciplinary approach, combining field ecology with pioneering genetic methods, provide new insights into the biology and conservation of wild parrot populations?

a) *How do ecological factors, such as nest site selection and parasitism, influence the breeding success and survival of wild macaws?*

   Our work showed that artificial nest boxes for Scarlet Macaws had similar reproductive parameters to natural nests, which has important implications for conservation management where natural nest hollows have been removed. Additionally, we described a new technique for removing bot fly larvae from nestlings, which can be widely applied by veterinarians and scientists.

b) *How can genetic methods be applied to understand the population structure, movements, and demography of macaws in challenging tropical environments?*

A key methodological contribution was the development and validation of non-invasive genetic tagging on macaws, a technique that proved to be a reliable method for studying parrot populations without the need for capturing individuals. We applied this technique to show that Red-and-green Macaw communities at different clay licks were not genetically structured and consisted of individuals that moved frequently between sites. Finally, our research used landscape genetics to provide baseline information on natural dispersal barriers, revealing that high elevation might act as a natural barrier to gene flow.

Part III: Conservation genetics of parrots

This part detailed my methodological contributions and their direct application to parrot conservation.

How can advanced genetic and genomic techniques be used to effectively assess the viability of threatened parrot populations and provide data-driven recommendations for conservation management?

a) What is the role of genetic tools in advancing our understanding of the biology of threatened parrot species and conservation?

Our comprehensive review of genetic techniques in parrot conservation highlighted how the field has transformed the study of parrots and proposed a consortium of scientists to share expertise in conservation genomics. Our studies on the Critically Endangered Swift Parrot confirmed severe extinction risk and small effective population size. We showed how sex-biased mortality from an introduced predator reduced individual fitness and population viability, providing a crucial link between anthropogenic threats and their less obvious demographic consequences.

b) Which is the best method to estimate effective population size based on sibling data, and how do different molecular markers influence population genetic estimates?

Our work provided important guidance for conservation practitioners by demonstrating that genetic estimates of population size can be derived from even a few microsatellite markers, and that a shift toward SNPs in the literature does not necessarily make existing knowledge from microsatellites obsolete for conservation purposes. Our research advanced knowledge by comparing effective population size estimates between different markers in threatened wildlife, providing a valuable contribution to the field. We revealed a worryingly small N_e and cautioned against over-reliance on expert opinions when empirical genetic estimates were obtainable. This work provided practical recommendations for estimating N_e and effective number of breeders (N_b) from imperfect genetic data.

Conclusions and contributions to research and wider public

This thesis represents a unified and substantial body of work that collectively advanced the field of conservation biology, particularly in the realm of parrot conservation. Through the synthesis of 16 peer-reviewed publications, my research systematically addressed critical gaps in our understanding of parrot extinction risks and provided innovative, empirically grounded

solutions. The primary intellectual contribution of this thesis lies in its pioneering application of an interdisciplinary approach, which synergistically combined large-scale comparative meta-analyses, intensive field ecology, and cutting-edge conservation genetics to tackle complex, real-world conservation challenges.

This work diagnosed the global plight of parrots, confirming their disproportionately high extinction risk relative to other avian groups, and identified the intricate web of intrinsic biological factors and anthropogenic pressures—from habitat loss and fragmentation to the dynamics of illegal wildlife trade—that drive their decline. This comprehensive understanding of 'what' threatens parrots and 'where' these threats are most pronounced laid the essential groundwork for targeted conservation interventions.

Crucially, this thesis moved beyond diagnosis to develop and apply novel methodological frameworks, thereby addressing the 'how' of effective parrot conservation. My research pioneered the validation and application of non-invasive genetic tagging from moulted feathers in tropical environments, a significant advancement given the challenges of studying wild parrot populations. This method, applied for the first time on non-invasively sourced parrot DNA in such challenging conditions, proved to be important for understanding population structure, estimating local population sizes, and exploring dispersal barriers in previously hardly tractable species. Furthermore, our work demonstrated the utility and limitations of various molecular markers, providing critical guidance for conservation practitioners navigating the rapidly evolving landscape of genomic tools. By integrating these advanced genetic techniques with extensive field-based observations in diverse ecosystems—from the Peruvian Amazon to Tasmania, and the trade hotspots of Indonesia—this thesis offered a holistic perspective essential for informing conservation strategies. For instance, the Salmon-crested Cockatoo was uplisted to Endangered (BirdLife International 2025) based on our results from Chapter 5.

The international recognition of this research is evidenced by its significant impact within the scientific community and beyond, with publications appearing in leading international journals and several papers achieving high citation counts, including one ranking in the top 1% of cited papers in Environment/Ecology. The work has garnered substantial media attention, a testament to its broader relevance and my commitment to science communication. For many of the papers serving as the basis of this thesis I have created video abstracts to enable better visual understanding (see Publications serving as the basis for the thesis). My science communication strategy is further exemplified by the production of three documentary films that transmit the findings of these studies to the broader public:

- The Macaw Project (2006; https://youtu.be/xBrrnbvD5Wc),
- The Macaw Kingdom (2018; https://youtu.be/3ieppWouPxk),
- The Indonesian Parrot Project (2019; https://youtu.be/x9PJVypHXBw).

Outlook and future directions

The present thesis has contributed significantly to the growing body of knowledge in conservation genetics, landscape ecology, and avian conservation. By integrating these disciplines, our work provided a holistic framework for understanding and addressing the complex threats faced by parrots. Considering this, the following new research directions are

summarized, highlighting opportunities for the broader scientific community to advance conservation efforts.

Our work on the global extinction risks of parrots demonstrated the critical role of both biological and socioeconomic factors in determining the conservation status of a species. While we identified key drivers of decline, a more nuanced understanding is needed on the interactions between these factors. Future research should investigate how specific socioeconomic trends, such as shifting global markets for illegal wildlife, interact with intrinsic biological traits to influence vulnerability. This would not only enhance our understanding of anthropogenic threats but also contribute to the developing field of conservation criminology (Moura *et al.* 2018).

In Peru, we showed the benefits of artificial nest boxes for the breeding success of large macaws. Our published nest designs have been used successfully for macaws in other regions, e.g. in Mexico, where natural nest hollows became scarce due to habitat destruction, and these nest boxes should be also tested in other populations. We also highlighted the potentially negative overheating effect of the current nest designs to parasitism in macaw nestlings. Based on our findings, new studies should develop these further, with better thermoregulations and collect nest success and temperature data from previous and new nest designs.

This thesis pioneered the use of non-invasive genetic methods to study parrots in challenging field environments. While our work validated these techniques for population-level studies, there is a clear opportunity for future research to apply them at a larger scale. For instance, our landscape genetics study on macaws provided baseline information on natural dispersal barriers. Future studies should utilize these techniques to analyse how increasing fragmentation and anthropogenic dispersal barriers, such as deforestation, affect gene flow in other widespread parrot species. This would provide valuable insights into landscape-level ecological processes and better inform the design of protected area networks (Keller *et al.* 2015).

Our research on the Swift Parrot highlighted the direct link between genetic parameters, breeding systems, and population viability. The genetic data our work provided can serve as a crucial baseline for conservation breeding programs. Future studies should focus on how the gained genetic information can be used to establish a genetically managed back-up population, as has been successfully done for other parrots like the Kakapo (Clout 2006). This would help to preserve genetic diversity and mitigate the risk of extinction from stochastic events in the wild (Faust *et al.* 2025), until proper forest protection measures are established at their key habitats in Tasmania (Webb *et al.* 2018).

Finally, this thesis demonstrated the power of next-generation sequencing and genomic data for conservation. As sequencing costs continue to fall, future research should focus on developing standardized genomic toolkits and databases for threatened species. My new research, supported by the Australian Research Council, aims to build such a forensic genomic toolkit to track the illegal parrot trade. This work will not only provide a powerful model for law enforcement but also advance fundamental research on population genetics, molecular forensics, and disease ecology, serving as a springboard for similar efforts on other taxa. These ideas offer opportunities for future study designs and may help to build more effective, data-driven conservation schemes.

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